Research Article

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Microbial Phosphatase Activity as an Indicator of Water Quality in Tributaries and Littoral Area of Lake Ohrid in the Period 2013–2014

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Abstract: Inducible enzymes of microorganisms play the most important role in degradation of polymer compounds in water. Recently, water enzymatic activity has been emphasised as a new, potentially good indicator for water saprobity and trophic state of ecosystems. The aim of this work was to determine the levels of phosphatase activity (on p-nitrophenyl phosphate as substrate) in the rivers Cherava and Velgoshka and the littoral area of Lake Ohrid, where the rivers inflow into the lake. The enzymatic activity and the number of organotrophic bacteria were studied in the period from September 2013 to September 2014. The water at the studied localities was characterised from satisfactorily clean to slightly and very polluted. The statistically significant correlation between phosphatase activity and microbiological parameters revealed the phosphatase activity in the water as a good indicator of the available organic matter and the degree of eutrophication.

Key words: Water phosphatase activity, heterotrophic, oligotrophic bacteria, eutrophication

Introduction

Phosphatases as enzymes that hydrolyse high-weight organic phosphorus compounds to orthophosphates have an essential function in lake nutrient dynamics (Jansson et al. 1988, Chróst 1994). These enzymes are important not only for the phosphorus cycle, but also have strong effect on carbon mobility in aquatic ecosystems. According to Hoppe (2003), the phosphatases can manifest multiple functions in aquatic ecosystems by their simultaneous supplying with phosphorus, organic carbon and nitrogen. The occurrence of the phosphatases in substrate depleted water ecosystems is of special interest, since the phosphatase activity can be induced by the carbon limitation (Hoppe 2003) and is a convenient indicator of phosphorus deficiency (Cembella et al. 1982).

In addition to the classical microbiological parameters for assessment of water quality, modern biochemical methods have also been frequently used (Matavulj et al. 1989, 1990). One of those methods is the estimation of enzymatic activity in water as a result of the activity of organotrophic microorganisms, which as a main component of aquatic ecosystems have a major role in controlling the degradation of most organic components.

The long-term investigations and analyses of the water in Lake Ohrid have supported the conclusion that this aquatic ecosystem has an oligotrophic character with a unique ecological complex, which provides conditions for forming a special endemic and relict living world (Stanković S. 1960, Albrecht & Wilke 2008). The natural eutrophication has been slow and balanced, but recently, the lake has been subjected to high anthropogenic influence (Kostoski et al. 2010). The aim of this work was to determine the levels of phosphatase activity (on p-nitrophenyl phosphate as substrate) in the rivers Cherava and Velgoshka and the littoral areas of Lake Ohrid, where these rivers inflow into the lake.

Materials and Methods

Lake Ohrid is a cross-border lake located in the south-western part of the Republic of Macedonia. It has a total surface area of 358 km^2 (Matzinger et al. 2007), of which 118.9 km^2 belong to the Republic of Albania. Lake Ohrid is the largest lake in the Dessarete Lake group, with a maximal depth of 288.7 m (Matzinger et al. 2007). The largest tributaries of the lake are the rivers Sateska, Koselska and Velgoshka, all of which are on the Macedonian side, as well as the Cherava River, which upper course belongs to the Albanian territory.

The samples for the microbiological study were collected in the period from September 2013 to September 2014. Four localities were sampled: two tributaries of Lake Ohrid, the rivers Velgoshka and Cherava, and the littoral areas, where these tributaries inflow into the lake.

The acid, neutral and alkaline phosphatase activities were estimated in water samples on p-nitrophenyl phosphate as a substrate, and the index of phosphatase activity of water (IFA) was calculated according to the method of Matavulj (1986). Besides the phosphatase activity, we determined the number of heterotrophic (saprophytic) bacteria (H) on meat peptone agar (MPA) as a media, and the number of facultative oligotrophic bacteria (FO) on diluted MPA 1:10 (Petrović et al. 1998). We also estimated the ratio FO/H, which is a very good indicator of water self-purification ability. Spearman's non-parametric test (Statistica 7.0; Statsoft Inc.) was used to find the possible correlation between alkaline phosphatase and heterotrophic bacteria and between alkaline phosphatase and facultative oligotrophic bacteria.

The classification of water quality was made according to Matavulj et al. (1989).

Results and Discussion

Permanently high values of the phosphatase activity were recorded in the waters of the Velgoshka River and the littoral area of the lake in front of its inflow. In the Velgoshka River, the alkaline phosphatase activity had an average value of 5.150 μmol/s/dm^3; the neutral phosphatase demonstrated temporally its own activity (1.950 μmol/s/dm^3); and the acid phosphatase was incidentally present in the water (Fig. 1).

The maximal activities of all phosphatases (acid, neutral and alkaline) were recorded in August 2014 and those activities were probably generated by bacterial cells. The number of bacteria was almost 120,000 CFU.ml^{-1}. This high number of bacteria caused a decrease in the FO/H index to 0.533. The results were due to the increased content of dissolved organic matter in the water (26.040 mg.L^{-1}) and the high temperature, a factor which is in close relation to the nutritional conditions in the ecosystem (Carlsson & Caron 2001).

The comparative analysis of the three phosphatases in the littoral area of Lake Ohrid in front of the inflow of the Velgoshka River, showed that the most abundant was the alkaline phosphatase, with an average activity of 1.014 μmol/s/dm^3; the neutral phosphatase had an average activity of 0.792 μmol/s/dm^3; the acid phosphatase was incidentally present in the water and its average activity was 0.404 μmol/s/dm^3 (Fig. 2).

The permanently high values of phosphatase activity in the waters of the Velgoshka River and the littoral area of the lake in front of its inflow indicated that this river had strong negative influence on the littoral zone of Lake Ohrid. Previous results obtained from a two year-long investigation (2008–2010) of Lokoska (2011, 2013) demonstrated that the number of heterotrophic bacteria in the Velgoshka River was very high (over 100,000 CFU.ml^{-1}). This tributary has reduced water content and limited capacity for auto-purification because of the permanent input of waste industrial water, which contains inorganic and organic chemical pollutants.

The average activities of the three phosphatases in the water of the Cherava River were almost equal. The alkaline and neutral phosphatases were steadily active, while the acid activity occurred occasionally, but with a sufficiently high activity, which determined their average activity value of 2.020 μmol/s/dm^3 (Fig. 3).

The maximal phosphatase activity of both the Cherava River and the littoral area in front of its inflow were recorded in September 2013. In the Cherava River, the acid phosphatase activity was 8.170 μmol/s/dm^3, the neutral phosphatase activity was 6.570 μmol/s/dm^3, and the alkaline phosphatase activity was 5.120 μmol/s/dm^3, while in the littoral area, the same activities had values of 2.676 μmol/s/dm^3, 1.061 μmol/s/dm^3, and 0.538 μmol/s/dm^3, respectively (Fig. 4). Most likely, the high phosphatase activity was a result of the high content of dissolved organic matter in the Cherava River in that period (42.700 mg.L^{-1}), as well as the human impact during the touristic season as the camp 'Ljubanista' is located nearby.

The comparative analysis of the acid, neutral and alkaline phosphatase activities indicated that the highest activity was manifested by the alkaline phosphatase and this activity was permanently present in both the river and littoral lake sites. Jansson et al.

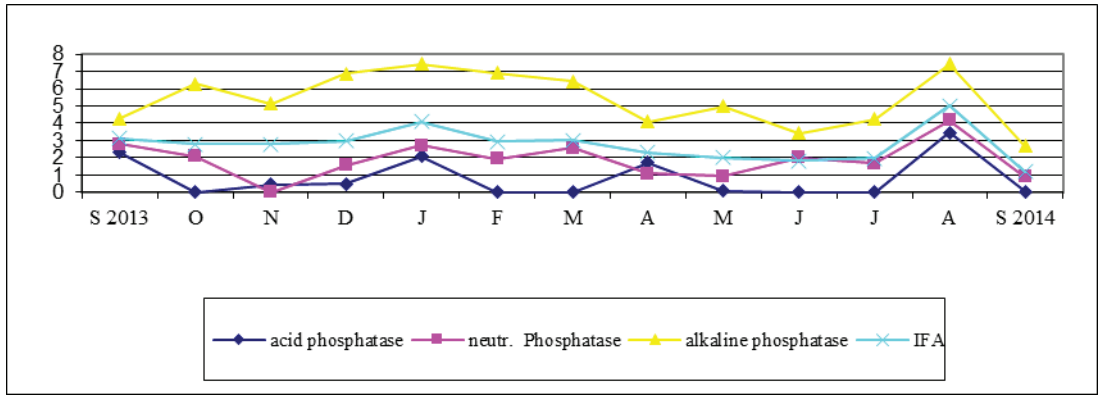

Fig. 1. Dynamics of acid, neutral and alkaline phosphatase activities in the water of the Velgoshka River, Lake Ohrid watershed, in the period September 2013 – September 2014; IFA – index of phosphatase activity of water

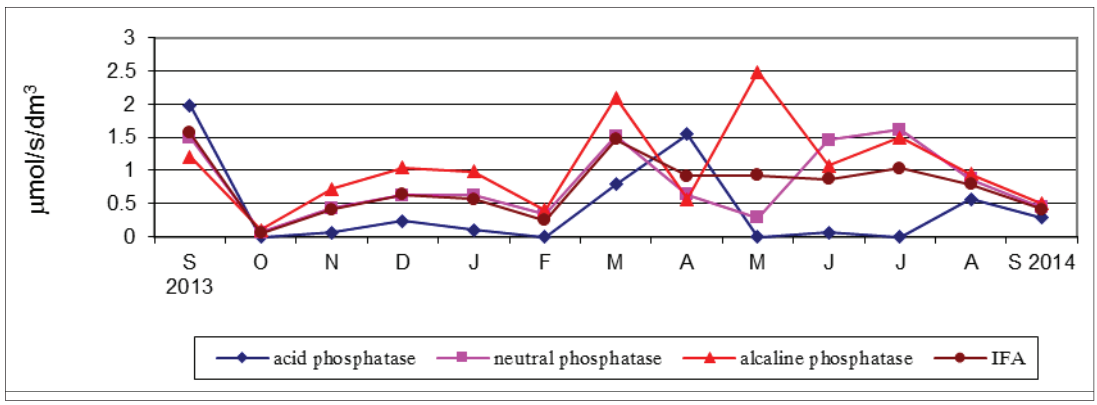

Fig. 2. Dynamics of acid, neutral and alkaline phosphatase activities in the littoral area of Lake Ohrid in front of the inflow of the Velgoshka River, in the period September 2013 – September 2014; IFA – index of phosphatase activity of water

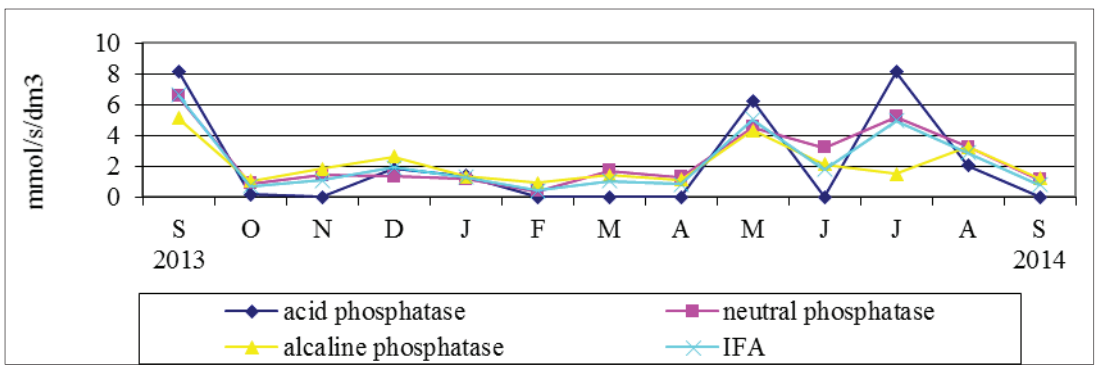

Fig. 3. Dynamics of acid, neutral and alkaline phosphatase activities in the water of the Cherava River, Lake Ohrid watershed, in the period September 2013 – September 2014; IFA – index of phosphatase activity of water

(1988) assumed that the conditions that are favourable for the expression of acid phosphatase activity are not characteristic of the most natural lakes. Actually, it has been confirmed that bacteria and algae generally grow and develop better in alkaline environmental conditions, and therefore, they produce more alkaline than acid phosphatase with external functions.

Based on the received results and the average values of the index of phosphatase activity of water (IFA), the water quality of the studied sites was assessed according to the classification of Matavulj et al. (1989). The average phosphatase activity in the water of the Velgoshka River was 2.632 µmol/s/dm^3, which corresponded to average water quality of IIIA class (polluted), while the average phosphatase activity in the Cherava River was 2.020 µmol/s/dm^3, corresponding to II–III classes (moderately polluted). The average value of the IFA index in the littoral

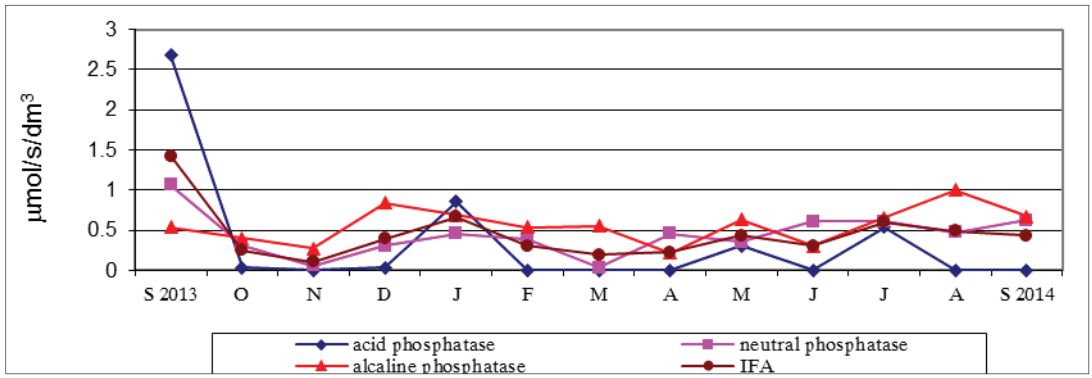

Fig. 4. Dynamics of acid, neutral and alkaline phosphatase activities in the littoral area of Lake Ohrid in front of the inflow of the Cherava River, in the period September 2013 – September 2014; IFA – index of phosphatase activity of water

Table 1. Correlation (Spearman's nonparametric test, $p<0.05$) between the alkaline phosphatase and the heterotrophic and facultative oligotrophic bacteria in the littoral area of Lake Ohrid, in the period September 2013 – September 2014

Period of sampling	Correlation coefficient (r) for heterotrophic bacteria and alkaline phosphatase	Correlation coefficient (r) for facultative oligotrophic bacteria and alkaline phosphatase
September 2013	0.981	0.880
November 2013	0.912	0.940
December 2013	0.912	0.880
January 2014	0.990	0.990
February 2014	0.991	0.830
March 2014	0.960	0.880
April 2014	0.940	0.860
May 2014	0.870	0.860
June 2014	0.780	0.810
August 2014	0.910	0.640
September 2014	0.645	0.920

area in front of the inflow of the Cherava River was 0.429 µmol/s/dm^3, IIA class (satisfactorily clean), while that in the littoral area in front of the inflow of the Velgoshka River was 0.707 µmol/s/dm^3, IIB class (slightly polluted) (Matavulj et al. 1989).

Our results showed a comparatively high correlation between the total enzymatic activities of all three phosphatases (in the original samples) and the classic microbiological parameters (number of heterotrophic and facultative oligotrophic bacteria). During the entire studied period, we found a high degree of positive correlation between the alkaline phosphatase and the number of heterotrophic (organotrophic) bacteria (Table 1). An exception from this finding was recorded in September 2014 when the minimal value was r=0.645. The highest correlation value (r=0.991) was observed in February 2014. In general, the average correlation value between the organotrophic bacteria and alkaline phosphatase during the studied period was r=0.887.

The facultative oligotrophic bacteria were the dominant autochthonous bacteria that mineralise dissolved organic matter of autochthonous origin. The correlation coefficient between the facultative oligotrophic bacteria and alkaline phosphatase ranged from r=0.640 in August 2014 to r=0.990 in January 2014 (Table 1). In September 2014, there was a high correlation between the facultative oligotrophic bacteria and alkaline phosphatase (r=0.920), although the correlation between the heterotrophic bacteria and alkaline phosphatase was very low (r=0.645). The average correlation value between the facultative oligotrophic bacteria and alkaline phosphatase was r=0.855.

Jones (1972) was one of the first authors who noticed a positive correlation between the enzymatic activity of alkaline phosphatase and biomass of microorganisms. The mentioned author determined the total phosphorus content, total dissolved phosphorus, orthophosphates and alkaline phosphatase activity in 16 regions of English Lake District and found a significantly high correlation between the enzymatic activity and total phosphorus content, as well as the bacterial biomass. The high

degree of correlation of mentioned parameters show that enzymes are good indicators of eutrophication. The high positive correlation between the alkaline enzymatic activity and classic biological indicators found by us during the present study also indicated that the enzymatic activity can be used as an additional parameter in the assessment of water quality in the Lake Ohrid watershed.

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