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Original paper

Extracellular enzymatic activities in the aquatic ecosystems of the Danube Delta. 2. Alkaline phosphatase activity

IOAN PĂCEŞILĂ[1,2*], EMILIA RADU[1]

[1]Institute of Biology Bucharest, Spl. Independentei, 296, 060031, Bucharest, Romania
[2]The Research Institute of the University of Bucharest, ICUB, Str. Dr. Dimitrie Brîndză 1, Bucharest, Romania

Abstract Phosphorus is one of the most important inorganic nutrients in aquatic ecosystems, the development and functioning of the phytoplankton communities being often correlated with the degree of availability in assimilable forms of this element. *Alkaline phosphatase* (AP) is an extracellular enzyme with nonspecific activity that catalyses the hydrolysis of a large variety of organic phosphate esters and release orthophosphates. During 2011-2013, AP Activity (APA) was assessed in the water column and sediments of several aquatic ecosystems from Danube Delta: Roșu Lake, Mândra Lake and their adjacent channels – Roșu-Împuțita and Roșu-Puiu. The intensity of APA widely fluctuated, ranging between 230-2578 nmol p-nitrophenol L-1h-1 in the water column and 2104-15631 nmol p-nitrophenol g-1h-1 in sediment. Along the entire period of the study, APA was the most intense in Roșu-Împuțita channel, for both water and sediment samples. Temporal dynamics revealed its highest values in summer for the water column and in autumn for sediment. Statistical analysis showed significant seasonal diferences of the APA dynamics in spring vs. summer and autumn for the water column, and any relevant diferences for sediment.

Keywords Dicistronic operon, Pharmaceutical proteins, Plastid transformation.

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*Corresponding author: IOAN PĂCEŞILĂ, Institute of Biology Bucharest, Spl. Independentei, 296, 060031, District 6, Bucharest, Romania, Phone + 4 021 2219202, Fax + 4 021 2219071
E-mail: ioan.pacesila@gmail.com

Introduction

Phosphorus is an essential element for the growth and development of the living organisms, being a component of various macromolecules such as phospholipids, nucleotides and nucleic acids (WORSFOLD & al [1]; MIHALACHE & al [2]). Therefore, alongside with nitrogen, sustain the biological communities in waters, and thus, the natural aquatic ecosystems life and functionality. It is recognized as the primary limiting nutrient in fresh waters, where its presence in small quantities inhibits the development of the primary producers (especially phytoplankton), but in excess can lead to the acceleration of the eutrophication process (BRANOM & SARKAR [3]; SCHELDE & al [4]; YANG & al [5]; WITHERS & JARVIE [6]).

The phosphorus concentration in an ecosystem is controlled by physical, chemical or biological mechanisms (XIA & al [7]). For a long time, researches about the influence of this element above the phytoplankton or microorganism communities in the aquatic ecosystems have been focused only on the dissolved reactive phosphorus (DRP), especially of inorganic origin. But gradually, more and more studies began to prove the importance of the dissolved organophosphoric compounds (DOP), as a source of phosphorus for the phytoplankton communities and different groups of heterotrophic microorganisms. In order to access phosphorus from the organic molecules, these organisms synthesize extra-cellular enzymes with hydrolytic activity – called phosphatases, able of delivering it (from DOP) in assai-lable inorganic forms.

Alkaline phosphatase (AP, E.C. 3.1.3.1) is an enzyme involved in the cycle of phosphorus in nature, one of the most widespread and studied extracellular enzyme in the aquatic ecosystems (REN & al [8]). AP catalyses the hydrolysis of a large variety of phosphate esters (e.g. esters of the primary and secondary alcohols), cyclic alcohols, phenols and amines. Also, actions on polyphosphates, releasing the orthophosphate compound. It is not implied in the catalysis of the phosphodiesters. Due to its activity, AP is recognized as one of the drivers that can sustain the eutrophication process of an aquatic ecosystem (SONG & al [9]).

AP is a dimeric molecule composed of two subunits, each containing two ions of Zn^{2+} – one necessary for maintaining the structural integrity of the enzyme, and the other one – for conducting its catalytic activity. The activity of this enzyme is stimulated by the presence of Mg^{2+} in the environment. These ions accelerate the dephosphorylation process, binding not to the active site of AP (allosteric regulation). On the other hand, high concentrations of Zn^{2+} can inactivate AP, due to their competition with Mg^{2+} for linking to the same site on the enzyme. The most common inhibitors of phosphatase activity are chelators of divalent ions (BRETAUDIERE & SPILLMAN [10]). Similar with other extracellular enzymes, the catalytic activity of the alkaline phosphatase is inhibited by the accumulation in the reaction medium of its final product, the orthophosphate. The decrease of the extracellular concentration of orthophosphate, stimu-late bacterial and algal cells to access their intracellular stores of substance. Therefore, the microplankton synthetize AP not simultaneously with the decreasing of the orthophosphate loadings in the environment, but only after the microorganisms deplete their own intracellular concentrations of it (JANSSON & al [11]; CHRÓST & al [12]). Frequently, APA values are used as a tool that indicate the phosphorus levels in the aquatic ecosystems (STOBER & al [13], SUZUMURA & al [14), but as well, nowadays the enzyme has many utilizations in various fields of the human activity.

It is used in the molecular biology studies, for DNA sequencing and molecular cloning. AP hydrolyzes the phosphate groups of the oligonucleotides or protein fragments and, therefore, frequently facilitates the remo-ving of the 5' monophosphate group of DNA fragments, in order to prevent DNA cyclisation. Another important AP application is in the food industry, where indicate the efficiency of the milk pasteurization process. Some studies suggest as well, that this enzyme could be used as a biosensor for the heavy metals or pesticides detection in environment, as these compounds inhibit its activity. Furthermore, due to its substrate non-specificity, the assessment of AP activity could provide an indicator of the soil quality in agriculture (NALINI & al [15]).

Most likely, as a result of the new technologies development in order to meet the human population needs, AP will expand its role in many more areas of activities in the future.

The present study it is part of a broader, integrative and long term research theme, focused on the assessment of the natural aquatic ecosystems from Danube Delta in terms of their structural and functional characteristics nowadays, but as well as ecological value, or possible impact drivers that direct their evolution over the time. Well known, the Danube Delta is one of the main natural regions worldwide, with numerous unique attributes regarding the spatial heterogeneity of its wetlands or the biodiversity these inhabit (GÂŞTESCU & ŞTIUCĂ [16]).

In this paper, we aimed to present the spatio-temporal dynamics of the alkaline phosphatases activity (APA), in order to complete the framework of the enzymatic activity investigated along 2011-2013 period of time in the water column and sediments of several natural ecosystems of the Danube Delta.

Two natural lentic ecosystems with different trophic states, and their connective channels, were selected to be investigated. **The intensity of the decomposition processes of the detrital organic matter was assessed through the extracellular activity of two enzymes – β-glucosidase** (PĂCEŞILĂ [17]) **and alkaline phosphatase.** These enzymes are involved in the mineralization process of the organic macromolecules, and can provide an important amount of nutrients for various heterotrophic microorganisms and phytoplanktonic organisms in the aquatic ecosystems. Thus, the necessity of the present study appeared to be as well justified in a larger context, of the anthropogenic alterations in the Danube Delta ecosystems along the time (VĂDINEANU [18], VĂDINEANU [19]).VĂDINEANU & al [20]).

Materials and Methods

Eight sample sites were established in four aquatic ecosystems from Roşu-Puiu lake complex of Danube Delta (Romania): five in Roşu Lake (R_1-R_5), one in Mândra Lake (M) and one in each of Roşu-Împuţita (C_1) and Roşu-Puiu (C_2) channels (PACEŞILĂ [17]).

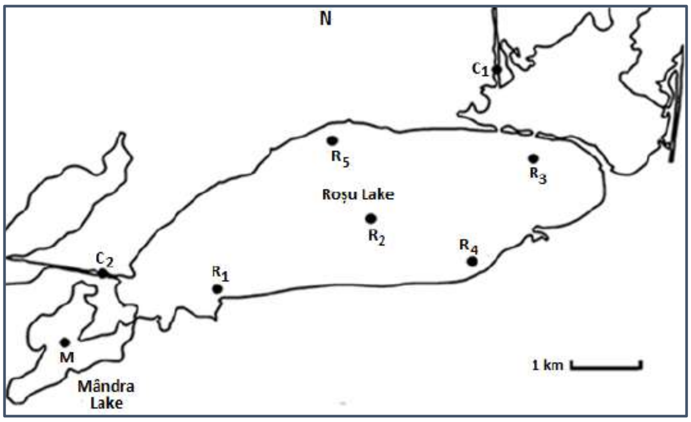

Figure 1.
Location of the sampling stations (PĂCEŞILĂ, [15])

Roşu Lake (R)
Mândra Lake (M)
Roşu-Împuţita channel (C_1)
Roşu-Puiu channel (C_2)

Samples were taken seasonally during 2011-2013 period of time, from water and at the water-sediment interface, in May, Jully and October. AP activity was evaluated using p-nitrophenyl-phosphate consumption (OBST [21]), and the results were reported to a liter of water, or a gram of dry silt. Variations were discussed in terms of seasonal and annual avarege values of APA in the studied sites.

All variables supported ln (x) transformation and all statistical analyses were performed using Paleontological Statistics (PAST) software (HAMMER & al [22]).

Results and Discussion

AP activity revealed a complex dynamic fluctuation, its values ranging between 230-2578 nmol p-nitrophenol $L^{-1}h^{-1}$ in the water column and 2104-15631 nmol p-nitrophenol $g^{-1}h^{-1}$ in sediment.

Spatially, APA intensity fluctuated between the sites during the study. Its variations could be explained by the spatial heterogeneity of the assessed ecosystems and by their temporal changes in the physico-chemical and biological parameters (WETZEL [23]; JANSSEN & al [24]).

The most intense APA was registered in Roșu-Împuțita channel, for both water (with an average value in the study period of 1079 nmol p-nitrophenol $L^{-1}h^{-1}$, Fig. 2a) and sediment (10016 nmol p-nitrophenol $g^{-1}h^{-1}$, Fig. 2b). This channel is known for the intense decomposition processes that are taking place in its substrate, particularly of the sulphur compounds [BOTNARIUC & VĂDINEANU [25]). The lowest AP activity was detected in the water column of the Mândra lake (a shallow, mesoeutrophic lentic ecosystem, PARPALA & ZINEVICI [26]) (782 nmol p-nitrophenol $L^{-1}h^{-1}$, Fig. 2a), and in the sediment of the Roșu lake (6515 nmol p-nitrophenol $g^{-1}h^{-1}$). Lake Roșu is a large one (14,5 km^2), with frequently occurring algal blooms (MOLDOVEANU & FLORESCU [27]). In its water column the highest average value of APA, was measured in R5 station (1381 nmol p-nitrophenol $L^{-1}h^{-1}$, Fig. 2a) and the lowest, in R2 (505 nmol p-nitrophenol $L^{-1}h^{-1}$, Fig. 2a), while within the sediment samples, the highest APA average value in R1 (10803 nmol p-nitrophenol $g^{-1}h^{-1}$, Fig. 2b) and the lowest in R4 (2453 nmol p-nitrophenol $g^{-1}h^{-1}$, Fig. 2b).

In the Roșu-Puiu channel, the average APA value in the water column was 998 nmol p-nitrophenol $L^{-1}h^{-1}$ (Fig. 2a) and in sediment, 9392 nmol p-nitrophenol $g^{-1}h^{-1}$ (Fig. 2b).

Thus, APA intensity dynamic revealed location specificity in the most of the sampling points. However, a high similarity were observed between several sampling points, such as R1 - R4 and C1 - C2 in the water column (Fig. 3A), and R3 - R5 and C1 - R1 in the sediment (Fig. 3B). Noteworthy, at the sediment level, the APA dynamic in R4 differed completely by the other stations.

The seasonal variation of the APA did not showed a clear trend in the evaluated ecosystems. In the majority of the sampling sites, AP activity was more intense in summer, in the water column, (Fig. 2a - A), excepting R3, C2 and M, where the highest values of this enzyme were recorded in autumn (Fig. 2a - A). Unlike the water column, in the sediment samples the maximum AP values were detected in September, in almost all the cases (Fig. 2b - A).

The One Way ANOVA results, established that APA dynamic recorded significant differences between seasons for the water column (n=24; p<0,001; F=14,11). According to the Tukey post-hoc test, the differences were between spring and summer seasons, but also between spring and autumn. This suggests that the changes that occurred in the dynamics of the environmental factors following the transition from spring to summer, have influenced to a greater extent the pattern of APA variation, most likely due to the increase in the need for phosphorus nutrient for the plankton communities. In the sediment temporal pattern of samples, were not confirmed differences (n=24, p=0,356, F=1,056).

The NMDS graphic representation enabled as well the observation of a seasonal grouping tendency of the APA values for the water column (Fig. 4A.). In sediment, this trend could be observed especially for the autumn samples (Fig. 4B.).

The results also reveal that 2013, generally offered the most favorable environmental conditions for APA, being the year with the highest intensity values of the enzyme activity (excepting R2 station for the water column, and C1 for the sediment samples – where the intensity peaks appeared in 2012). The lowest AP intensity was detected in 2011 (Fig. 2B).

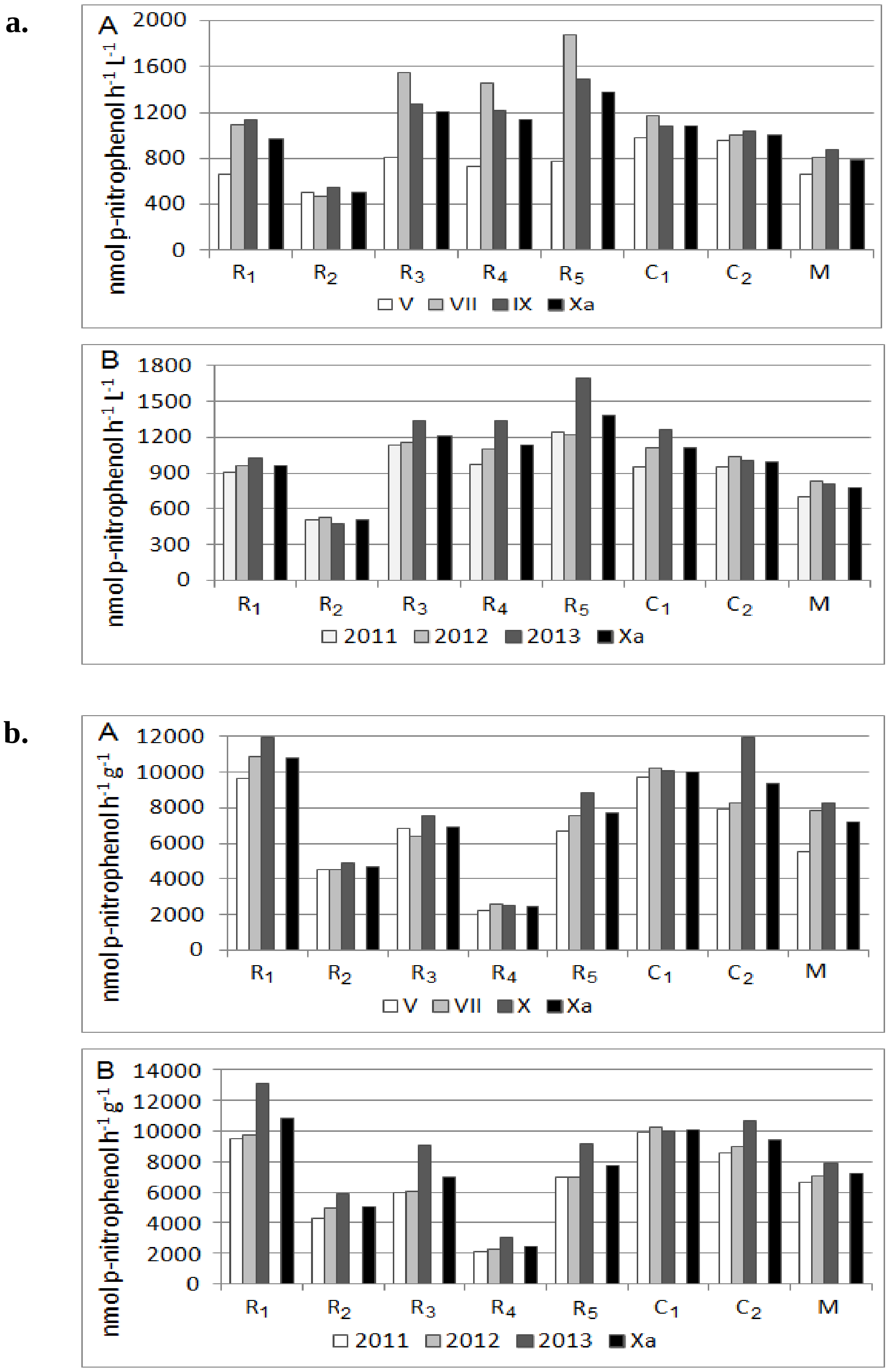

Figure 2. The seasonal **(A)** and annual **(B)** dynamics of the alkaline phosphatase activity (APA) in the water column **(a)** and in the sediment samples **(b)**.

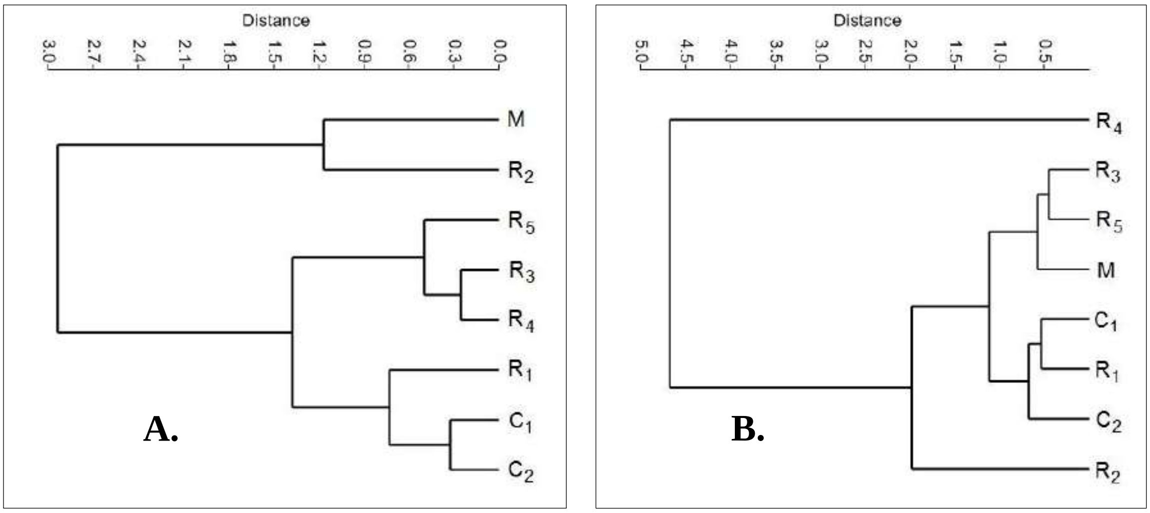

Figure 3. The cluster analysis of similarity (Ward's method) of the APA spatial dynamics across the sapling sites in the water column **(A)** and in the sediment **(B)**.

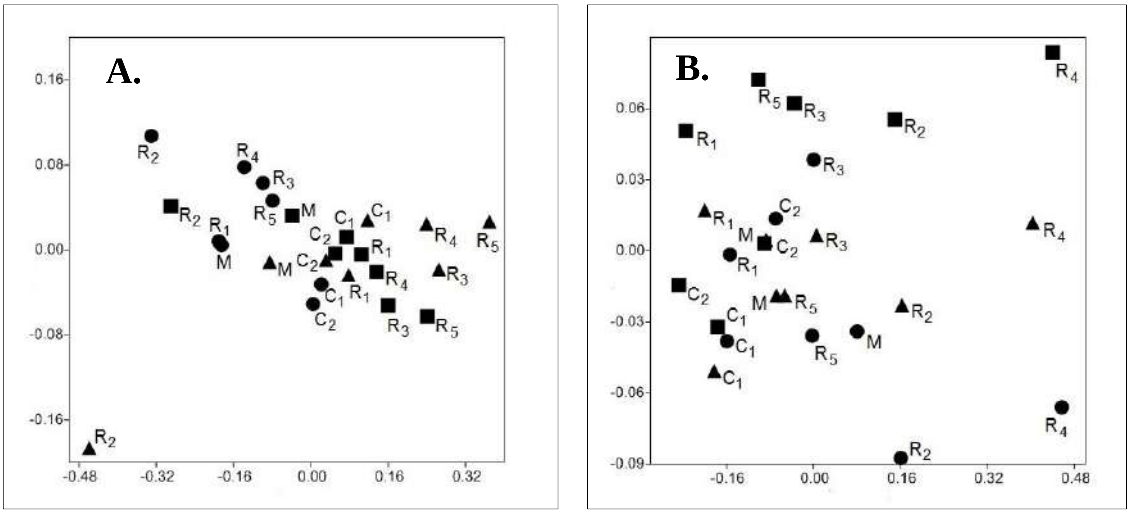

Figure 4. The non-metric multidimensional scaling analysis of APA temporal dynamics, for the water column (**A.** NMDS, Euclidian similarity index, 2D, R2: OX=0.82, OY=0.09, stress: 0.018) and in the sediment samples (**B.** NMDS, Euclidian similarity index, 2D, R2: OX=0.79, OY=0.11, stress: 0.03). Symbols on the plots: triangles – Lake Roşu (R_1-R_5) samples, circles – Roşu-Împuțita (C1) and Roşu-Puiu (C2) channels, and squares – for the Mândra Lake (M) samples.

Conclusions

- Our results have shown that Alkaline Phosphatase Activity (APA) has been present throughout the whole period of the study in the investigated ecosystems, indicating that the planktonic and benthic communities actively used the organophosphorus compounds (DOP) |as a source of phosphoric nutrient, participating to the phosphorus recycling in these aquatic ecosystems.
- APA intensity fluctuated significantly and recorded its highest values at the water-sediment interface.
- Across the deltaic ecosystems in our study area, APA presented spatial variations. Its highest values were found in Roșu-Împuțita channel for both types of substrate, and the lowest in the water column of the Mândra Lake, and in the sediment of the Roşu Lake.
- APA revealed a low degree of similarity between the sampling sites, that could indicate a large heterogeneity of the environmental parameters values in the investigated area.
- The dynamics of APA had shown its highest intensity in 2013 and the lowest in 2011 for all ecosystems, in both, the water column and sediments.
- Regarding the temporal variations, APA intensity in the water column was higher, mainly in the summer season, and in autumn only for the sediment samples.
- NMDS plot seasonally grouped a part of the samples. The pattern appeared more obvious for the water column APA, and suggested that the seasonally fluctuation

of the physico-chemical parameters played an important role in the variability of this enzyme intensity.

- One way ANOVA proved as significant only the differences between APA dynamics in spring, compared to the other seasons.

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