BIOTIC AND ABIOTIC FACTORS CONTROLLING ORGANIC MATTER DECOMPOSITION IN AQUATIC ECOSYSTEMS OF SFÂNTU GHEORGHE BRANCH, THE DANUBE DELTA

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Abstract. Decomposition of organic matter by heterotrophic microorganisms is a key process to ecosystems survival. Through this process the mineral elements present in the composition of the organic matter are released and reintroduced in the biogeochemical cycles. Thus, it is prevented an excessive accumulation of organic matter and, also, nutritional sources of primary producers are recycled. In the aquatic ecosystems most part of the organic matter consists of polymeric macromolecules which cannot be decomposed directly by microorganisms. In this case, the first stage of decomposition occurs outside of microbial cells under the action of extracellular enzymes synthesized by heterotrophic microorganisms. These enzymes hydrolyse polymeric macromolecules into monomers, directly usable by microbial cells. For this reason, nowadays, measuring extracellular enzymatic activity represents an important tool in the evaluation process of decomposition of organic matter in the aquatic ecosystems. This paper presents some characteristics of decomposition processes of organic matter in water and sediment samples from the riverine ecosystems of Sf. Gheorghe branch, the Danube Delta, assessed through the hydrolysis rate of extracellular enzymes and, also, evaluates the interaction of these processes with different biotic and abiotic factors. α-amylase, alkaline phosphatase and β-glucosidase extracellular enzymatic activities were determined using specific fluorogenic substrates. The water and sediments samples were collected in spring, summer and autumn between 2008 and 2010. Simple linear regression method was used to estimate the significance degrees between enzymatic activity and the environmental factors.

Keywords: extracellular enzymatic activity, aquatic ecosystems, the Danube Delta.

Rezumat. Factorii biotici şi abiotici care controlează procesele de descompunere a materiei organice în ecosistemele acvatice de pe braţul Sfântu Gheorghe, Delta Dunării. Descompunerea materiei organice de către microorganismele heterotrofe reprezintă un proces cheie care contribuie la supravieţuirea în timp a ecosistemelor. Prin acest proces elementele minerale din compoziţia materiei organice sunt eliberate şi reintroduse în ciclurile biogeochimice. Este astfel evitată acumularea în exces a materiei organice şi de asemenea, este reciclată sursa nutriţională a producătorilor primari. În ecosistemele acvatice, cea mai mare parte a materiei organice este alcătuită din macromolecule de natură polimerică, fapt pentru care nu pot fi preluate direct de către microorganisme. În acest caz, prima etapă a procesului de descompunere are loc extracelular sub acţiunea enzimelor extracelulare sintetizate de celulele microbiene care, prin hidroliză, disociază macromoleculele polimerice în monomeri direct utilizabili. Din această cauză, astăzi, măsurarea activităţii enzimatice extracelulare reprezintă un instrument important în evaluarea proceselor de descompunere a materiei organice în ecosistemele acvatice. Lucrarea de faţă prezintă unele caracteristici ale proceselor de descompunere a materiei organice din apa şi sedimentele ecosistemelor acvatice de pe braţul Sf. Gheorghe, Delta Dunării, estimate prin rata de hidroliză a enzimelor extracelulare, şi, de asemenea, evaluează interacţiunea acestor procese cu factorii biotici şi abiotici determinaţi. Enzimele extracelulare măsurate au fost: α-amilaza, fosfataza alcalină şi β-glucozidaza, utilizând substrate enzimatice specifice. Studiul a avut loc în perioada 2008-2010 în sezoanele de primăvară, vară şi toamnă. Compararea rezultatelor obţinute cu parametrii biotici şi abiotici determinaţi a fost realizată utilizând metoda regresiei liniare simple.

Cuvinte cheie: activitate enzimatică extracelulară, ecosisteme acvatice, Delta Dunării.

INTRODUCTION

Detrital organic matter, resulting from the activity of living organisms (excretion) or after their death, is subject to decomposition and mineralization processes by heterotrophic microorganisms; through these processes, the mineral constituents of the organic matter are released in the aquatic environment and recycled by the living organisms (BOTNARIUC & VĂDINEANU, 1982; SIMON-GRUIŢĂ, 2000; ZARNEA, 1994). Consequently, the decomposition of detrital organic matter is one of the key processes that contribute to ecosystem survival (SULKAVA & HUHTA, 1998), avoiding the excessive accumulation of nutrients and organic matter that could lead to oxygen depletion and death of oxyphilic organisms.

The decomposition is a biological process that includes the physical breakdown and biochemical transformation of complex organic molecules of dead material into simpler organic and inorganic molecules (JUMA, 1998). In aquatic environments, most part of the organic matter (>95%) is composed of polymeric, high-molecular-weight compounds (CHROST & OVERBECK, 1990). In this case, the first stage of the decomposition process usually takes place outside the microbial cells, under the action of extracellular enzymes. Extracellular enzymes hydrolyse large organic molecules, such as polymers, leading to the formation of compounds with simpler structure that can be taken further by microbial cells (WETZEL, 1991). Extracellular enzymatic hydrolysis is the first step in mineralization of most of the polymeric constituents included in the composition of the organic matter (polysaccharides, proteins, organo-esters) by heterotrophic microorganisms, playing therefore an important role in the transfer of matter and energy through aquatic ecosystems (HARBOTT *et al*., 2005).

Between 2008 and 2010, the structural and functional parameters of the aquatic communities were evaluated in Sf. Gheorghe branch, the Danube Delta, the southernmost of the three main branches through which the Danube flows into the Black Sea. This branch was subject of channelization in the 80's, when six meanders were cut to shorten the

navigation route; consequently, different types of sections were formed in the river branch: the free-flowing sector (FS), the meanders section (MS) and the newly built channel (NBC) (GÎŞTESCU & ŞTIUCĂ, 2006).

This paper presents the activity of three extracellular enzymes in water and sediment samples from Sf. Gheorghe branch: α-amylase, alkaline phosphatase and β-glucosidase and, also, evaluates the interaction of these processes with different biotic and abiotic factors. α-amylase and β-glucosidase are specific enzymes that act on polysaccharides starch and cellulose (in the final phase of degradation) (NICOLESCU *et al*., 2000), while alkaline phosphatase is a nonspecific enzyme that catalyses the hydrolysis of a large variety of phosphate esters (JANSON *et al*., 1988). The enzymatic activity was assessed together with other ecological characteristics, in order to emphasize the differences between the three sections.

MATERIAL AND METHODS

Samples were taken from the water column and water-sediment interface in April, July and October 2008-2010, from six stations (S2-S7) (Fig. 1) corresponding to the three sectors mentioned above, as it follows:

- the free-flowing sector (FS): stations S4 and S7
- the meanders section (MS): stations S2 and S5
- the newly built channel (NBC): stations S3 and S6.

Water samples were collected on water column using a modified Patalas device and kept in sterile bottles until the analyses. Sediment samples were collected from the top layer (the sediment-water interface) with a Corer device and stored in plastic bags. After sampling, both water and sediment samples were introduced in freezing bags and kept at 4^{o}C for transport to the laboratory, where they were processed in short time to avoid major changes of enzymatic activities.

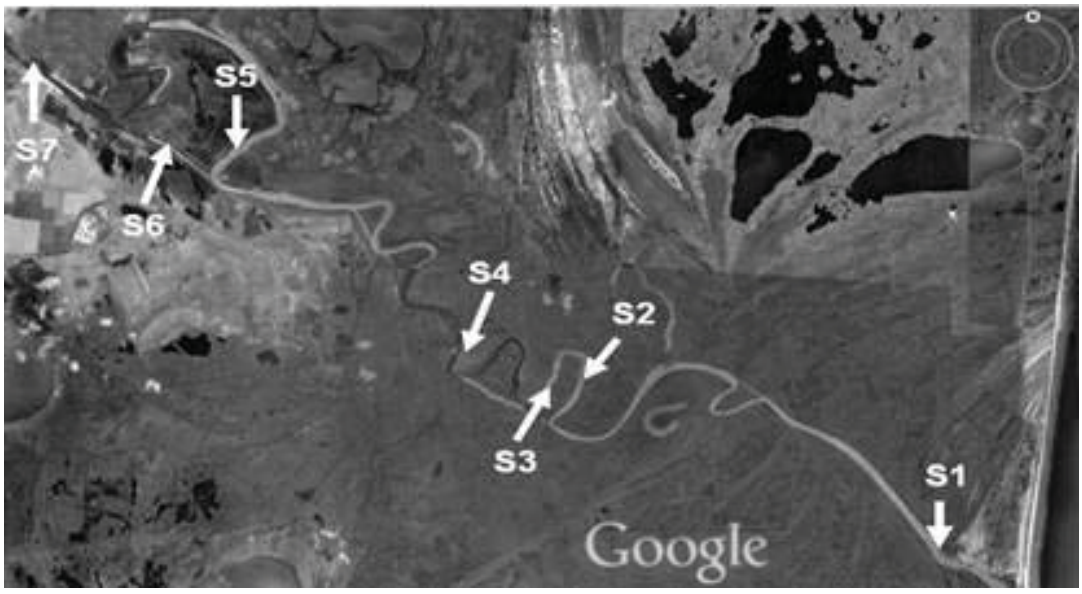

Figure 1. Location of sampling stations along Sf. Gheorghe branch (image from Google Earth).
Figura 1. Locaţia staţiilor de prelevare a probelor de pe braţul Sf. Gheorghe (imagine după Google Earth).

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The intensity of the enzymatic activities was evaluated based on the estimation of substrate consumption by the existing enzymes; the absorbance of the reaction product was measured spectrophotometrically and its concentration was estimated by extrapolating the standard curve. The enzyme substrates used in the assessment were: 4-nitrophenyl phosphate for alkaline phosphatase, p-nitrophenyl-α-D-glycopyranozide for β-glucosidase and Amylopectin Azzure B-chloride for α-amylase (OBST, 1985). Water samples were used without processing, while sediment samples were dissolved in sterile tap water, the supernatant beings used for the enzymatic analyses. The incubation time for alkaline phosphatase and β-glucosidase activities was 6 h at 28^{o}C for water samples and 24 h at 28^{o}C for sediment samples, while for α-amylase both the water and sediment samples were incubated for 24 hours at room temperature under continuous shaking. After incubation, the enzymatic reactions were stopped and products determined spectrophotometrically. Alkaline phosphatase and β-glucosidase reaction product (nitrophenol) shows maximum absorbance at 405 nm wavelength, while the α-amylase catalysed reaction product (Azzure-B-Chloride) has maximum absorbance at 595 nm. All samples were analysed in triplicates.

The following physico-chemical and biological parameters were also determined:

- in water column: microbial biomass, pH, temperature, transparency, depth, concentrations of oxygen, total organic carbon, nitrite, nitrate and ammonium, dissolved inorganic nitrogen, organic and total phosphorous;
- in sediment: pH, temperature and organic matter concentration.

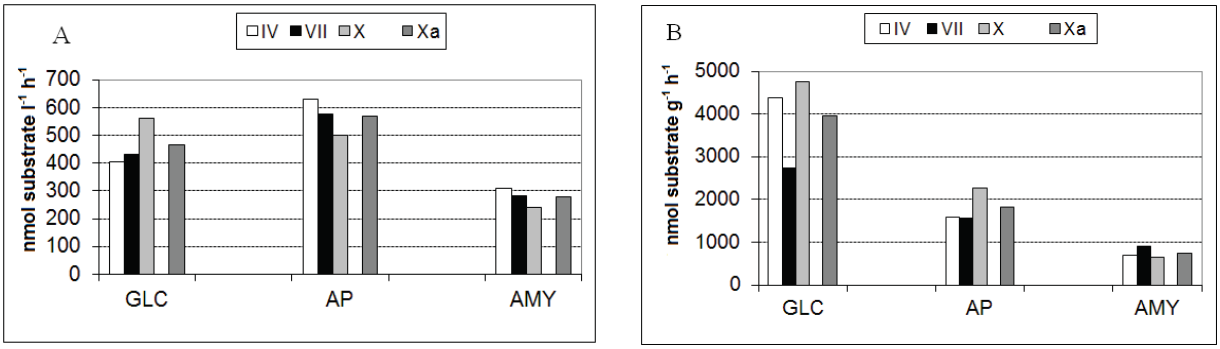
RESULTS AND DISCUSSIONS

The study of extracellular enzymatic activity was performed throughout the entire research program, except for α-amylase activity that was not measured in April 2008 in the water column. Hydrolysis rates of the studied enzymatic activities fluctuate in a wide range, recording different average values between the three sectors and in the same sector in different seasons or in different years.

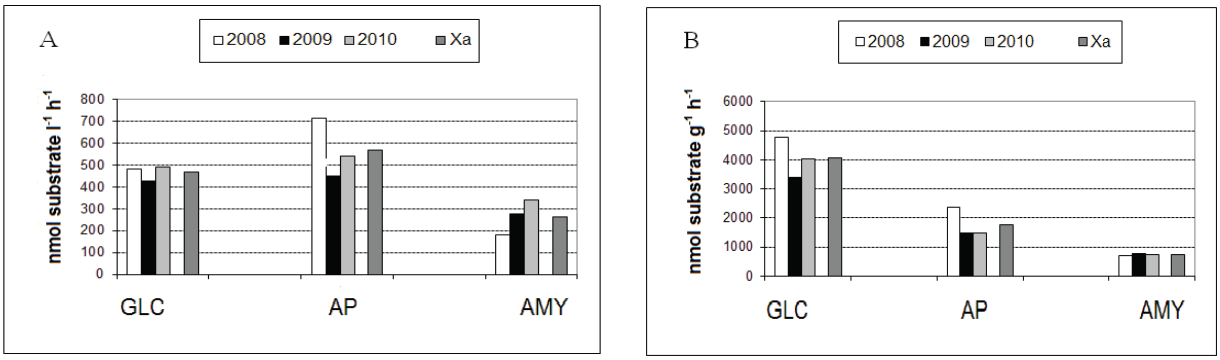
Recorded enzymatic activity fluctuated within the following limits:

- in water column: - amylase : 137 – 396 nmol Azure-B-chloride/l/h
  - alkaline phosphatase : 190 – 920 nmol p-nitrophenol/l/h
  - glucosidase: 190 – 925 nmol p-nitrophenol/l/h
- in sediment: - amylase: 355 – 1269 nmol Azure-B-chloride/g/h
  - alkaline phosphatase : 908 – 6117 nmol p-nitrophenol/g/h
  - glucosidase: 1731 – 9336 nmol p-nitrophenol/g/h

a)



b)



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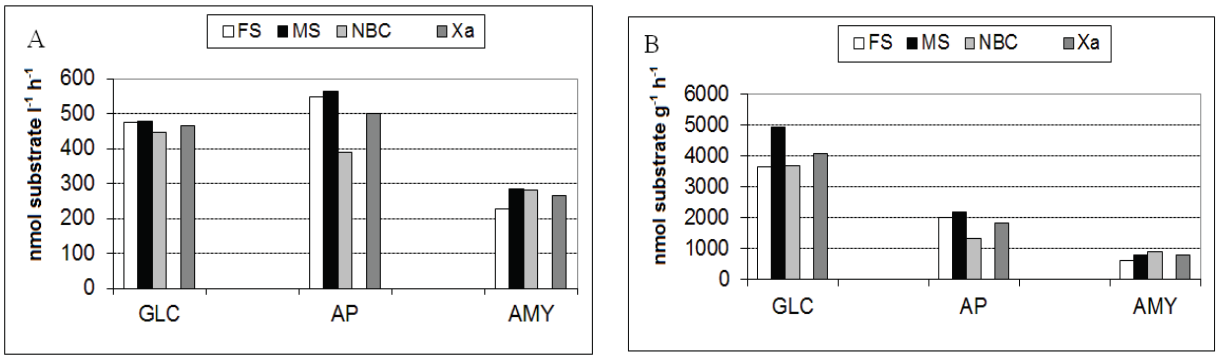

Figure 2. Seasonal (a), annual (b) and spatial (c) dynamics of amylase (AMY), glucosidase (GLC) and alkaline phosphatase (AP) activities in water column (A) and sediments (B). / Figura 2. Dinamica sezonieră (a), anuală (b) şi spaţială (c) a activităţilor amilazică (AMY), glucozidazică (GLC) şi fosfatazică (AP) în coloana de apă (A) şi în sediment (B).

In the water column, the most intense was phosphatase activity, which recorded a multiannual average value of 566 nmol p-nitro-phenol/l/h, while in the sediment was glucosidase activity with a multiannual average value of 4070 nmol p-nitrophenol/g/h.

Seasonal dynamics did not follow a common pattern for the investigated enzymatic activities in the water column or in the sediment. The amylase activity recorded the highest values in summer season, both in the water column and sediment (Fig. 2a), probably due to higher temperatures and substrate abundance, especially of algal origin; amylase is an enzyme that breakdown starch into glucose, a compound easily assimilated by microbial cells (IONICĂ *et al*., 2006).

Glucosidase activity has shown maximum values during autumn in the water column, except for 2008 in the MS and NBC. Usually, the highest values of β-glucosidase activity were recorded during the decline of phytoplankton due to the release of large amounts of polysaccharide substrate from the dead algae (CHRÓST & OVERBECK, 1990). Similar results were recorded by WILCZECK *et al*. in 2002 in a study on the dynamics of extracellular enzyme activities performed on the Elbe River. (WILCZECK *et al*., 2005). The highest intensity values in sediment were recorded in the spring season in 2008 and 2010 and in the autumn season in 2009.

The dynamics of phosphatase activity in water and sediment did not show a clear pattern, probably due to the changes occurred in the quantity and quality of the substrate, this enzyme catalysing the hydrolysis of a large variety of phosphate esters (JANSON *et al*., 1988). In the water column, the highest average seasonal values were recorded in summer for 2008, while in 2009 and 2010 the maximum occurred in the spring season. In the sediment, the maximum seasonal average values were recorded in autumn for 2008, in summer for 2009 and in spring for 2010.

Like seasonal dynamics, the annual dynamics did not show a common trend for the studied enzymatic activities (Fig. 2b). Amylase activity has shown an upward trend in water column in all the three sectors, with minimum values in 2008 and maximum in 2010, while in sediment, the maximum values were recorded in 2009 and minimum in 2008.

For glucosidase activity, the annual average values did not show a clear dynamics in the water column and sediment in the three sectors. Most likely this is due to substrate availability and fluctuation of physico-chemical parameters during the study period. The highest annual average values were recorded in 2010 for the water column and in 2008 for the sediment, while the lowest annual average was recorded in 2009 in both water and sediment.

Generally, the highest intensity was recorded in the MS, except for phosphatase activity in the water column and amylase activity in sediment (Fig. 2c).

Since the dynamics of enzymatic activity is strongly correlated with the dynamics of other environmental parameters (NENIŢESCU, 1974; NELSON & COX, 2004), relationships between enzymatic activities and physico-chemical and biological parameters were investigated using mathematical modeling. Nowadays mathematical modelling is an important tool in studying a wide range of environmental areas (MOLDOVEANU & FLORESCU, 2011). The simple regression equations and the statistical significance between pairs of biotic and abiotic parameters of the obtained correlation matrices were screened in order to identify the parameters with the highest influence on the enzymatic activity.

For α-amylase activity, highly significant linear correlations were obtained with several physico-chemical parameters, emphasizing their role in controlling the enzymatic activity in the investigated ecosystems (Table 1). The intensity of α-amylase activity in the water column was dependent on the variation of pH and oxygen concentration, amylolitic microorganisms - the main group of organisms responsible for the synthesis of α-amylase – acting in the presence of oxygen (IONICĂ, 2006). An increase of pH above 9 caused a significant decrease of the intensity of this enzymatic activity, suggesting that this parameter may become a limiting factor of enzymatic activity under certain conditions such as powerful algal blooms. Highly significant correlations were also obtained with the concentration of dissolved inorganic nitrogen, an important source of nutrients for phytoplankton, as well an important source of polysaccharide compounds in the water column.

Another significant correlation was obtained between α-amylase and the transparency of the water column: in general, the highest values of intensity of amylase activity were recorded in areas with lower transparency, probably due to the presence of high amounts of dissolved and particulate organic matter, derived from the death of aquatic organisms or their excretes, which are the main substrate source for α-amylase (SIVARAMAKRISHNAN *et al*., 2006). Between α-amylase activity and the microbial biomass in the water column a low significant linear correlation was identified, which may suggest that the biomass of the microorganisms that synthesize extracellular amylase have no significant quantitative contribution to the total microbial biomass.

In the sediment, several correlations were identified between α-amylase activity and temperature, the amount of organic matter and the microbial biomass (Table 2) indicating the dependence of this enzymatic activity of temperature fluctuation, the quantity and quality of the substrate and also a quite high contribution of microorganisms that synthesize extracellular α-amylase to the total microbial biomass.

Table 1. Linear correlations between the intensity of amylase activity and physico-chemical parameters from the water column for which the null hypothesis was rejected ($p < 0.05$). / Tabel 1. Corelaţiile liniare obţinute între intensitatea activităţii amilazice şi parametrii fizico-chimici din coloana de apă faţă de care ipoteza nulă nu a fost respinsă ($p < 0.05$).

Physico-chemical parameters	n	r	p	Significance degree
pH	48	0.45	< 0.01	***
Transparency (m)	48	0.44	< 0.01	***
O_2 (mgO/l)	48	0.449	< 0.01	***
NH_4 (mgN/l)	48	0.47	< 0.01	***
NO_3 (mgN/l)	48	0.48	< 0.01	***
DIN (mgN/l)	48	0.32	< 0.05	**

Between phosphatase activity and microbial biomass in the water column there was a highly significant linear correlation, indicating that in general, high phosphatase activity values correspond to high microbial biomass values. This suggests a high abundance of groups of microorganisms involved in the mineralization of phosphorus compounds in Sf. Gheorghe aquatic ecosystems. A very important factor that influenced the overall dynamics of phosphatase

activity was the concentration of hydrogen ions present in the water column (Table 3); increasing the intensity of the enzymatic activity is in general proportional with the increase of pH value. Most intense activity of this enzyme was recorded in the 8-10 pH range.

Table 2. Linear correlations between the intensity of amylase activity and physico-chemical and biological parameters from sediment for which the null hypothesis was rejected ($p < 0.05$). / Tabel 2. Corelaţiile liniare obţinute între intensitatea activităţii amilazice şi parametrii fizico-chimici şi biologici din sediment faţă de care ipoteza nulă a fost respinsă ($p < 0.05$).

Physico-chemical parameters	n	r	p	Significance degree
Microbial biomass (mgC/l)	54	0.331	< 0.05	**
Temperature (m)	54	0.45	< 0.01	***
Organic matter %	54	0.378	< 0.01	**

It was found that high depth did not lower the intensity of enzyme activity by the dilution phenomenon of the substrate (NICOLESCU *et al*., 2000). Enzyme substrate is present, therefore, throughout the water column.

Nitrate and nitrite concentrations in the water column influenced also the dynamics of phosphatase activity. These compounds are an important nutritional source for phytoplankton (MARTENS, 1989), which together with heterotrophic microorganisms is responsible for the presence in the water column of alkaline phosphatase, this enzyme being involved in the organophosphorus ester metabolism of algal cells (JANSON *et al*., 1988).

Table 3. Linear correlations between the intensity of phosphatase activity and physico-chemical parameters of the water column for which the null hypothesis was rejected ($p <0.05$). / Tabel 3. Corelaţiile liniare obţinute între intensitatea activităţii fosfatazice şi parametrii fizico-chimici şi biologici din coloana de apă faţă de care ipoteza nulă a fost respinsă ($p <0.05$).

Physico-chemical parameters	n	r	p	Significance degree
Microbial biomass (mgC/l)	54	0.431	< 0.01	***
pH	54	0.62	< 0.001	****
Depth (m)	54	0.35	< 0.01	**
NO_2 (mgN/l)	54	0.435	< 0.01	***
NO_3 (mgN/l)	54	0.28	< 0.05	*
Org P (µg/l)	54	0.46	< 0.001	***
Total P (µg/l)	54	0.434	< 0.01	***

Alkaline phosphatase is an enzyme responsible for the release of phosphorus from organic compounds and the reintroduction of this element in the biogeochemical cycles. The intensity of alkaline phosphatase synthesis is dependent on the amount of phosphorus present in aquatic ecosystems. This was also confirmed in St. Gheorghe ecosystems, where changes in the concentration of organic phosphorus were positively correlated in most cases with changes in the intensity of phosphatase activity. In sediment, a moderate correlation with the amount of organic matter was obtained, indicating the dependence of this enzymatic activity on the quantity and quality of the substrate (Table 4).

Table 4. Linear correlations between the intensity of phosphatase activity and physico-chemical parameters from sediment for which the null hypothesis was rejected ($p < 0.05$). / Tabel 4. Corelaţiile liniare obţinute între intensitatea activităţii fosfatazice şi parametrii fizico-chimici din sediment faţă de care ipoteza nulă a fost respinsă ($p < 0.05$).

Physico-chemical parameter	n	r	p	Significance degree
Organic matter %	54	0.34	< 0.05	**

Between β-glucosidase activity and microbial biomass in the water there was a moderately significant linear correlation (Table 5) during the study period, suggesting that the biomass of microorganisms that synthesize extracellular β-glucosidase had an important quantitative contribution to total microbial biomass, respectively that the polysaccharidic substrate was abundant. This confirms the results from the literature showing that, usually, the intensity of β-glucosidase activity in the water column is associated with the dynamics of microbial biomass (CHRÓST & OVERBECK, 1990).

A highly significant correlation and similar dynamics were obtained with transparency, probably due to increasing phytoplankton photosynthesis and productivity in these areas, respectively the increased number of algal organisms leading to the release of large amounts of phytoplanktonic polysaccharidic exudates – an important substrate source for this enzyme (FAJON *et al*., 1999). Therefore, increasing the number of algal organisms lead to an increased rate of "sloopy feeding" of phytophagous zooplankton and, therefore, to the release of intracellular polymeric compounds in the environment (including the polysaccharidic compounds) which also contribute to increase the glucosidase activity (BOCHDANSKY *et al*., 1995). A low significant correlation was recorded between the β-glucosidase activity and total phosphorus concentration, phosphorus being a mineral element essential in the development of phytoplankton (SYLVAN *et al*., 2006).

Compared with other enzymatic activities, β-glucosidase activity showed the highest significant correlation with the amount of organic matter in the sediment; this may suggest that glucosidase activity represents an important factor contributing to the decomposition of the organic matter from the sediments of the investigated ecosystems. A significant correlation was also obtained with pH and temperature (Table 6).

Table 5. Linear correlations between the intensity of glucosidase activity and physico-chemical and biological parameters from the water column for which the null hypothesis was rejected ($p < 0.05$). / Tabel 5. Corelaţiile liniare obţinute între activitatea glucozidazică şi parametrii fizico-chimici şi biologici din coloana de apă faţă de care ipoteza nulă a fost respinsă ($p < 0.05$).

Physico-chemical parameters	n	r	p	Significance degree
Microbial biomass (mgC/l)	54	0.383	< 0.01	**
Transparency (m)	54	0.493	< 0.001	****

Table 6. Linear correlations between the intensity of glucosidase activity and physico-chemical parameters from sediment for which the null hypothesis was rejected ($p < 0.05$). / Tabel 6. Corelaţiile liniare obţinute între intensitatea activităţii glucozidazice şi parametrii fizico-chimici din sediment faţă de care ipoteza nulă a fost respinsă ($p < 0.05$).

Physico-chemical parameters	n	r	p	Significance degree
pH	54	0.426	< 0.01	***
Temperature ^{o}C	54	0.346	< 0.05	**
Organic matter %	54	0.404	< 0.01	***

CONCLUSIONS

The intensity of enzymatic activities evaluated in the water column and sediments of the aquatic ecosystems of Sf. Gheorghe branch, varied significantly in terms of seasonal, annual and spatial dynamics. In the water column, the most intense was phosphatase activity, while in sediment was glucosidase activity. Also, the intensity of the enzymatic activity was higher in sediment compared with the water column. Although a clear pattern of seasonal and annual dynamics for the three enzymatic activities could not be revealed, the spatial dynamics has shown in general the highest values in the meanders section. The calculation of linear regression between the enzymatic activity, the microbial biomass and different physico-chemical parameters revealed the significant role of the environmental factors (pH, temperature, transparency of the water column, microbial biomass, nutrients and oxygen concentration in the water column and temperature and organic matter in sediment) in controlling the enzymatic activity, respectively the organic matter decomposition, in the investigated ecosystems.

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