EVALUATING THE POLLUTION FROM MUREŞ RIVER ON ARAD-PECICA SECTOR BASED ON ENZYMATIC ACTIVITIES FROM SEDIMENTS (WESTERN ROMANIA)

Sebastian TREITLI*, Mărioara Nicoleta FILIMON*, Claudia PETRUCEAN*

*West University of Timişoara, Faculty of Chemistry-Biology-Geography, Department of Biology, Timişoara, Romania
Corresponding author: Sebastian Treitli, West University of Timişoara, Faculty of Chemistry-Biology-Geography, Department of Biology, 16 Pestalozzi, 300115 Timişoara, Romania, Phone: 0040256592646, Fax:0040256592622, e-mail: sebastian.treitli@yahoo.com

Abstract. Seven sediment samples were collected from the river Mureş from the Arad-Pecica sector and were measured the following enzymatic activities: catalase, actual and potential dehydrogenase, urease and reduction of trivalent iron (Fe^{3+}). All this activities were detected in all analyzed samples. Based on relative values of the enzymatic activities, the enzymatic indicator of the sediment quality (EISQ) was also calculated. The lowest value of EISQ was observed in point P6 in close proximity to the discharge area of waste water resulted from water treatment plant of the Pecica town, which could indicate a heavy pollution from the water treatment plant of the town. The pollution of Mureş river on the analyzed sector can be determined by livestock farms, gravels and water treatment plants which do not work properly.

Keywords: sediment, enzymatic activity, Mureş

INTRODUCTION

Microorganisms are, without doubt, the most abundant organisms in the world and form the basis of most food webs [14]. They may be characterized as the driving force for the cycle of elements [17]. In the sediment they play a major role in decomposition and mineralization of organic matter because they have the ability to metabolize certain compounds by enzymatic luggage which they have it. These enzymes can act intracellular, and extracellular, when break down complex substances that cannot pass the cellular membrane. In addition to the capacity of mineralization of organic substances the microorganisms can neutralize certain toxic compounds in the sediment, compounds that could be potentially toxic to other aquatic organisms. This process is very important in the aquatic environment because releases mineral substances necessary for primary producers.

Bacterial enzymatic activities ensure the decomposition of vegetal or animal organic debris and allow the optimal evolution of biogeochemical circuits of the main chemical elements like C, N, S, P, Fe [15].

Enzymatic activities are considered sensitive to pollution and have the advantage that they are easy to determine without sophisticated and expensive instruments [25].

In many studies the enzymatic activity is used to evaluate the quality of the environment the water toxicity [6, 28]. Recently, fundamental views of the capacities of the bacterial communities inhabiting sediments to cope with pollutants have been given [3], but relatively little is known about these bacterial communities [1].

Sediments have a fundamental role in biogeochemical circuits in the aquatic environment. Here is completed the mineralization processes of organic substances which were not decomposed in the water column [21].

The importance of microbial and enzymatic activity was frequently emphasized by many authors [19], but still insufficiently studied in our country.

Mureş the second longest river in Romania after Danube situated in the central-western part of the Romania. The basin has a length of 761 km in Romania and 42 km in Hungary.

This is one of the most important rivers of Romania but is has a lot of sources of pollution like cities and mining activity or chemical industry. Several studies show the anthropogenic effect on Mureş river [27, 24, 26, 22]

In this paper we proposed to track the influence on enzymatic activity of microorganisms in sediments at confluence of different tributaries with Mureş especially with potentially polluting tributaries.

Thus were selected seven sampling sites before and after the confluence of Mureş with different brooks or canals that we have considered having a pollution potential.

MATERIALS AND METHODS

The samples were collected in November 2009 from the Mureş river bed at 50 cm from the bank using Ekman grab, the sampling point locations are indicated in Fig. 1.

The collected samples were placed in plastic containers and transported laboratory for further analysis of them. The following enzymatic activities were quantitatively established for the sediment samples: catalase (CA), urease (UA), reduction of trivalent iron (RFE) and dehydrogenase: actual dehydrogenase (ADA) and potential dehydrogenase (PDA).

The catalase activity (CA) is performed expressing the decomposition intensity of H_2O_2 which is formed in the aerobic microorganism breathing process [2]. Catalases are enzymes that are found in almost every aerobic and facultative aerobic organism [29]. The microbial peroxidases are involved in decomposition of a large number of aromatic compounds including potentially toxic compounds [18].

The reaction mixture for CA consisted of 3 g sediment + 10 mL phosphate buffer + 2 mL solution H_2O_2 3%. The incubation was carried out at room temperature for 1 h. Then the reaction was stopped by adding 10 mL de H_2SO_4 4N and 78 mL distilled water and the solution obtained was filtered through filter paper. From the obtained filtrate is taken 25 mL and is introduced in an Erlenmeyer glass. Then is added 2.5 mL H_2SO_4 4N and the solution is titrated with a solution of $KMnO_4$ 0.05 N until appears a weak pink coloration [9]. CA was expressed as mg H_2O_2/g.f.s.(gram fresh sediment)

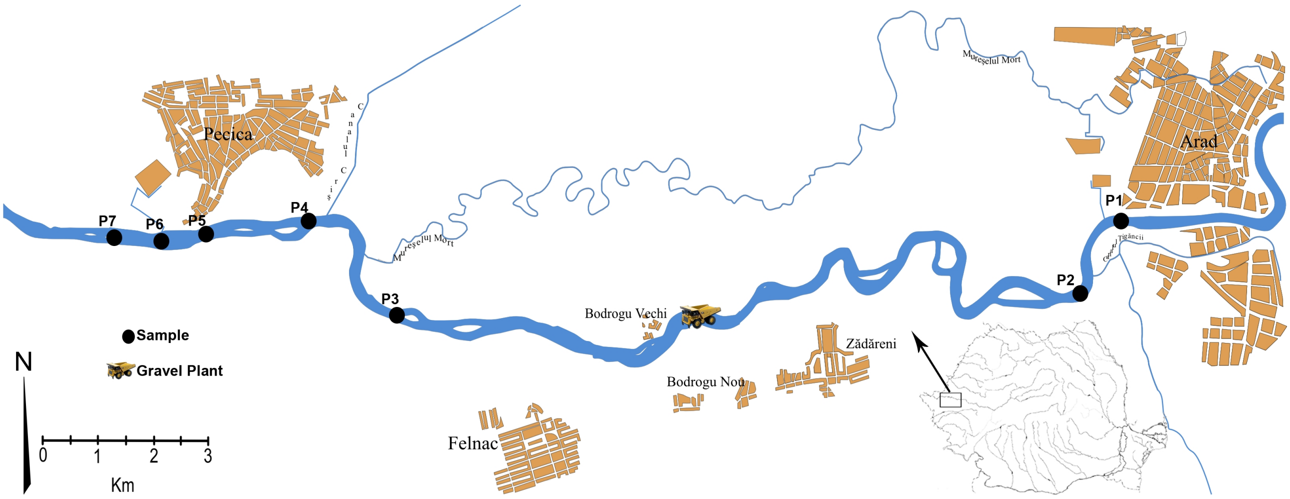

Figure 1. Map of Mureş on Arad–Pecica sector and the sampling points location. P1- Downstream Arad, P2- Downstream Canalul Ţigăncii, P3 - Upstream Mureşelul Mort, P4 – Downstream Mureşelul Mort, P5 - Pecica Town, P6 – Water treatment plant Pecica, P7 – Downstream Water treatment plant Pecica.

Actual dehydrogenase activity (ADA) and potential (PDA) were determined using described methods [5]. The reaction mixture consisted of 3 g sediment + 0.5 mL TTC 3% solution + 1mL distilled water and for PDA is added 1 mL glucose solution 3%. The incubation was carried out at 37^o C for 48 h. ADA and ADP were represented as mg formazan/g.f.s. measuring absorbance at 485 nm.

Dehydrogenase is an enzyme produced by microorganisms in the soil which acts on organic compounds in soil and releases hydrogen which is transferred to compounds that accept H^+.

Urease activity (UA) is expressing the decomposition intensity of urea in NH_3 + CO_2. The reaction mixture consisted of 5 g sediment + 2 mL toluene + 5 mL phosphate buffer + 5mL urea solution 3%. The mixture was incubated at 37^o C for 24 h. The formed ammonia is extracted with a KCl solution and is determined by nesslerization, and is measured the absorbance at 445 nm [9].

Trivalent iron reduction activity (RFE) can be used as ecotoxicological test to evaluate the pollutants' effect over the microbiota of the soil or sediments.

To determine this activity the following reaction mixture was used: 3 g sediment + 5 mL glucose solution 4% + 0.5 mL Ferric chloride solution ($FeCl_3$) and 5 mL distilled water. The incubation was carried out at 28^o C for 48 h.

After incubation in each tube was added 5 mL solution KCl 2M, after which the content is filtered through filter paper. From filtrate is taken 7 mL and added 1mL of α,ά-dipiridil solution. For the obtained solution is measured the absorbance at 240 nm [11].

The quality of the studied sediment was enzymologically characterized by the intensity of the enzymatic activities defined by the values of the enzymatic indicator of the sediment quality (EIQS) which is calculated on the formula issued by Muntean in 1996 [20].

RESULTS

The dehydrogenase activities (Fig. 2) presented variations along the investigated area, higher values of actual dehydrogenase activity were found in sampling point P2 (Downstream Canalul Ţigăncii) (19.583) and P5 (Pecica Town) (8.708), these values may be attributed to additional contribution of organic substances provided by human activity.

Results of the catalase activity (Fig. 3) show the decomposed hydrogen peroxide in sample. The catalase activity does not have a large variations along the sampling points. 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.

Urease activity (Fig. 4) does not show large variations in analyzed samples however the biggest value of this activity was found downstream of confluence with Mureşelul Mort and Canalul Criş (P4), which could indicate a fecal pollution due to the presence of a large number of farms along Canalul Criş, farms with numerous stalls which leads to increase of the necessary substrate for this microorganisms.

Zootehnical farms are known as polluters of aquatic environment, most of the times here are discharged animal sewage, which contains a high quantity of nitrogen compounds, or most of the times these sewages are improperly stored, these infiltrate in groundwater and from here those compounds get in the river. This situation can appear when the storage is near the river, when precipitation stimulates the infiltration process and the nitrogen compounds reach the river. The fecal pollution is betrayed by the presence of an intense urease activity.

The same problem was observed in the sample point P2 what can be explained by discharge of Canalul Ţigăncii which brings wastewater from Arad town.

The trivalent iron reduction activity (Fig. 5) does not have a large variations. The highest values can be found in sampling point P3 (Upstream Mureşelul Mort) which could indicate an increase of the substrate on which these microorganisms act in this sampling point.

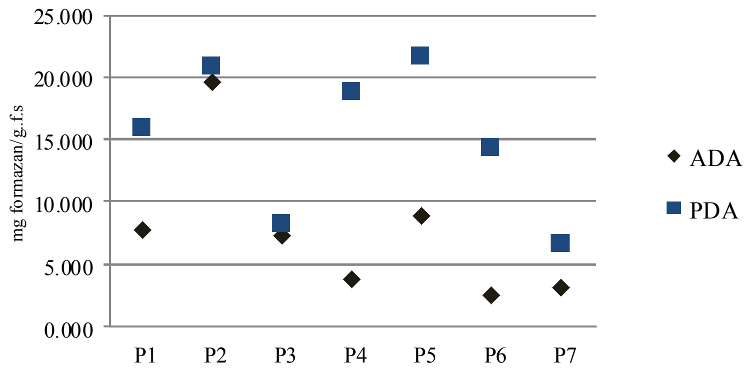

Figure 2. The intensities of actual and potential dehydrogenase activities in Mureş in 2009. P1- Downstream Arad, P2- Downstream Canalul Ţigăncii, P3 - Upstream Mureşelul Mort, P4 - Downstream Mureşelul Mort, P5 - Pecica Town, P6 – Water treatment plant Pecica, P7 – Downstream Water treatment plant Pecica.

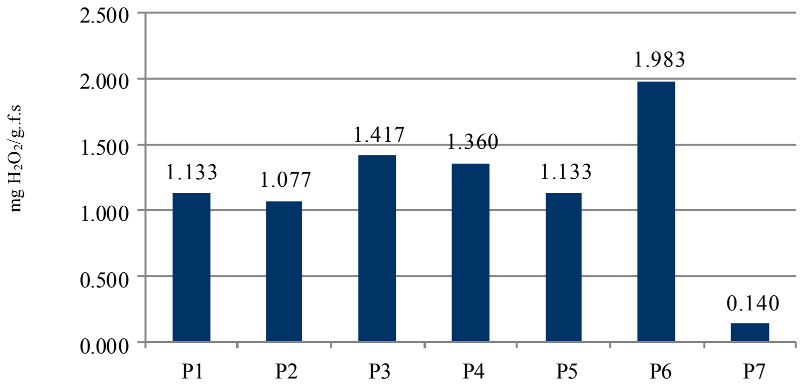

Figure 3. The intensities of catalase activity (CA) in Mureş in 2009. P1- Downstream Arad, P2- Downstream Canalul Ţigăncii, P3 - Upstream Mureşelul Mort, P4 - Downstream Mureşelul Mort, P5 - Pecica Town, P6 – Water treatment plant Pecica, P7 – Downstream Water treatment plant Pecica.

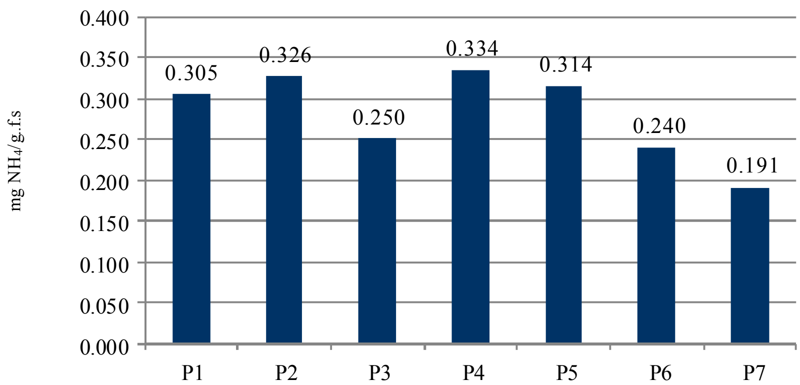

Figure 4. The intensities of urease activity (UA) in Mureş in 2009. P1- Downstream Arad, P2- Downstream Canalul Ţigăncii, P3 - Upstream Mureşelul Mort, P4 - Downstream Mureşelul Mort, P5 - Pecica Town, P6 – Water treatment plant Pecica, P7 – Downstream Water treatment plant Pecica.

EISQ provides an overview of enzymatic potential of sediments [12], decreased values of EISQ downstream can be explained by the pollution brought by tributaries.

The lowest value of EISQ (Fig. 6) was observed in the sample P6, just at the discharge of wastewater originating from the water treatment plant of Pecica town, which indicates a strong pollution made by the waters originating from the water treatment plant, inefficiently purified waters. This problem is because this station is being upgraded, temporary running on compact modules of water treatment which does not cover the real city needs. The highest value of EISQ was observed in P2 (Downstream Canalul Ţigăncii) and P3 (Upstream Mureşelul Mort), which shows higher enzymatic activities in this point. In P2 a reason for high enzymatic activity could be the discharge of wastewater which comes directly from Arad, and brings organic compounds which stimulate the enzymatic activity.

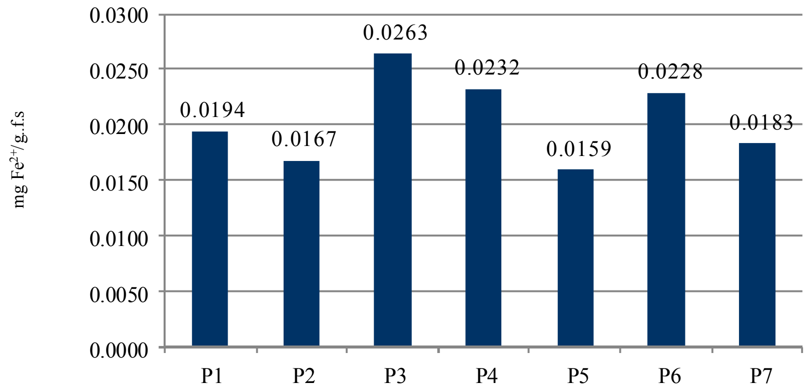

Figure 5. The intensities of trivalent iron reduction activity(RFE) in Mureş in 2009. P1- Downstream Arad, P2- Downstream Canalul Ţigăncii, P3 - Upstream Mureşelul Mort, P4 - Downstream Mureşelul Mort, P5 - Pecica Town, P6 – Water treatment plant Pecica, P7 – Downstream Water treatment plant Pecica

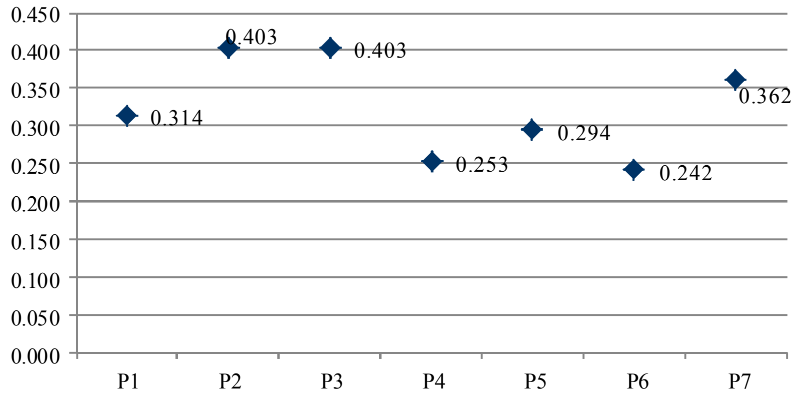

Figure 6. The enzymatic indicator of sediment quality from the Mureş river sediment in 2009. P1- Downstream Arad, P2- Downstream Canalul Ţigăncii, P3 - Upstream Mureşelul Mort, P4 - Downstream Mureşelul Mort, P5 - Pecica Town, P6 – Water treatment plant Pecica, P7 – Downstream Water treatment plant Pecica

DISCUSSIONS

The enzymatic activities showed variations of their intensity, these changes were according to the sampling point locations and type of enzymatic activity analyzed. Several studies [10, 13, 16] showed that the level of particular enzyme activity in water basins depends on the quantity and quality of organic compounds

Dehydrogenase activity (Fig. 2) was found in all analyzed samples, this activity accurately reflect the intensity of respiration [4] If the difference between ADA and PDA is small this illustrates the existence of an intense respiration process in sediments without an additional nutritive input.

The samples P2, P3 at a relative distance from Arad town and before confluence with brook Mureşelul Mort the actual dehydrogenase activity has values close to the potential dehydrogenase activity indicating a high respiratory intensity.

Decreased actual dehydrogenase activity after confluence with brook Mureşelul Mort can be explained through possible pollution brought by Mureşelul Mort and Canalul Criş waters which flows in Mureş.

The slump of dehydrogenase activity downstream of Bodrogu Vechi town may be due ballast exploitation from the river bed; exploitation which disturbs the sediment lowers the organic matter from the sediment and disrupts the activity of microorganisms in sediment.

Lower values of dehydrogenase activity close to Pecica town can be caused by sandy consistency of sediments from this area which is poorer in organic matter and does not allow the proliferation of microorganism. Studies that show the effect of sediment consistency on microorganism communities was detected in the literature [2]

Far less catalase activity was found in sample P7 downstream Pecica town and water treatment plant of town Pecica, here the remaining amount of H_2O_2 undecomposed was much higher than all other samples which indicate a lower number of microorganisms with a reduced enzymatic activity**.** Similar situations were reported in paper presented by Bodoczi and collaborators [2].

In some samples can be observed an increased trivalent iron reduction activity which demonstrates in the same time an increase of the substrate on which these microorganisms act, the main source of iron could be the water treatment plants which discharge their waters in this area. It is known that the trivalent iron is used in various salts like ferric sulfate ($Fe_2(SO_4)_3$), or ferric chloride $FeCl_3$ [7], this ferric compounds are used to remove phosphorus from water [8], this method is more used because of low price of chemicals needed. The trivalent iron salts does not precipitate sufficient or this salts are used in excess leading to their occurrence in the environment.

However the values of these activities are lower this may be explained by the cold season in which the samples were collected, knowing that the activity of these bacteria is more intense in summer and autumn seasons [12].

The EISQ value downstream of the water treatment plant shows an increase in enzymatic activity which shows a high auto regenerative capacity of the sediments [4].

Similar studies were made by other authors on Mureş river but upstream of our studied area [24, 23]. If we compare the data from our work with the literature [23] we can observe that the catalase activity has higher values that upstream of Mureş. Comparing our EISQ with the same study we can see that our values are close to the values present upstream in autumn.

The highest value of EISQ was observed in P2 (Downstream Canalul Ţigăncii) and P3 (Upstream Mureşelul Mort), which shows higher enzymatic activities in this point. In P2 a reason for high enzymatic activity could be the discharge of wastewater which comes directly from Arad, and brings organic compounds which stimulate the enzymatic activity.

Knowing the intensity of the enzymatic activities represents an important trend in the protection activity of the aquatic environment, by characterizing the degree of contamination of the aquatic ecosystems.

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