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Original article

Microbial Extracellular Enzymatic Activity Unveils the Anthropic Impact on Riparian Ecosystems From Southern Romania

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Abstract

The anthropization processes can cause complex, often irreversible changes in natural ecosystems. Several decades ago, on the upper course of the Argeș River flowing in the Southern Romania, a series of anthropic interventions took place, which resulted in several new accumulation lakes. Above the socioeconomic benefit to the local communities along this watercourse, their construction also influenced the structure and functioning of the existing ecosystems. In the present study, we have analysed the rate of the detrital organic matter decomposition processes achieved by the heterotrophic microbial communities in several ecosystems located on the upper course of the river. The obtained results revealed that the intensity of these processes was influenced both by the type of the anthropic area the sampled ecosystems attain - rural or urban, and by the sampling time.

Keywords

extracellular enzymatic activity, detrital organic matter mineralisation, dam reservoirs, Total Dissolved Solids

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Introduction

The riparian ecosystems are often the subject of the anthropic exploitation regarding their wealth in goods and services, perceived as a benefit for the development of human communities – especially the urban ones. Usually, they are characterized by a high degree of biodiversity, specifically harboring numerous endemic species. Their development and spreading are influenced by the presence of watercourses that often isolate them naturally from other types of aquatic ecosystems. Therefore, human interventions on these types of ecosystems can significantly harm the local biodiversity, endangering populations of different plant or animal species. However, the authorities mainly consider riparian ecosystems as important in the socio-economic systems development (based on their exploitation) and rarely impose concrete protection measures, such as the creation of protected areas along the water streams (MAAß et al., [1]; ARIFJANOV [2]; SANTIAGO et al. [3]; DAMO & ICKA [4]). Thus, the assessment of human activities that impact these categories of ecosystems is considered a necessity of high importance in our present context.

Argeș River is a left tributary of the Danube, which flows through the S-SE part of Romania. It has a length of 350 km and the surface of its river basin of 12.550 km^2. The river springs from Făgăraș Mountains, at the confluence of two rivers originating in glacial lakes - Capra and Buda - currently forming the artificial lake Vidraru, from which the river presently flows. It has numerous tributaries with an asymmetrical lateral distribution, the debit of the tributaries on its left side being six times higher in comparison with the right-side ones. It crosses numerous rural and urban localities, the most important in its upper course, being the cities Curtea de Argeș and Pitești. It also serves as an important source of tap water supply for the localities along its course (ION et al. [5]).

Several decades ago, following a national development plan that considered the economic interest of local communities, several dams were built along the Argeș River flow, including: Vidraru, Oești, Cerbureni, Curtea de Argeș, Zigoneni, Vâlcele, Budeasa, Bascov and Golești. These important anthropic habitat alterations, along with the exploitation of the riverbed substrate, poaching, intensive agriculture in the meadows near the stream and different improperly managed household discharges, generated significant changes in the species composition of the previous (unaltered) natural ecosystems. Due to these changes in the structure and functions of aquatic ecosystems, some species of fish disappeared, while other populations developed in the dam areas with the new ”favourable” spawning conditions (TRUŢĂ & DUMITRU [6]; CONETTE [7]; DIACONU & MAILAt [8]; IONESCU [9]).

Monitoring the intensity by which decomposition processes take place can provide important information about the quality and availability of the existing nutritional resources in an ecosystem (FRAINER et al. [10]; HARBOURD et al. [11]) and, indirectly, about its structure.

Microbial extracellular enzymes are involved in the mineralization of detrital organic matter (DOM) and refilling the biogeochemical circuits with chemical elements. Most of the DOM is made up of large polymeric macromolecules, which cannot be introduced directly in the microbial metabolism. As a result, microorganisms synthesize extracellular enzymes that hydrolyze the organic macromolecules into simple molecules that could be then internalized, and thus, the extracellular enzymes play an important role in the transfer of matter and energy through the ecosystem. Taking into account this aspect and the fact that extracellular enzymes are sensitive to anthropogenic changes of the ecosystems (especially pollution), their analysis can provide valuable information about the trophic state of an ecosystem and the impact of the human activity. Nowadays, the activity of the extracellular enzymes is evaluated in most of the studies which assess the ecosystem state (DANG et al. [12]; LEE et al. [13]; KOHLER et al. [14]; STADLER et al. [15]).

Considering the mentioned contextual aspects, we decided to assess the decomposition activity of the aquatic microbial communities along the upper course of the Argeș River, as far as downstream Pitești city. The aim of the present study was to determine whether DOM decomposition processes vary significantly along the reservoirs constructed in the upper course of the Argeş River. Related to the increase of the localities number that the river crosses, they are expected to vary regarding the mineralization of the organic matter. Thus, through this study we want to see if these differences show a gradient pattern from upstream to downstream of the river, or if they have a random variation.

Materials and methods

Sample collection

The area where the study was conducted is geographically located in the central-southern part of Romania, in Argeș County. It is characterized by the presence of a mountainous region in its northern part, that is often populated with mixed and conifer forests. In the southern part of the study region can be found the Subcarpathian hills, mainly dominated by mixed forests.

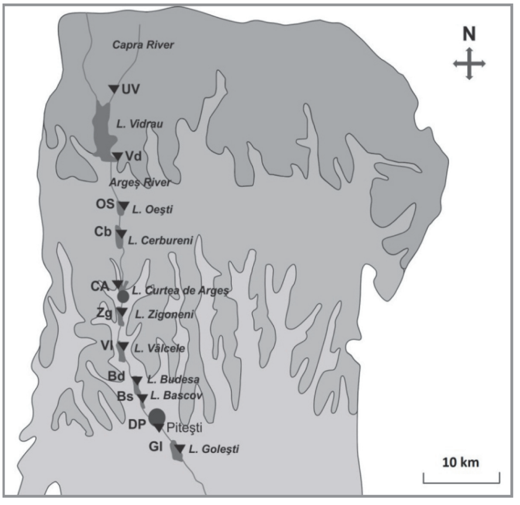

Figure 1. Sampling site locations along the Argeş River (Argeş County, România): UV – upstream Vidraru, Vd – Vidraru Lake, Os – Oesti Lake, Cb – Cerbureni Lake, CA – Curtea de Argeş Lake, Zg – Zigoneni Lake, Vl –Vâlcele Lake, Bd –Budeasa Lake, Bs –Bascov Lake, Gl –Golești Lake, DP – downstream Pitești.

Water samples were taken from 11 stations, the first of them located in a natural ecosystem - the Capra River - upstream the Lake Vidraru (UV). The other ten sampling stations were located along the upper course of the Argeş River - 9 of them corresponding to dam locations: Vidraru Lake L. (Vd), Cerbureni L. (Cb), Oeşti L. (Os), Curtea de Argeş L. (CA), Zigoneni L. (Zg), Vâlcele L. (Vl), Budeasa L. (Bd), Bascov L. (Bs), Goleşti L. (Gl) and one of the river course, downstream Pitești city (DP) (Fig. 1). Vidraru L. and Capra River sample stations are located in the mountain area, while the sampling points are located in the Subcarpathic Hills area. In order to assess the variation of the enzymatic activity along the areas that were subjected to the anthropic changes, we measured the dynamics of the extracellular enzymatic activity in the accumulation lakes built along the river.

Surface water samples were collected over a 12-month period (September 2017 to September 2018) from all 11 stations, to cover four different seasons. The sampling was done from the upper layer of the water column – in the first 50 cm below the surface, and from the immediate vicinity of the shore. After that, the samples were stored in a refrigerated bag during transportation to the laboratory, where they were subsequently stored at 4°C. Before processing, the samples were filtered through a zooplankton filter of 40 μm, in order to remove the predatory zooplankton organisms.

Measurement of Extracellular Enzymatic Activities

In the present study, we analyzed the activity of several enzymes - α and β glucosidase, alkaline phosphatase and alanine aminopeptidase - involved in the decomposition of some categories of macromolecules present in various types of habitats and involved in biogeochemical circuits of C, P and N, in order to highlight the differences between the selected site regions.

The α glucosidase enzyme (EC 3.2.1.20) catalyzes the hydrolysis of α-1-4-glycosidic bonds in polysaccharides such as starch, a widespread molecule in natural environments (DA COSTA et al. [16]). Instead, β glucosidase catalyzes the hydrolysis of β-1-4-glycosidic molecules, being considered a key enzyme in the mineralization of cellulose, one of the most common molecule on Earth used by many organisms (especially plants and green algae) (KAUSHAL et al. [17]; BRIGHAM [18]). Alkaline phosphatase has a non-specific activity, hydrolyzing a wide range of organic phosphoesters with the releasing of orthophosphate, while alanyl aminopeptidase hydrolyzes the alanyl peptides (PĂCEȘILĂ & RADU [19]; PĂCEŞILĂ et al. [20]).

The activity of four extracellular microbial enzymes involved in the hydrolysis of some organic substrates frequently found in many habitat types was analyzed: α-glucosidase (EC 3.2.1.20), β-glucosidase (EC 3.2.1.21), alkaline phosphatase (EC 3.1.3.1) and alanine-aminopeptidase (EC 3.4.11.2) (STADLER et al. [15]; PĂCEȘILĂ & RADU [19]; HANC et al. [21]).

The extracellular enzymatic activity was determined colorimetrically, by evaluating the substrate (p - nitrophenyl - α - D – glucopyranoside for α – GLC, p - nitrophenyl - β - D – glucopyranoside for p - nitrophenyl - β - D – glucopyranoside, 4 - nitro phenyl – phosphate for AP and L - alanine - 4 - nitroanilide – hydrochloride for AMP) consumption (PĂCEȘILĂ et al. [22]). Each 0.5 mL of water samples was mixed with 0.5 mL of enzyme substrate solution and incubated for 6 h at 30°C. For each enzyme the substrate solution had a concentration of 1m/mL, dissolved in a 0.14M NaCl solvent solution. After incubation, the enzymatic reactions were stopped by adding 0.5 mL 1M of Na_2CO_3 solution. Prior to spectrophotometer reading, the samples were centrifuged at 2000 rpm for 5 minutes in order to remove any impurities, and the supernatant was used for spectrophotometer reading, using a CECIL CE 1010 spectrophotometer. The absorbance of enzymatic reaction products - p-nitrophenol for the first three enzyme or p-nitroanilline for AMP – was read at 405 nm wavelength, and product concentration was determined by extrapolation on the standard curve. The samples were processed in triplicate.

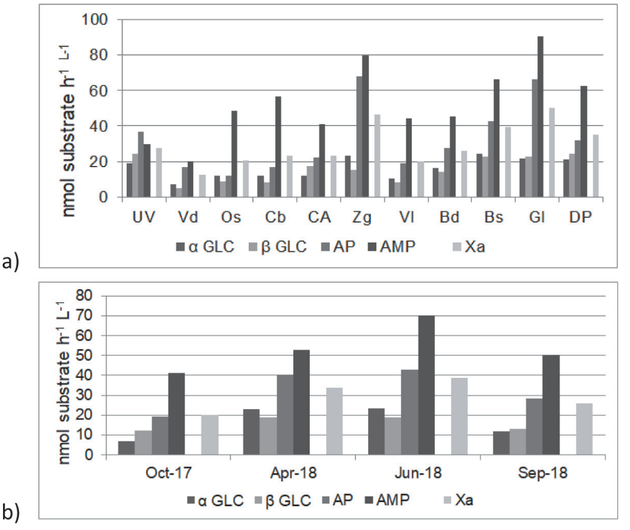

Figure 2. The spatial (a) and seasonal (b) dynamic of the microbial extracellular enzymatic activity in the studied sites (Argeș River, România).

Total Dissolved Solids

Total Dissolved Solids (TDS) was measured directly in the water column using a Sheenzy TDS-3 tester device.

Statistical analysis

The PAST software (Paleontological Statistics) (HAMMER et al. [23]) was used to analyze the logarithm transformed data.

Results

Extracellular Enzyme Activity

The spatial and temporal dynamics of the enzymatic activities intensity along the site locations during the study period is shown in Figure 2.

The α glucosidase activity (α GLC) was not detected in September 2018 in the Cerbureni lake (Cb), but its highest value of intensity was registered in Lake Golești (Gl) in June 2018 (38.45 nmol p-nitrophenol h-1L-1). In the case of β glucosidase activity (β GLC), the minimum value was recorded in Lake Cerbureni (Cb, 1.55 nmol p-nitrophenol h-1L-1) in September 2018, and the maximum - in the station located downstream the Pitesti city (DP, 46.98 nmol p-nitrophenol h-1L-1), in June 2018. The phosphatase activity presented a minimum intensity in Lake Cerbureni (Cb, 5.13 nmol p-nitrophenol h-1L-1), in September 2018, and the highest in Lake Zigoneni (Zg, 105.28 nmol p-nitrophenol h-1L-1), in June 2018. Regarding the aminopeptidase activity, the lowest value was found in the station located upstream of Lake Vidraru (Vd, 10.63 nmol p-nitroanilline h-1L-1), in September 2018, and the highest in June 2018, in Lake Golești (Gl, 163.03 nmol p-nitroanilline h-1L-1) (Fig. 2a).

Also, the enzymatic decomposition processes appeared to be the most intense in June 2018, and the lowest in September 2017 (Fig. 2b). It can be noted as well that α-GLC and β-GLC were also intense upstream the Vidraru L. (UV, on the Capra natural river), which suggests a more abundant presence of polysaccharide molecules in the water column in this area, compared to the following anthropized stations from the upper part of the upper course of the Argeș River (Fig. 2a).

TDS (Total Dissolved Solids) values in the studied ecosystems

In order to enrich the enzymatic activity assessment meaning frame, we also measured the TDS – that include dissolved solids of organic or inorganic nature, present in a molecular, ionized or colloidal state. It is mainly represented by different types of ions (potassium, sodium, carbonates, sulfates, nitrates, etc.) and positively correlates with the water conductivity, influencing as well the pH values (ISLAM et al. [24]).

In the case of our sampled ecosystems, the TDS values varied between 10-89, corresponding to a high quality in terms of drinking water standards (MORAN [25]). The lowest value of this parameter was recorded in Curtea de Argeș L., in April 2017, and the maximum in Golești L., in the same period of time. A weak correlation was found between β GLC values and TDS during the analyzed period (N = 44,

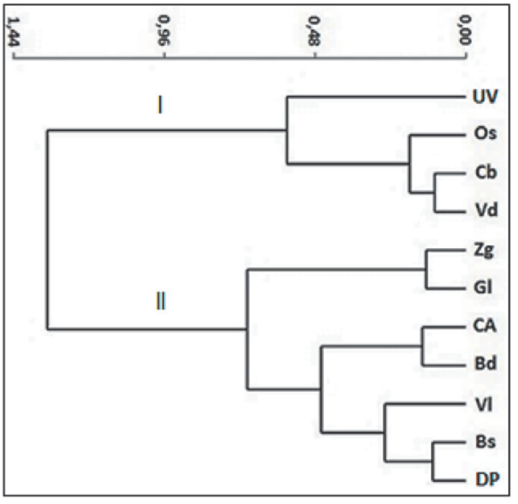

Figure 3. Cluster analysis of the enzymatic activity across the sampling stations (Ward's method, Coph. corr.: 0,7228).

$p < 0.05$), which suggests that the activity of these enzymes was also influenced by the amount of the dissolved particles present in the water column.

Cluster analysis

Using the cluster analysis, we compared the intensity of the hydrolysis processes within the sampling sites (Fig. 3). The obtained clusters (I and II) revealed that the association followed the anthropic area criteria type - the highest similarity being assessed between the urban (CA, BD, BS, Gl, DP) and rural (Os, Cb, Zg, Vl) sampling sites.

The first cluster (I) includes the stations located upstream of the Curtea de Argeș city. It can be observed that they are mainly grouped according to their geographical proximity. Also, the station positioned in the Capra river natural area, upstream the Vidraru dam, belongs to another cluster group. This confirms a different type of ecosystem, a lotic one, and at the same time, the absence of the anthropogenic alterations in the region. For the rest of the stations in this group, the similarity in terms of their bacterial enzymatic activity could be explained by a lower level of the anthropic impact in the territory.

In the second cluster (II), the aggregation based on proximity is less present, except Bs and DP stations. This aspect suggests that each studied lake has a characteristic dynamic of the mineralization processes determined by its unique environmental factors and anthropic influences. It should be noted in this case that Zg and Gl lakes, located downstream of an urban area, had a high degree of similarity in terms of the microbial enzymatic activity.

Discussions

Dams' construction along river courses is often considered a solution to problems related to drinking water supply, irrigation and electricity supply. For a long time, dams were considered an ecological solution, without too many negative effects on the environment. For this reason, their construction has increased in recent decades, including for the large rivers (BINNIE [26]; DOPICO et al. [27]; BIRNIE-GAUVIN et al. [28]).

The most intense enzymatic activity was recorded in Golești Lake which is known for its recreational role. Golești Lake is frequently the subject of eutrophication processes during the summer, with a greatly increased amount of organic matter. This aspect is reflected in the high values of the AP and AMP in this lake (Fig. 2a). In spring and at the beginning of the summer season there are frequent algal blooms occurring here (DUMITRAN & VUTA [29]). The algal blooms increase the amount of DOM, a source of nutrients for the heterotrophic microorganisms (WANG et al. [30]). DOM commonly contains biopolymers, such as polysaccharides and proteins, and humic like substances (YANG et al. [31]). Algal blooms increase the need for phosphorus - an essential element in the algae development, which leads to the synthesis of phosphatases - enzymes that release phosphorus from the organic compounds (ZHANG et al. [32]).

Also, an intense enzymatic activity was shown downstream of Pitesti (Fig. 2a), the most important city located on the upper course of the river, in the hilly region. The sewage water discharge from the settlements frequently determines the nutrient enrichment of water bodies, which leads to the growth of the aquatic organism communities and implicitly to the increase of DOM quantity in these ecosystems. These alterations are frequently associated with intense decomposition processes and the synthesis of extracellular enzymes by the inhabitant aquatic microbial communities (BERRIO-RESTREPO et al. [33]; PEARCE et al. [34]).

Argeș County is characterized by intensive agriculture practices, more than half of its surface being occupied by arrable land, and by horticulture and fruit trees growing. The meadows of the river are traditionally used in agriculture. The practice of intensive agriculture involves the use of artificial and natural fertilizers, but also of other chemical compounds such as pesticides and insecticides. One of the consequences of these treatments is the modification of the soil characteristics, including the quantity and quality of organic matter. Following the elution processes determined by the rain, floods, the organic matter and the chemical compounds existing in these soils reach the river water and influence its quality (MICU et al. [35]; TUDOR et al. [36]). Also, currently there are not many comprehensive studies about the direct influence of pesticides on the bacterial extracellular enzymatic activity in the aquatic environments. However, most likely, their presence in water does not exert a direct and significant influence on it (STANLEY et al. [37]). Another factor that can influence the extracellular enzymatic activity, and implicitly the rate of the decomposition processes, are represented by the presence of heavy metals (APONTE et al. [38]). Studies carried out along the river on heavy metal pollution have not revealed a very high amount of heavy metals in the water column, not even downstream of Pitești, an important industrial and commercial city (STOICA et al. [39]).

The polysaccharide macromolecules representing an important input of substrate (especially cellulose and starch) are coming most likely of the forest soils located close to the river. The area is largely populated with deciduous trees that sometimes fall in the river, and the organic matter from their litter is eluted by rain in the river water (IFTIMIE [40]; FIORI et al. [41]). It should be noted that in Vidraru Lake (having the largest surface and volume) the enzymatic decomposition processes take place with a lower intensity. In lentic ecosystems the surface water currents transport DOM and nutrients, thus diluting the amount of substrate available for the extracellular enzymes near the shores. In large lakes, the speed of the surface currents is stronger, due to a more powerful wind and to the vertical stratification present in this type of ecosystems (BENNINGTON et al. [42]; HUI et al. [42]).

Zigoneni Lake also recorded high values of the microbial enzymatic activity, compared with Golești Lake. Although no other studies have reported a high concentration of the detrital organic matter in this lake, the dynamics of the mcirobial enzymatic activities determined in the present study suggests that the decomposition processes take place with higher intensity near the lake shores.

Conclusions

The Argeș River is the largest river in the Argeș County that springs from the Vidraru dam reservoir. During the time, important anthropization processes occurred on its upper water course, including the building of several reservoir dams. One of the main occupations of the inhabitants of the county is represented by different forms of intensive agriculture, which are practiced in the meadows of the river (TRUŢĂ & DUMITRU [6]; CONETTE [7]; DIACONU & MAILAt [8]; IONESCU [9]). Despite the important anthropization of the area, the Argeș river water quality preserves high standards regarding its use for the domestic consumption (ION et al. [5]).

The extracellular enzymatic hydrolysis processes taking place at the upper layer of the water column were detected throughout the entire study period in all but one of the sampled stations. They were more intense in the summer, most likely stimulated by the higher temperatures.

The enzymatic activity has shown an increasing gradient from the upstream to the downstream of the river. Its highest values were often observed in the stations from the southern part of the studied area, which correspond to the highest anthropogenic influence exerted by the Pitești city - the most important urban settlement along the Argeș River.

The obtained data reflect the influence of the anthropogenic impact on the decomposition processes performed by the microbial communities present in the river water. This influence becomes more important as much as the river crosses the localities along its watercourse, especially in the urban areas.

The analysis of the mineralization processes carried out by the microbial communities from the Argeș River gives us a better understanding of the way in which the human activity affects the lotic ecosystems. For a better assessment of the anthropogenic impact on the role that heterotrophic microorganism communities play in this ecosystem, additional studies are needed in order to consider other factors, such as the structure of microbial communities and the influence of environmental factors.

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Conflict of Interest

The authors declare to have no conflict of interest.

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