

Article

Ecological Importance of Alkaline Phosphatase Activity and Acid Phosphatase Activity in Lakes with Different Catchment Use Structures

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Abstract: Surface waters in Europe and across other continents have been affected by anthropogenic activities, leading to changes in the ecological state of lakes due to the continuous phenomenon of eutrophication. This study assesses the activity of enzymes secreted in bottom sediments collected from two lakes, aiming to determine the interaction between bottom sediments and water based on samples collected from lake bottoms and overlying water. The study measured the production of secreted enzymes via alkaline phosphatase (ALP) and acidic phosphatase (ACP) in two distinct environments. Sample collection encompassed two lakes within two diverse catchment types (agro-forestry and forest) during spring and summer. The findings revealed higher ACP values than ALP in both lake environments, with higher enzyme values recorded in the lake with a typical forest catchment area. High values of enzymes were related to the pollutant's input into the lakes, suggesting that anthropogenic activities may have a substantial influence on the studied lakes. Principal component analysis (PCA) allowed the identification of a negative correlation between the enzymatic activity of phosphorus in bottom sediments and the content of organic phosphorus (org-P) in overlying waters only within the forest catchment, where the angle between the vectors representing primary variables is close to 180 degrees. The conducted statistical analysis demonstrated significant distinctions among the lakes concerning most of the examined parameters.

Keywords: phosphatase enzymes; organic phosphorus; land use; environmental impacts



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1. Introduction

Surface water from lakes in lowland areas can be used for recreational, agricultural, industrial, and other purposes. River–lake systems play an important role in nature and hydrology. Due to their specific location, most often in lowland areas, they are particularly exposed to the eutrophication process. The quality of surface waters is closely related to the negative phenomenon of eutrophication [1,2]. Eutrophication is linked to elevated levels of nutrients, specifically nitrogen (N) and phosphorus (P). P typically serves as the limiting factor in lake eutrophication, and when external P is appropriately managed, P released from lake sediments becomes the main contributor to water eutrophication. One of the factors of the trophic evolution of waters is the interaction between bottom sediments in water reservoirs and overlying waters. Bottom sediments can act as a trap and sometimes an emitter of P due to many biological, physical, and chemical processes taking place in water. Therefore, controlling and monitoring exogenous P is important to protect surface water quality, prevent eutrophication, and maintain a healthy aquatic ecosystem [3,4]. Research on the release of endogenous phosphorus from sediments has primarily focused on understanding the impact of environmental, physical, and chemical factors on morphological transformations and release quantities [5–8].

The European Commission's Standard Measurements and Testing (SMT) Program introduced a P fractionation method, classifying phosphorus in various forms, including total phosphorus (P), organic phosphorus (org-P), inorganic phosphorus (IP), reactive P sorbed on metal oxides (NaOH-P), and calcium-bound phosphorus (HCl-P). In lake ecosystems, org-P plays a pivotal role in the phosphorus balance, serving as a significant source of phosphorus released from sediments in eutrophic lakes. The fundamental processes governing org-P release in lakes are closely tied to mineralization and the breakdown of phosphatase enzymes, specifically acid phosphatase (ACP) and alkaline phosphatase (ALP). Acid phosphatase primarily originates from microorganisms and plant root secretions, while ALP is predominantly produced by microorganisms [9]. The form, like ALP, plays a role in dephosphorylating organic phosphorus compounds by disrupting the phosphodiester linkage, leading to the removal of phosphate groups and the formation of orthophosphates. Earlier studies have demonstrated that environmental factors, including land use type, climate, pH, and agricultural practices, play a substantial role in influencing the activities of phosphatase enzymes and the diversity of microorganisms that harbor these enzymes [4,10–12]. Notably, the pH in both soil and sediments stands out as a decisive factor, particularly for phosphatase, as it governs both the extent and nature of phosphatase activities [13].

The sediment–water interface in lakes is a specific connection important for the biogeochemical nutrient cycle [14]. Various physical, chemical, and biological reactions occur at the junction of two surfaces (water and sediment), which makes this place the basis for regulating and controlling the circulation of elements between sediments and overlying water [14,15]. The sorption and desorption of P and organic matter at the water–sediment interface may affect the bioavailability of P, such as organic carbon [16,17] and P [18–20], and may also increase the activity of phosphatase enzymes [21,22].

Phosphatase, a key enzyme for the internal circulation of phosphorus in lakes, is common in both water and sediments [23–25]. Its action can be attributed to phosphatases found on the surface of algae and bacteria cells and dissolved enzymes originating from autolysis or the excretion by algae, bacteria, or zooplankton [23]. Serving as an essential catalyst, phosphatase hydrolyzes organic P in soil and sediment, releasing available org-P for use by plants and microbes [26].

In lakes, both ALP and ACP are capable of hydrolyzing monoester P, except for phytate [23,25]. ACP is frequently found inside algal cells and is likely produced to modulate the internal metabolism of P [23].

The aim of the study is, therefore, to evaluate the activity of phosphatase enzymes in the two lakes characterized by different land use patterns in their immediate catchment areas. Additionally, the aim is to examine whether there is a reaction between org-P in overlying water and ALP and ACP in bottom sediments. We hypothesize that: (1) Different land uses determine the levels of both ACP and ALP. (2) In the case of the lake situated within a forested catchment, the values of ALP will be higher compared to the lake within an agroforestry catchment. (3) The primary factor influencing the levels of phosphatase enzymes is org-P content.

The findings of this study may contribute to a deeper understanding of the potential effects of catchment use on lake biogeochemistry.

2. Materials and Methods

2.1. Description of the Sampling Sites and Sediment Collection

The study area comprises two natural lakes within the river–lake system, specifically Lake Łekno and Lake Jeziory Wielkie, located in the central-western part of Poland (52°09'24" N 17°08'41" E and 52°10'28" N 17°07'48" E), belonging to the Głuszynka river–lake system (Figure 1). Lake Raczyńskie is a water source for lakes in this river–lake system, where lakes play a crucial role in retaining orthophosphates (V) in the water, as emphasized by Janicka et al. in their work [27]. Their observations highlight the significant impact of anthropogenic factors on the water quality of these lakes. Specifically, a decreasing trend in

orthophosphate (V) content was observed in the river–lake system, indicating the reduction potential. Conversely, sporadic peaks in orthophosphate content occurred, such as after the clearing of forests near the shore, underscoring the heightened sensitivity of the analyzed lakes to anthropogenic pressures. Lake Łekno has an average water depth of 2.2 m, with a maximum water depth of 5.0 m, while Lake Jeziory Wielkie has an average water depth of 3.0 m and a maximum water depth of 5.4 m. The study area has a moderate climate, with an average air temperature of 9.1 °C and an average precipitation of 580.4 mm [27]. In the hydrological year 2020, the sum of precipitation was 510 mm, and in 2022, 520 mm. According to the analysis of precipitation conditions for the 30th hydrological anniversary, it was discovered that the years 2020 and 2022 were dry.

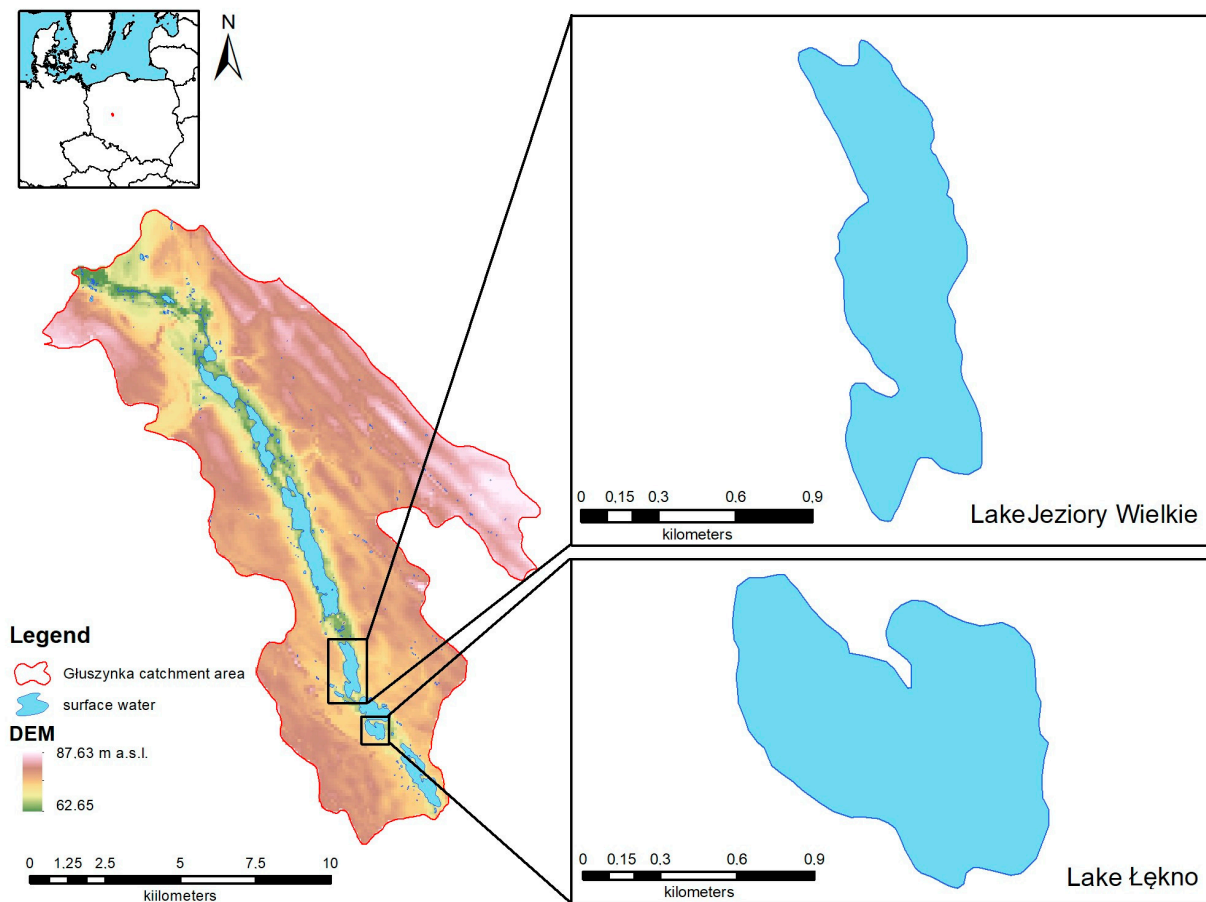


Figure 1. Study area.

The direct catchment area for Lake Łekno spans 1.42 km², while that of Lake Jeziory Wielkie covers 7.53 km². The research focused on non-stratified lakes, where there is no distinct thermal stratification. The direct catchment area of Lake Łekno has a forested character, with as much as 77.22% of this area covered by forests (with a predominance of mixed forests), while only 3.48% of the direct catchment area is anthropogenic. In contrast, the immediate catchment area of Lake Jeziory Wielkie has a different character (agroforestry). Agricultural areas dominate the catchment, accounting for 45.86% of the area, while forests still maintain a large share of the area, accounting for 42.05% (Figure 2).

Sampling points and their quantity were determined in proportion to the size of the reservoir, setting a strict grid of squares (FishNet) with a width of 200 m. There were 6 sampling points for Lake Łekno and 15 for Lake Jeziory Wielkie. The latitude and longitude coordinates of these sampling points were determined using a GNSS unit (Figure 3).

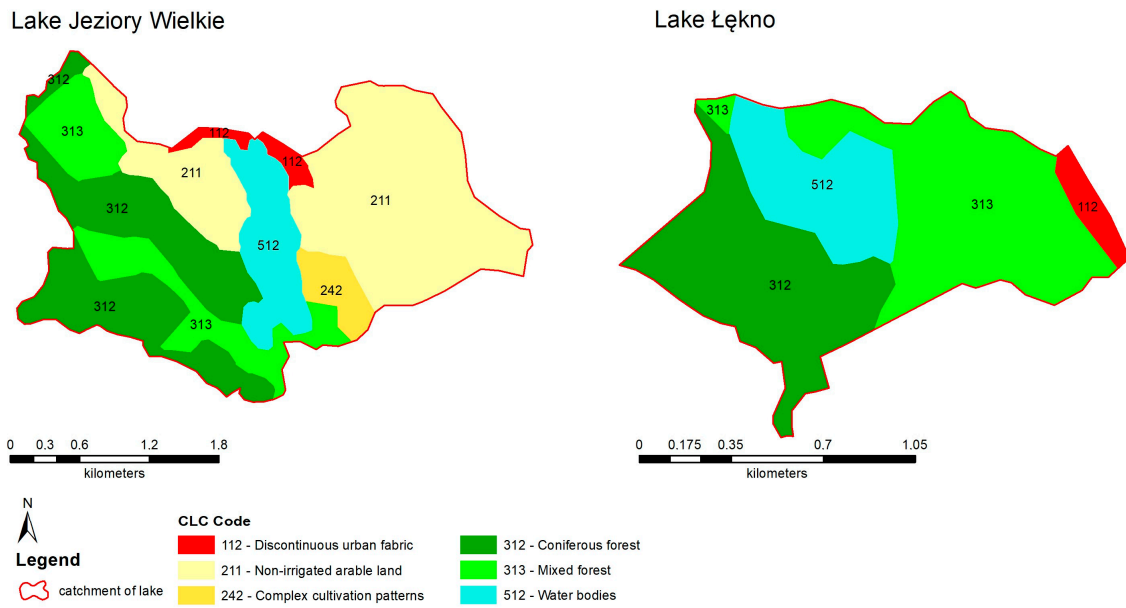


Figure 2. Land use of catchment area, source: CORINE Land Cover (CLC).

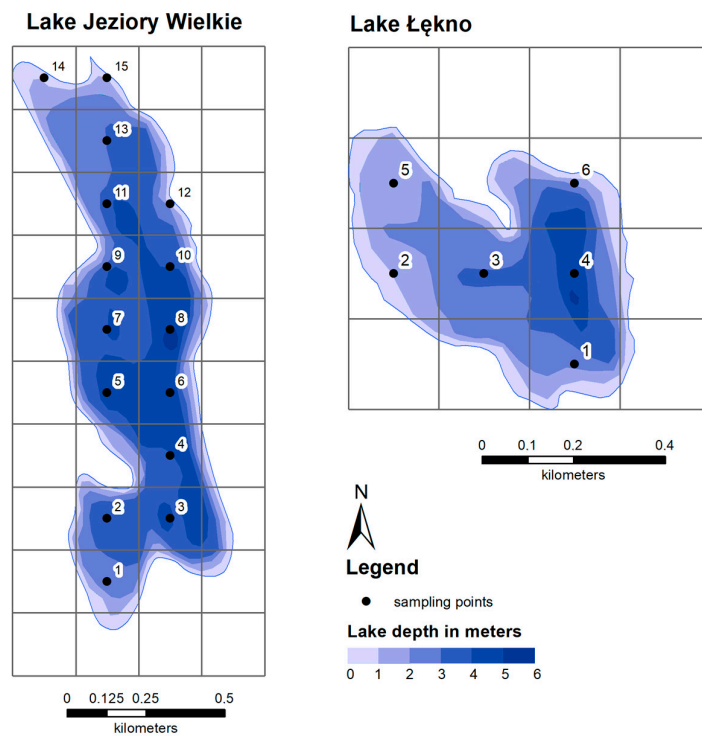


Figure 3. Sampling points.

At selected sampling points, as shown in Figure 3, the surface layer of sediments (0–20 cm) was collected from lake bottoms using a van Veen gripper, then sealed in polyethylene bags and immediately transported back to the laboratory in the spring of 2020 and the summer of 2022.

One portion of the samples was stored in a refrigerator at 4 °C to analyze the ACP and ALP activity test (within 24 h). The remaining portion of the samples was prepared to analyze the texture. Soil texture was determined using Casagrande’s areometric method, modified by Prószyński, using the PN-R-040032 standard [28]. The soil texture samples were classified according to the United States Department of Agriculture standards. During

the sampling of bottom sediments, overlying water was also collected from the bottom layer using a Van Dorn sampler. The concentration of org-P was determined using the EN ISO 6878 method [29]. The pH measurements were taken in situ using CP-105 ELMETRON.

2.2. Data Collection

We acquired the cartographic resources, serving as the foundational data for our study (data open access):

- The Map of the Hydrographic Division of Poland (MPHP) with a scale of 1:10,000 was collected by the Institute of Meteorology and Water Management (IMGW) in Poland (IMGW = Instytut Meteorologii i Gospodarki Wodnej);
- Climatic data (precipitation and air temperature) collected from IMGW;
- CLC vector layers derived from the Copernicus Land Monitoring Service (most recent data from 2018);
- Digital Elevation Model (DEM) with a grid size of at least 5 m for Poland provided by GUGiK. We used DEM to verify lake catchment boundaries and study land surface topography (slope);
- Bathymetric maps on paper of Lake Łęknio and Lake Jeziory Wielkie were vectorized.

All spatial analyses were performed using ArcMap 10.7.1. Inverse distance weighting (IDW) interpolation was used to estimate ACP and ALP enzyme values.

2.3. ACP and ALP Sediment Activity Test

ACP and ALP activity was assessed using the Tabatabai and Bremner [30] method, carried out as follows: 1.0 g of a freshly harvested sample was placed in a disinfected reaction tube, and 0.2 mL of toluene was added to the tube to create anaerobic conditions. Then, 4 mL of a tris(hydroxymethyl) aminomethane-based universal buffer (pH 6.5 for ACP samples and pH 11 for ALP samples) was added. Subsequently, 1 mL of a disodium p-nitrophenyl phosphate substrate was added and mixed well via vortexing. The samples were then incubated at 37 °C for 1 h. After the incubation period, a mixture of 1 mL of 0.5 mmol CaCl₂ and 4 mL of 0.5 mmol NaOH solutions was added and thoroughly mixed via vortexing. Next, the samples were filtered using specific filters (MN 616). Subsequently, absorbance readings were taken at 400 nm using the spectrophotometer 7315 Jenway and converted to a sediment activity intensity per gram (dry weight). Each sample underwent three replicates, and ALP and ACP activity were measured as µg p-nitrophenol produced per gram (dry weight) of sediment per hour. The final result represented the average of three trials.

2.4. Statistical Analysis

The multiple comparisons of Tukey's WSD test for unequal sample sizes were used to confirm the existence of various ($\alpha = 0.05$) groups of objects (lakes) concerning their phosphatase (ALP and ACP) levels in bottom sediments, org-P, and pH of samples overlying water, depth, and distance from the shore separately. A principal component analysis (PCA) was performed to show differences between objects regarding the content of all determining factors. Statistical analysis was carried out using the Statistica software (version 13.3).

3. Results and Discussion

The results of determining physicochemical factors in overlying waters in spring and summer are presented in Tables 1 and 2.

The overlying water of Lake Jeziory Wielkie was characterized by a high pH, with an average pH of 8.17. This pH level was slightly higher in spring, averaging 8.19, compared to the summer when it averaged 8.49. In the spring, the highest pH values were observed in the northern part of the lake (specifically at points 13, 14, and 15), which may have been influenced by dissolved organic compounds originating from surface runoff from agricultural areas. The content of org-P in the overlying water of Lake Jeziory Wielkie revealed an average org-P content of 0.53 mg dm⁻³. The org-P content was higher during

the spring period (0.69 mg dm^{-3}) compared to the summer period (0.38 mg dm^{-3}). The higher org-P content in bottom waters in spring could be caused by the runoff of pollutants from the catchment (agroforestry catchment) during spring rains. The sum of precipitation in 1 month before sample collection was 60 mm (IMGW).

Table 1. Physicochemical properties of overlying water in spring and summer in Lake Jeziory Wielkie.

Point	pH [-]		Org-P [mg dm^{-3}]	
	Spring	Summer	Spring	Summer
1	7.89	8.99	0.72	0.61
2	7.95	7.89	0.83	0.31
3	7.72	7.81	1.26	0.39
4	8.86	8.94	0.42	0.07
5	7.62	7.81	0.51	0.19
6	8.8	8.43	0.48	0.21
7	7.97	7.83	0.62	0.34
8	7.62	7.53	2.65	0.82
9	7.89	7.93	0.18	0.39
10	8.01	8.62	0.44	0.43
11	8.18	7.84	0.42	1.23
12	8.03	7.63	0.58	0.21
13	8.77	7.8	0.23	0.18
14	8.85	7.94	0.87	0.14
15	8.71	7.72	0.20	0.10

Table 2. Physicochemical properties of overlying water in spring and summer in Lake Łekno.

Point	pH [-]		Org-P [mg dm^{-3}]	
	Spring	Summer	Spring	Summer
1	6.10	6.30	0.08	0.05
2	6.78	6.85	0.07	0.03
3	7.21	7.35	0.07	0.04
4	7.65	7.42	0.09	0.04
5	7.35	7.40	0.05	0.03
6	7.38	7.43	0.07	0.05

This research examined the activity of phosphatases in the spring and summer, aligning with periods of heightened biological activity corresponding to phases of intense growth and potential environmental shifts. Other scholars exploring this subject similarly concentrate on these two study periods as indicative intervals [4,10–12,23–25,31–33]. The average content of ALP and ACP enzymes in Lake Jeziory Wielkie was 637.57 and $745.41 \mu\text{g}$ of p-nitrophenol $\text{g}^{-1} \text{h}^{-1}$. The content of the ALP enzyme in bottom sediment samples of this lake in spring ranged from 119.34 to $407.23 \mu\text{g}$ of p-nitrophenol $\text{g}^{-1} \text{h}^{-1}$. However, the content of the ACP enzyme was slightly higher in this period, ranging from 144.68 to $866.51 \mu\text{g}$ of p-nitrophenol $\text{g}^{-1} \text{h}^{-1}$. In the summer, the content of both enzymes was slightly higher and ranged from 180.87 to $2852.25 \mu\text{g}$ of p-p-nitrophenol $\text{g}^{-1} \text{h}^{-1}$ and from 329.36 to $1793.01 \mu\text{g}$ of p-nitrophenol $\text{g}^{-1} \text{h}^{-1}$, respectively, for spring and summer (Figures 4 and 5). Moreover, the average ALP value for Lake Jeziory Wielkie in summer was four times higher than in spring. Additionally, the average value of ACP was twice

as high in the same period (Table 1). Daniszewski [31] emphasized in his research that the enzyme values in the summer period are higher than in the colder periods. Similar conclusions were reached by Viaroli et al. [32], who confirmed that a greater amount of the ALP enzyme was observed in the summer. However, the study by Gałazka et al. [33] proved that the values of ACP enzymes were approximately 25% higher than the values of ALP enzymes. Many studies prove that the activity of soil enzymes is positively correlated with the soil's nutrient content. Our research confirms this statement, and additionally, having information about the texture of bottom sediments (sand and loamy sand), we know that they can contribute to the easy transfer of substances at the water–sediment interface (Figure 6). Moreover, the research of Janicka et al. [27] shows that the catchment area of the Głuszynka river–lake system comprises easily permeable formations with low retention capacity. This indicates that in the event of intensive fertilization of these soils, there exists a tangible risk of rapid substance transport to surface and groundwater, potentially posing an additional detrimental factor to the water quality of Lake Jeziory Wielkie.

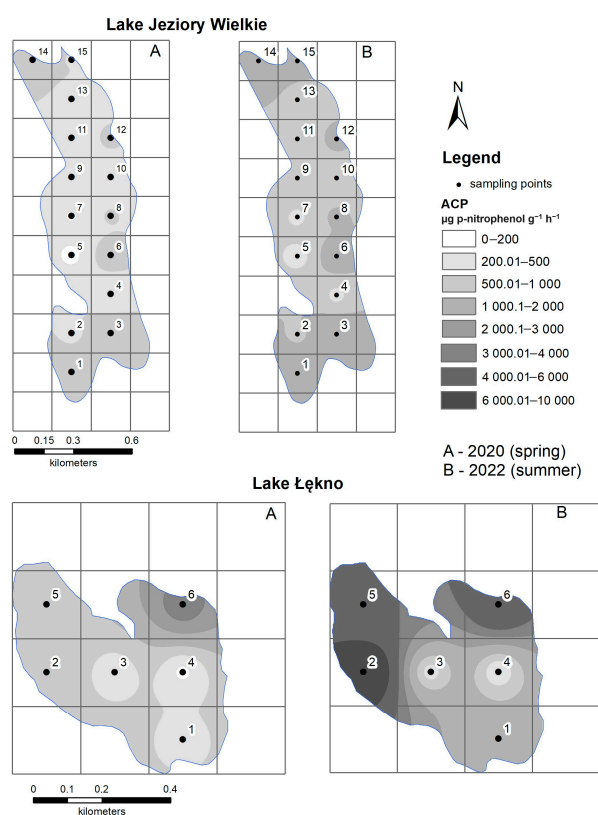


Figure 4. ACP enzyme in bottom sediments of Lake Jeziory Wielkie and Lake Łęčno during spring (A) and summer (B).

In our study, we also observed that the average ALP content was higher in measurement points located at greater depths (points 5 and 9). However, this relationship was not observed for ACP (Figures 4 and 5). These specific points, 5 and 9, located in Lake Jeziory Wielkie, are positioned in an area exposed to greater surface runoff due to the steepest declines occurring within the catchment area, which exceed 31° (Figure 7). An area with steeper slopes may have a more pronounced impact on pollutant runoff, potentially leading to adverse effects on water quality [34]. This pattern aligns with the findings of Li et al. [35], who, in their research on alkaline enzyme levels in Lake Sancha, noted that ALP enzyme content was higher in places of pollutant inflow. The parent materials of soils in these catchment areas are glacial till and loamy sand. This explains the higher enzyme contents observed in the case of points 5 and 9 in Lake Jeziory Wielkie, as this type of soil facilitates the penetration of pollutants from agricultural areas with steep slopes.

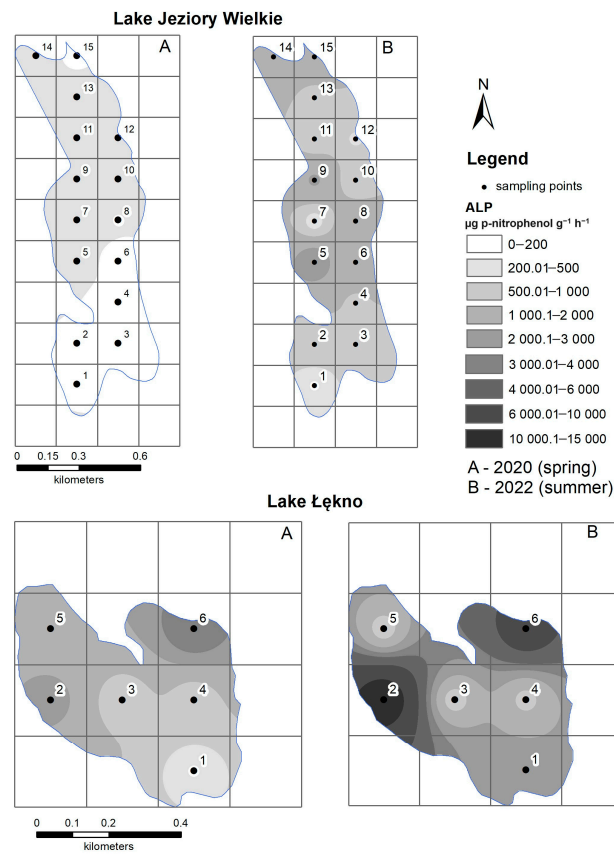


Figure 5. ALP enzyme in bottom sediments of Lake Jeziory Wielkie and Lake Łekno during spring (A) and summer (B).

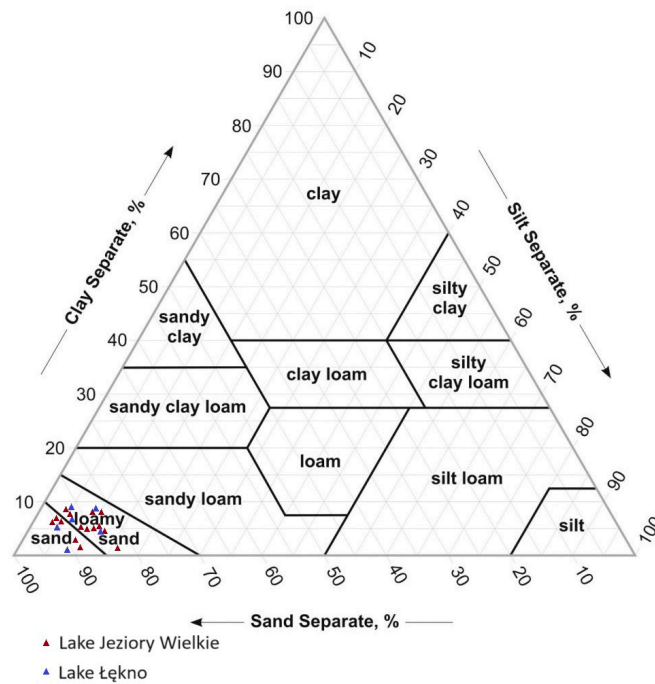


Figure 6. Analysis of the granulometric composition of soil samples in the lakes.

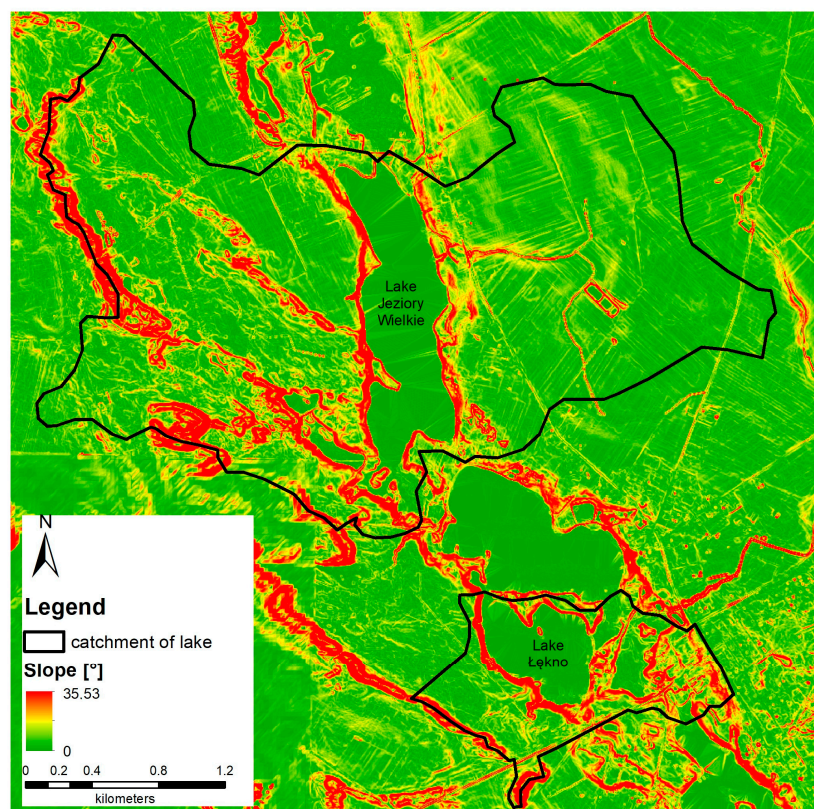


Figure 7. Slope in the catchment area of Lake Jeziory Wielkie and Lake Łekno.

In the case of Lake Łekno, a slightly lower pH was observed in overlying waters during both measurement periods. The average pH in this lake was 7.721. However, concerning org-P in overlying waters in spring, the content was nearly twice as high as during the summer. In summer, the average org-P content was 0.04 mg dm^{-3} , while in spring, it reached 0.07 mg dm^{-3} (Table 2). Upon analyzing the enzymatic activity of phosphatase, it was observed that Lake Łekno exhibited slight differences compared to Lake Jeziory Wielkie. In spring, higher ALP and ACP phosphatase contents were recorded in Lake Łekno than in Lake Jeziory Wielkie. The average ALP content in Lake Łekno was six times higher than in Lake Jeziory Wielkie, amounting to $1567.55 \text{ } \mu\text{g p-nitrofenol g}^{-1} \text{ h}^{-1}$. This relationship was also observed for ACP, but the increase in content was slightly lower. The ACP content in Lake Łekno was twice as high as that observed in Lake Jeziory Wielkie. Moreover, higher ALP and ACP values were observed in the summer period, reaching three times higher levels than in the spring. Such a high content of ALP and ACP in Lake Łekno can be associated with the specific land use in the catchment area. Forest catchments are characterized by a lower pH, which causes enzymatic processes to differ from those occurring in Lake Jeziory Wielkie (Figures 4 and 5).

The highest content of both ALP and ACP enzymes in Lake Łekno was recorded at points 2 and 6, irrespective of the season. These points are situated near water seepages, which are likely to supply the lake with phosphorus compounds that cause such high values of ALP and ACP enzymes. Physicochemical analysis of seepages is important from the point of view of supplying the lake with substances like N and P. The additional water supply can stimulate the growth of phytoplankton and subsequently lead to increased enzymatic activity. In their study, Corman et al. [36] proved that in lakes with seepages, the concentration of P in lake water samples was positively correlated with the water quality of the seepage.

The results of Tukey's WSD test for unequal sample sizes are presented in Table 3. The average depth of bottom sediment sampling was 3.14 m and 2.35 m for Lake Jeziory Wielkie and Lake Łekno, respectively. However, the distance of measurement points from

the shore was, on average, 94.60 m and 70.67 m for Lake Jeziory Wielkie and Lake Łekno, respectively (Table 3). These two variables did not show a statistically significant difference between the analyzed sizes for both lakes.

Table 3. Researched factors in Lake Jeziory Wielkie and Lake Łekno.

Factor	Tukey's WSD Test				
	Group 1: Lake Jeziory Wielkie		Group 2: Lake Łekno		
	Average of Group 1	Average of Group 2	t	df	p
Depth [m]	3.14	2.35	1.319	19	0.203
Distance from the shore [m]	94.60	70.67	0.985	19	0.337
pH average [-]	8.17	7.22	4.350	19	0.000 *
org-P average [mg dm ⁻³]	0.53	0.06	2.956	19	0.008 *
ALP average [μg p-n g ⁻¹ h ⁻¹]	637.57	2818.09	-2.894	19	0.009 *
ACP average [μg p-n g ⁻¹ h ⁻¹]	745.41	2188.69	-2.637	19	0.016 *
pH_spr [-]	8.19	7.08	4.666	19	0.000 *
pH_sum [-]	8.05	7.13	4.118	19	0.001 *
org-P_spr [mg dm ⁻³]	0.69	0.07	2.455	19	0.024 *
org-P_sum [mg dm ⁻³]	0.37	0.04	2.605	19	0.017 *
ALP_spr [μg p-n g ⁻¹ h ⁻¹]	236.41	1567.55	-3.788	19	0.001 *
ALP_sum [μg p-n g ⁻¹ h ⁻¹]	1038.73	4068.64	-2.401	19	0.027 *
ACP_spr [μg p-n g ⁻¹ h ⁻¹]	483.60	989.87	-1.549	19	0.138
ACP_sum [μg p-n g ⁻¹ h ⁻¹]	1007.21	3387.51	-2.656	19	0.016 *

n = 15 in group 1; *n* = 6 in group 2; and * denotes significant differences between means in columns according to the post hoc Tukey's WSD test.

Additionally, Tukey's WSD test analyzed parameters like pH, org-P, ALP, and ACP as average values and about the spring and summer seasons. Of these mentioned parameters, there was no statistically significant difference between the two analyzed lakes for ACP in spring only. In contrast, the remaining parameters are characterized by statistically significant differences between Lake Jeziory Wielkie and Lake Łekno. Thus, it can be concluded that, taking into account several factors, these lakes significantly differ statistically.

Furthermore, analyzing the PCA performed for Lake Jeziory Wielkie accounted for 57.43% of the data variability, which shows that factor 1 was most strongly related to the value of ACP in both spring and summer (ACP_spr and ACP_sum). Interestingly, these ACP values (ACP_spr and ACP_sum) are negatively correlated with the value of ALP in the summer (ALP_sum). Measurement points 5, 7, and 9 were most closely related to factor 1, which means that in the case of Lake Jeziory Wielkie, the indicators determining phosphorus enzymatic activity were mainly related to ALP. Factor 2 was responsible primarily for org-P content, which was negatively correlated with pH (Figure 8). However, in the case of Lake Łekno, which has a forest catchment area, a different relationship was noted. In the case of this lake, PCA accounting for 73.61% of the variability in the data showed that factor 1 was most strongly related to the amount of ALP in the spring period (ALP_spr), which was positively correlated with the values of ACP_sum and ALP_sum (summer). Measurement points 2 and 6 were most strongly associated with factor 1, which means that for these points, the greatest significance was observed for ALP (point 6) and ACP (point 2). Factor 2 was primarily responsible for pH, although the pH of the samples was not correlated with enzymatic activity and org-P (Figure 8). The PCA analysis showed a negative correlation between phosphorus enzymatic activity in bottom sediments and the

organic phosphorus content in the overlying water only in the type of forest catchment. An analysis of the projection of variables onto the factor plane revealed a negative correlation between these parameters, where the angle between the vectors representing the primary variables is observed to be close to 180 degrees. This analysis highlights that an increase in org-P content in a lake within a forest catchment area (with an autochthonous source of org-P) will lead to a decrease in the enzymatic content of ALP and ACP.

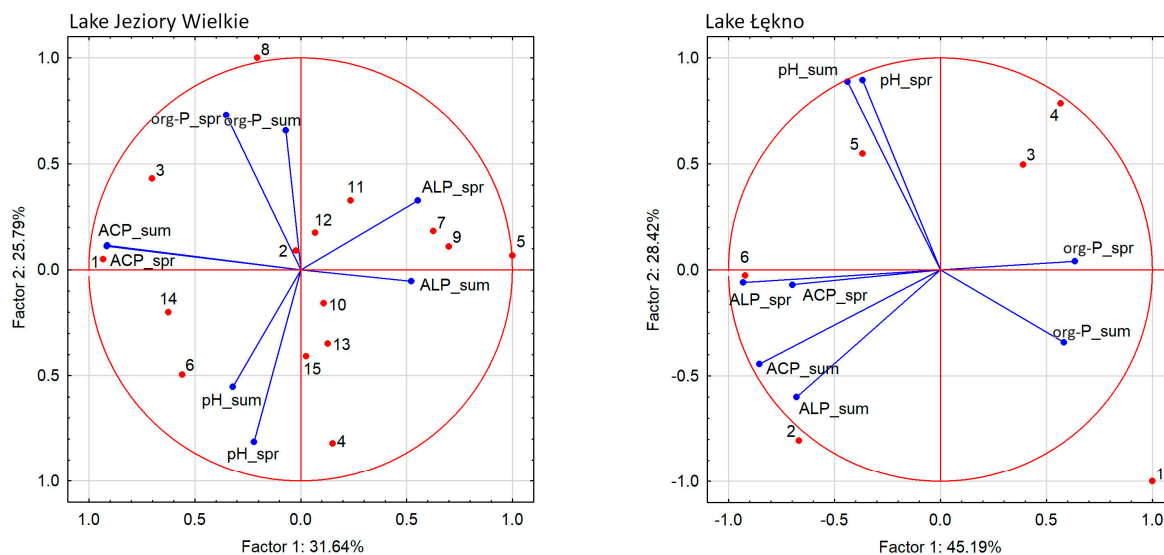


Figure 8. The PCA of the physicochemical properties of sediments and overlying water of Lake Jeziory Wielkie and Lake Łęčno about measurements points.

In the study by Ivančić et al. [37], higher levels of ALP were shown to occur during nutrient delivery from rivers, indicating that changes in anthropogenic nutrient loads can significantly influence the phosphorus status in water. This finding suggests that the number of org-P may affect the levels of the alkaline enzyme. Additionally, Nicholas et al. [38] showed a strong correlation between the content of phosphorus and ALP in soil samples.

4. Conclusions

Analyzing the enzyme values in spring and summer, it was shown that in the case of Lake Jeziory Wielkie, characterized by an agroforestry nature of the catchment area, higher values of the ACP enzyme were observed compared to ALP, with ACP enzyme values being higher in the summer season. For this lake, the highest enzyme contents were observed at measurement points 5 and 9, which may have been influenced by the specific land use patterns within the catchment area, the depth of the lake at these two measuring points, and the granulometric composition of the samples.

In the case of Lake Łęčno, with a typical forest catchment area, higher enzyme contents were observed, which confirms the hypothesis that in the case of a lake located in a forested catchment, ALP values will be higher compared to a lake located in an agroforestry catchment. Moreover, very high enzyme values were recorded in two points located near the seepage. It would be advisable to carry out physicochemical analyses of water samples originating from seeps to determine the impact of the quality of these inflows (seepage) on the quality of the lake.

The conducted statistical analysis demonstrated significant distinctions among the lakes concerning the majority of the examined parameters. However, no statistically significant differences were identified in sampling depth and the distance of the sampling points from the shore. However, the hypothesis is that different land uses determine the levels of both ACP and ALP.

The levels of ACP and ALP can be influenced by point sources of pollution and the type of catchment area. The hypothesis regarding org-P content being the primary factor influencing the levels of phosphatase enzymes has not been proven; org-P is not strictly correlated with the values of ALP and ACP enzymes.

Additionally, due to the selection of two representative seasons for the analysis of phosphatase changes in lakes with different catchment uses, in the future, it will be good to perform the same analyses for the other two seasons, autumn and winter.

In summary, this study has enhanced our understanding of how the type of lake catchment area use affects phosphatase enzyme activity.

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