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Microbiological research on the enzymologic potential of Arieş river (Romania) sediments

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Abstract. Ten sediment samples from Arieș river were collected and these samples have been analyzed qualitatively enzymologically. In the sediment samples, the following enzymatic activities have been qualitatively determined: four oligase activities: maltase, saccharase, lactase and cellobiase and three polyase activities: amylase, dextranase and inulinase. The studied activities were determined in each samples and displayed variations in the intensities of the processes depending on the sampling place. Generally, the highest intensity of qualitative enzimatic activities were registered in case of the oligases.
Key words: sediment samples, oligase activities, polyase activitites, Arieș river.

Abstrait. Dix échantillons de sédiments ont été prélevés dans les rivière Arieș et ont été soumis l'analyse enzymologie qualitative. Dans les échantillons de sédiments ont été déterminées par les suivantes activité l'enzymatique quantitatives: quatre activité oligazique: les activité malatsique, saccharasique, lactasique et celobiazique, et trois activité polyazique: amilazique, dextarnazique at 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 si les activités oligazique.
Mot clés: rivière Arieș, sediment, activités oligazique, activités poliazique.

Rezumat. Zece probe de sediment prelevate din râul Arieș au fost supuse analizelor enzimologice calitative. In probele de sediment au fost determinatie cantitativ următoarele activiăţi enzimatice: 4 activitati oligazice: activitatea matazică, zaharazică, lactazică şi celobiazică, respectiv, trei activităţi poliazice: activitatea amilazică, dextrranazică şi inulinazică. Activităţile enzimatice studiate au fost evidenţiate în fiecare din probele analizate, manifestând variaţii ale intesnităţii lor în funcţie de punctul de prelevare a probelor. Cele mai intesne activităţi enzimatice au fost înregistrate in cazul activităţilor oligazice.
Cuvinte cheie: sediment, activităţi oligazice, activităţi poliazice, râul Arieş.

Introduction. 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 collumn is finalized (Munteanu et al 2001).

The ecologica succession and the evolution of the aquatic ecosistems in time, is the result of the complex interactions between biocenotic communities, respectivley between therse and abiotic characteristics of the life media, being in a constant modification (Burian 2002).

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 fazes (solids, liquids and gases), biotic components (numerous microorganisms), small organisms (like enzymes) and the abiotic elements (minerals, humus, organo-mineral agregates) are part of the physical, chemical and biological processes that take place in this media. All biochemical transformations on sediment level depend on the enzyme presence in this media (Gianfreda & Bollag 1996).

The microorganisms' action on the substrate is made on enzymatical way, being realized by oxidoreduction and hydrolisis, as a result of some final products of microbial metabolism, respectively (Muntean et al 2004).

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 2004).

The aim of our study was to establish the enzymatic potential of the Arieș river sediments which indirectly reflect the microbiota activity.

Material and Methods. The enzymological analysis of the sediments in Arieș river was performed during the summer of 2009 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 upstream and downstream of the main towns the river passes through and taking into consideration the main pollutants in the area.

From the sediment samples which were qualitatively analysed the following enzymatic activities resulted: 4 oligase activities – maltase (MA), saccharase (invertase) (SA), lactase (LA), and cellobiase (CeloA) and three polyase activities – amylase (AA), dextranase (DA) and inulinase (IA).

In order to establish the enzymatic activities, the paper circular chromatography technique was used. The reaction mixtures consisted of 3 g sediment + 2 ml toluene (for preventing the proliferation of microorganisms) + 5 ml 2% enzymatic substrate (maltose, saccharose, lactose, cellobiose, starch, dextrane and inulin)/ 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). The intensity of the enzymatic activities established by the spots' colour was marked with "+" signs and it is represented in Table 1 for oligase activities and in Table 2 for polyase activities.

Results and Discussion. The qualitative analysis of oligase and polyase activities makes the appreciation of the enzymatic potential of sediments more complex. The results of the qualitative analisys confirm the quantitative one (Bodoczi & Dragan-Bularda 2008).

Oligases were well represented in all the analysed sediment samples, but their intensity was higher downstream of Abrud, Sălciua, Turda and Luncani.

Table 1

The qualitative enzymatic activities evolution (oligase) on the sediments of the Arieș river in summer of 2009

The enzymatic activity	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*
MA	+++	+++	++	++	++	++	+++	+++	+++	+++
SA	+++	++	++	++	++	++	++	++	++	+
LA	+++	++++	++	+	++	+++	+++	+++	+++	++++
CelloA	++	+	++	++	++	++	+++	++	+++	+++

MA – maltase activity; SA –saccharase activity; LA – lactase activity; CeloA- cellobiase activity.
Sampling points: 1-Abrud upstream; 2-Abrud downstream; 3- Baia de Arieș upstream; 4- Baia de Arieș downstream; 5- Salciua upstream; 6- Salciua downstream; 7- Turda upstream; 8- Turda downstream; 9- Luncani upstream; 10- Luncani downstream.

Table 2

The qualitative enzymatic activities evolution (polyase) on the sediments of the Arieș river in summer of 2009

The enzymatic activity	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*
AA	++	+++	++	++	++	++	+++	++++	++	+
DA	+	+/-	-	+/-	-	-	+/-	++	+/-	+/-
IA	+	+	++	+	+/-	+	+	-	-	+/-

AA- amylase activity; DA- dextranase activity; IA- inulinase activity.
Sampling points: 1-Abrud upstream; 2-Abrud downstream; 3- Baia de Arieș upstream; 4- Baia de Arieș downstream; 5- Salciua upstream; 6- Salciua downstream; 7- Turda upstream; 8- Turda downstream; 9- Luncani upstream; 10- Luncani downstream.

Lactase activity (LA)

Lactase activity (B-galactosidase) it is less intense downstream of Baia de Arieș where the waste water from Sartas Valey overflow (Fig. 1). This water is the result of Rosia Montana mining activities, a fact that seems to have negative influence on the lactase activity. The maximum level of lactase activity was observed in the sampling point downstream of Luncani.

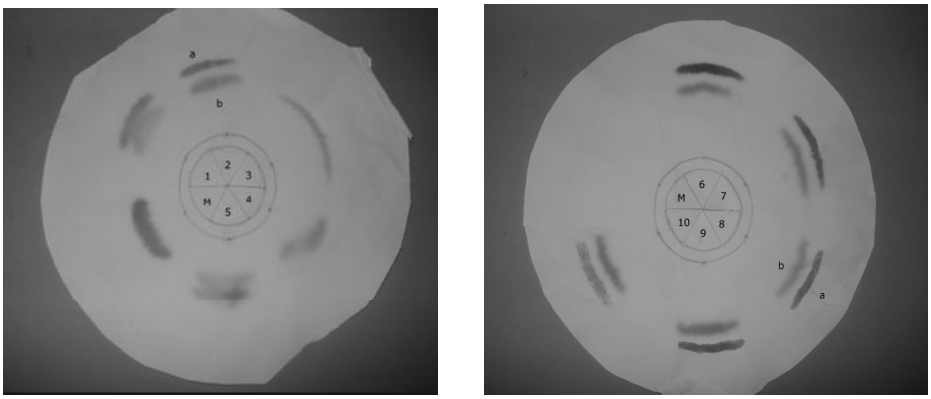

Figure 1. Lactase activity (LA). 1-10: sediment samples (see Tabel 1) + enzymatic substrate (lactose 2%); M – control – lactose solution 2%; a = glucose spot; b = lactose spot

Saccharase activity (SA)

The presence of saccharase (invertase) in sediments could be correlated with microbial activity and could be used as an „infertility index" (Gianfreda & Bollag 1996). Saccharase activity is dependent on existing microbiota and it is well correlated with the humus concentrantion of soil (Carpa and Drăgan-Bularda 2008) and of sediments as well (Eliade et al 1975).

This indicates that the vegetal residues incorporated in the soil and sediments and transformed by the microorganisms have led to the synthesis of the saccharase under the action of the specific substrate.

One can notice that the level of saccharase activity (Fig. 2) is similar in all the sediment samples, indicating that the accumulated saccharase constitutes one of the key enzymes in the carbon cycle.

Saccharase is present in all the analyzed sediment samples, but it is less intense than the rest of the studied oligase. It is also poorly represented in the sampling point dowsntream of Luncani, probably due to the existence of severe pollution of the river water in this area, with a negative influence on this enzymatic activity.

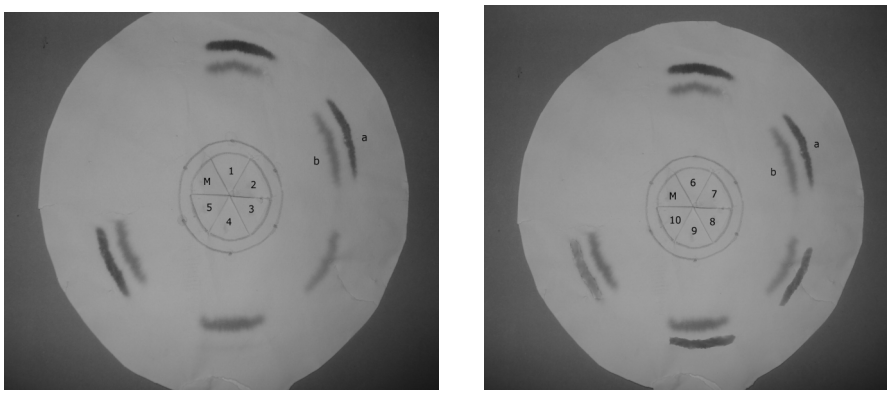

Figure 2. Saccaharase activity (SA) 1-10: sediment samples (see Tabel 1) + enzymatic substrate (saccharose 2%); M – control – saccharose solution 2%; a = glucose spot; b = fructose spot

Maltase activity (MA)

The maltase activity of the soil reflects the transformation of the starch in the vegetal residues by the intermediary maltose - glucose stage.

The most important sublayers of this enzyme are maltose and zaccharose. Maltose is strongly inhibited by glucose, while fructose inhibits it only weakly.

The maltose and cellobiase activities (Fig. 3 and 4) are relatively intense in the majority of the sampling points. One exception was, however, registered regarding a less intense level of cellobiase activity downstream of Abrud, probably due to the confluence of Arieș river with Abrud stream, the latter of which contains great quantities of waste water resulted in the mining exploitation by "SM. Abrud SA."

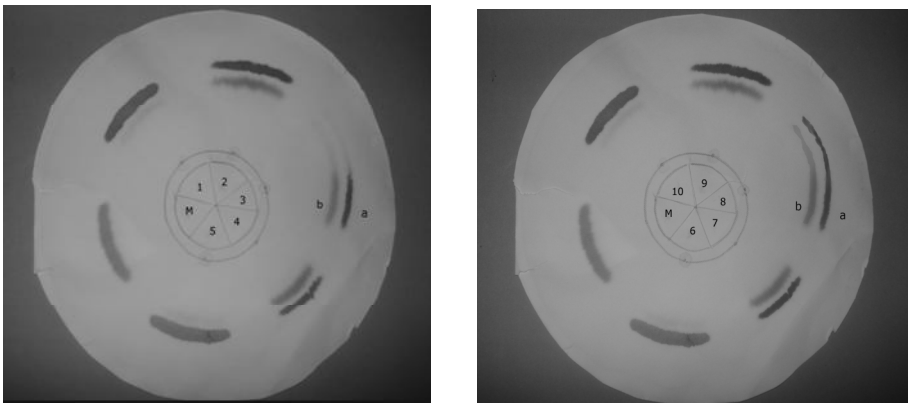

Figure 3. Maltase activity (MA). 1-10: sediment samples (see Tabel 1) + enzymatic substrate (maltose 2%); M – control – maltose solution 2%; a = glucose spot; b = maltose spot

Cellobiase activity (CeloA)

Cellobiase is another oligase involved in the carbon cycle in nature. It represents the sign of enzymatic transformation of the cellulose under the action of the accumulated cellulose. The presence of cellobiase as an accumulated enzyme represents significant proof regarding its accumulation in sediments as a result of the cellulose residues' degradation. Cellulose is the most important vegetal polysaccharide.

Cellobiase is a disaccharide resulting from the degradation of cellulose. The celobiase activity (β-glucosidase) is stimulated by different ions, heavy metals (Ag, Cu and Hg) acting like reversible inhibitors (Vasilescu 1961).

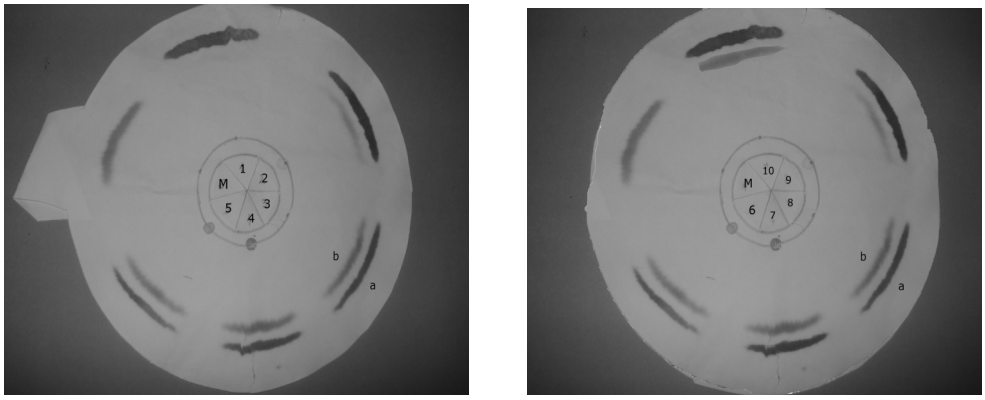

Figure 4. Cellobiase activity (CeloA). 1-10: sediment samples (see Tabel 1) + enzymatic substrate (cellobiose 2%); M – control – cellobiose solution 2%; a = glucose spot; b = cellobiose spot

Polyase activities of sediments represent the main component of the enzymatic potential of a soil, as polysaccharides represent the most important substances in the vegetal residues that find their way into the soil.

Polyase activities were weakly represented or even undetectable (the DA, IA) in some sections (upstream of Baia de Arieș, downstream of Sălciua and Turda). The fact that not all polyase present detectable values in some of the analyzed sections (DA - Abrud, Baia de Arieș, Luncani, and IA – Luncani) was also observed.

Amylase activity (AA)

The most intense activity was registered in the case of AA (Fig. 5), which was detected in all the sampling points. The most intense levels of activity were registered downstream of Abrud, upstream of and downstream of Turda, respectively. The AA activity rises proportionally to humus content and to the capacity for cationic change (Eliade et al 1975).

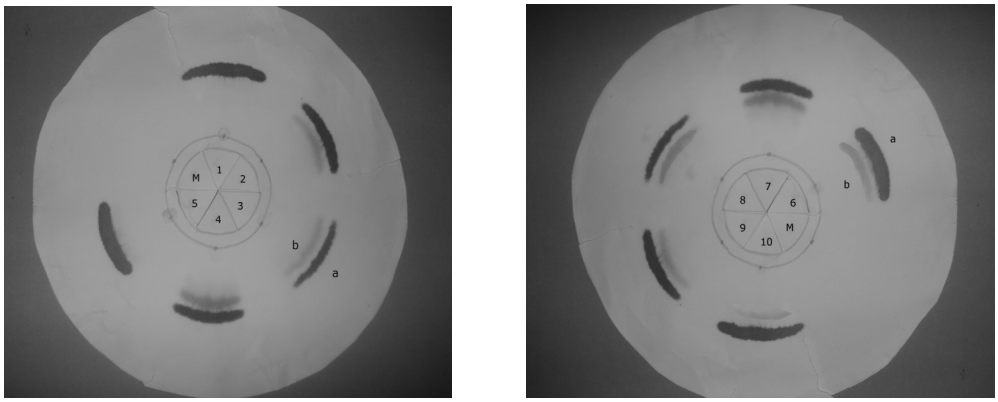

Figure 5. Amylase activity (AA). 1-10: sediment samples (see Tabel 2) + enzymatic substrate (starch 2%); M – control – starch solution 2%; a = glucose spot; b = maltose spot

Dextranase activity (DA)

Dextranase catalyze dextran hydrolisis, as they are extracellular, induced enzymes (Drăgan-Bularda 1972). Dextran is a polyglucoside which is not strictly related to the vegetal residues. The synthesis of dextran into sediments is achieved by microorganisms, starting from saccharase under the action of dextran-sucrase (Drăgan & Kiss 1972).

Dextranase activity is poorly represented. Its values are higher upstream of Abrud and downstream of Turda (see Figure 6). In the other sampling points the DA it is either undetectable or absent.

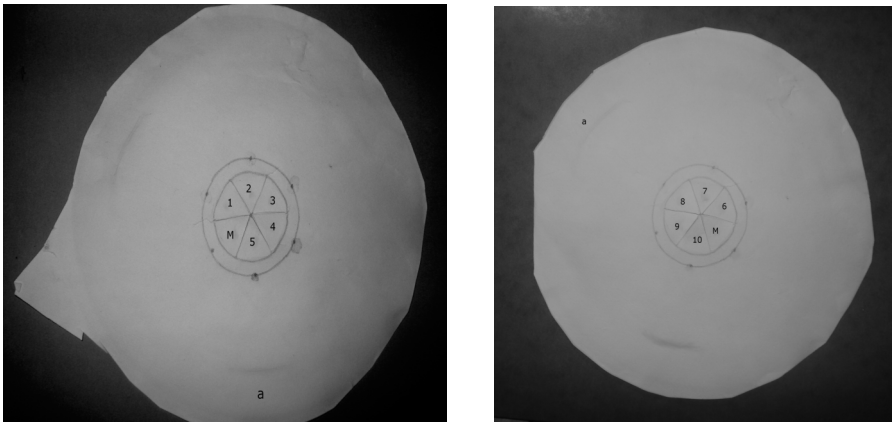

Figure 6. Dextranase activity (DA). 1-10: sediment samples (see Tabel 2) + enzymatic substrate (dextran 2%); M – control – dextran solution 2%; a – glucose spot

Inulinase activity (IA)

Inulinase is a polysaccharidase which specifically hidrolyses the inulin (polyfructoside).

From the analysis of the chromatogram (Figure 7), one can notice that the hydrolysis of the inulin was not total in all the analysed samples.

There is an intensification of enzymatic activity downstream, due to the sandy nature of the underlayer and to the water flow speed, higher than in upstream. In downstream, water flow speed is reduced, the river bed widens and the substrate becomes muddy, the density of microorganisms growing, their activity becoming more intense.

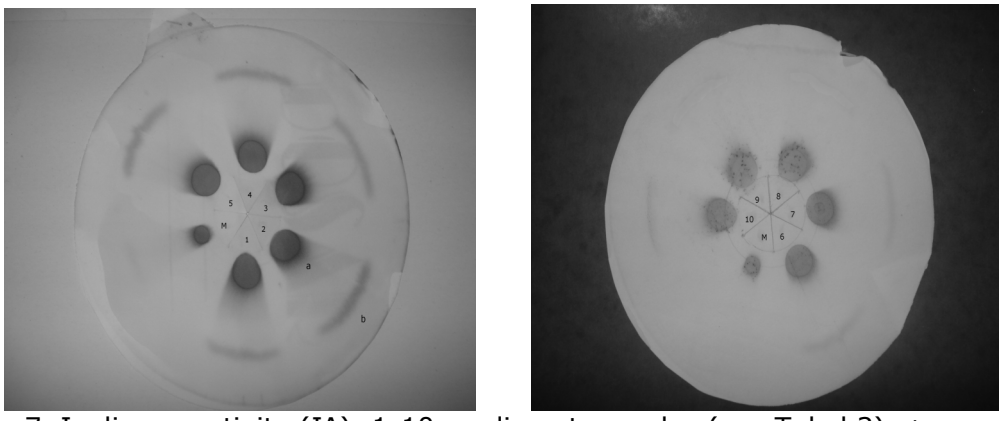

Figure 7. Inulinase activity (IA). 1-10: sediment samples (see Tabel 2) + enzymatic substrate (inulin 2%); M – control – inulin solution 2%; a = inulin spot; b = fructose spot

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

The oligase activities (maltase, saccharase, lactase and cellobiase) were significant in all the analyzed sediment samples. Maximum intensity was, however, registered in the 4 downstream sampling points.

The polyase activities (amylase, dextranase and inulinase) are present in almost all the sampling points, excepting the dextranase activity, which has the lowest intensity.

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 5th sample.

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