**Introduction**

Water, which is one of the main elements of the natural environment and occupies about 75% of the earth surface, is used in every area of human life. It is used not only for living purposes, but also in agriculture, industry and transport.

Natural water, mainly surface water, is exposed to numerous contaminants. These pollutants have become an increasing threat to human society and natural ecosystems in recent decades [1]. Frequently, although not the most important, sources of pollution of natural waters are thermal inflows and precipitations [2-4]. Very often effluents from farms, allotment gardens and communal and industrial sewage [5-8] wastewater are discharged into surface water [9]. Also, fertilizers used in agriculture are one of the main sources of water pollution, mainly by nitrogen-containing compounds [10]. This leads to an increase in concentrations of nitrogen compounds ($\text{NO}_3^-$, $\text{NO}_2^-$ and $\text{NH}_4^+$) in water reservoirs and adjacent areas. At the same time, nitrogen compounds are essential for the development of living organisms, and their presence in the aquatic environment determines the state of the ecosystem [11, 12].

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**Applying Chemometric Techniques to Biogenic Substances as a Source of Pollution in Lake Surface Waters**

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**Abstract**

During spring 2008 and 2013, the concentrations of nitrogen substances ($\text{NO}_3^-$, $\text{NO}_2^-$ and $\text{NH}_4^+$) were investigated against the background of changes in other selected physical and chemical water quality indices in the surface waters of Lake Warnowo located in Wolin National Park on Wolin Island. It was found that the investigated waters were additionally contaminated by nitrogen compounds. This is illustrated by increased values of $\text{NO}_3^-$, $\text{NO}_2^-$ and $\text{NH}_4^+$ concentrations of the investigated waters in 2013 to max. values 0.28 ($\text{NO}_3^-$/dm$^3$), 0.103 ($\text{NO}_2^-$/dm$^3$) and 0.34 ($\text{NH}_4^+$/dm$^3$), causing water quality deterioration in the lake by one class of quality compared to 2008 (where the max. values amounted to 0.39 ($\text{NO}_3^-$/dm$^3$), 0.016 ($\text{NO}_2^-$/dm$^3$) and 0.13 ($\text{NH}_4^+$/dm$^3$), which led to accelerated water eutrophication in the lake in 2008-2013. This paper performs one of the chemometric techniques – principal component analysis (PCA) – used to classify water quality indices.

**Keywords:**   PCA, nitrogen compounds, eutrophication, water pollution, water quality

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The consequence of excessive nitrogen compounds in the ecosystem is excessive, uncontrolled development of phytoplankton and aquatic vegetation [13, 14] leading to faster eutrophication of the water reservoir.

Thus, water bodies very often serve as temporary or final receptors of a diverse variety of contaminants, which consequently cause the water to become toxic. The rapid stride in industrialization is a major concern and at present is a severe threat to the survival of living organisms, jeopardizing the ecological balance [15]. In the world literature, results for pollution of various types of water (surface waters, groundwater) in various areas are comparable [16-19].

Based on the data collected during investigations in 2008 and 2013, an attempt was made to assess if and to what degree nitrogen compounds (NO\textsubscript{3} \textsuperscript{-}, NO\textsubscript{2} \textsuperscript{-} and NH\textsubscript{4} \textsuperscript{+}) and other selected general water quality indices affecting water quality in a water reservoir influenced the process of eutrophication of the investigated ecosystem.

**Material and Methods**

Warnowo Lake (Fig. 1; 53°56,1’ latitude and 14°32,8’ longitude [20] is located on Wolin Island in the territory of Wolin National Park (WNP) in the Warnowo Protected Area, in the northeastern region of WNP between towns Międzyzdroje, Warnowo and Wiselka. To the south and east of this lake is the village of the same name.

Lake Warnowo is the first gutter lake of several lakes connected by Lewińska (Pojezierna) Stream in Wolin Island, which flows from Lake Warnowo through lakes Rabiąż, Czajcze, and Domysłowskie, and through other lakes of the Warnowski-Kołczewskiego Lake District not lying on the territory of WNP, up to Kamieński Lagoon [21].

Water mirror of Lake Warnowo is located at an altitude of 1,4 m above sea level. The surface of this lake is 0,127 km\textsuperscript{2}, the maximum depth is 2,1 m, the average depth is about 1,3 m, and volume is 160700 m\textsuperscript{3} [20, 22]. Cryptodepression is small at 0,7 m. The small surface and depth of this lake and the average wind hooding allow for constant mixing of waters to the bottom. In the eastern part, the reservoir is connected by a canal (mostly overgrown with water vegetation), which is the first section of Lewinska Struga with a depth of about 0,7 m, a width of 3,5 m and a length of about 10 m – with Lake Rabiąż.

Water samples for investigation were collected [after 23] at the water measuring sampling station (Fig. 1) from the surface layer of Lake Warnowo from a depth of about 25 cm below the water surface. Water samples were collected in spring once a month on arbitrarily selected dates: 23.03., 17.04., 18.05, and 25.06 in the year 2008; and 16.03., 13.04., 11.05, and 15.06 in the year 2013. Temperature [24] and the pH of water [25] were determined at the place of sample collection. Water samples were taken separately to determine dissolved oxygen concentration [26]. Water samples taken for determining concentrations of dissolved substances in laboratory analyses were fixed [27] at the place of sample collection. Water samples were investigated in the laboratory within 24 hours. Determined concentrations are COD-Mn, NO\textsubscript{3} \textsuperscript{-}, NO\textsubscript{2} \textsuperscript{-}, and NH\textsubscript{4} \textsuperscript{+} [26].

In order to determine which of the investigated water quality indices in Lake Warnowo had an impact on the eutrophication-investigated reservoir, the collected results of investigation were conducted with statistical analysis using the computer software Statistica.

**Results and Discussion**

The results of investigations of surface waters of Lake Warnowo in the place of the measuring station (Fig. 1) are presented in Tables 1-4 and Figs 2-4. Based on the analysis of collected data, a clear picture of the water quality of Lake Warnowo and changes of this water quality in years 2008 and 2013 were obtained.
Table 1. Results of the investigation of chosen water quality indices of Lake Warnowo in spring 2008 and 2013.

| No. | Indices (units) | Date of water sampling in years | Year 2008 | Year 2013 |
|-----|----------------|---------------------------------|-----------|-----------|
|     |                |                                 | 23.03.    | 16.03.    |
|     |                |                                 | 17.04.    | 13.04.    |
|     |                |                                 | 18.05.    | 11.05.    |
|     |                |                                 | 25.06.    | 10.06.    |
| 1   | NO$_3^-$ (mg N-NO$_3$.dm$^{-3}$) | 0,39 0,15 0,06 0,06 0,17 0,10 0,28 0,04 |           |           |
| 2   | NO$_2^-$ (mg N-NO$_2$.dm$^{-3}$) | 0,016 0,014 0,005 0,012 0,001 0,103 0,003 0,024 |           |           |
| 3   | NH$_4^+$ (mg N-NH$_4$.dm$^{-3}$) | 0,03 0,13 0,08 0,11 0,34 0,01 0,02 0,09 |           |           |
| 4   | Temperature (ºC) | 6,0 10,0 15,0 21,0 -2,0 5,0 19,5 23,3 |           |           |
| 5   | pH (pH units) | 7,70 7,86 8,29 8,07 7,92 8,17 7,86 8,31 |           |           |
| 6   | COD-Mn (mg O$_2$.dm$^{-3}$) | 8,0 9,2 10,0 10,4 14,2 9,7 7,9 10,1 |           |           |
| 7   | O$_{2,dis}$ (mg O$_2$.dm$^{-3}$) | 8,3 7,1 4,7 4,2 12,72 17,00 6,50 2,40 |           |           |

Table 2. Statistical characteristics – minimal values (Min.), mean values (Mean), maximum values (Max.), standard error of estimation (SD) and standard deviation (CV) – of investigated water quality indices of Warnowo Lake in spring 2008 and 2013.

| No. | Indices (units) | Statistical characteristic | Year 2008 | Year 2013 |
|-----|----------------|---------------------------|-----------|-----------|
|     |                |                           | Min. Mean Max. SD CV Min. Mean Max. SD CV |
| 1   | NO$_3^-$ (mg N-NO$_3$.dm$^{-3}$) | 0,06 0,17 0,39 0,16 0,94 0,04 0,15 0,28 0,10 0,70 |           |           |
| 2   | NO$_2^-$ (mg N-NO$_2$.dm$^{-3}$) | 0,005 0,012 0,016 0,005 0,41 0,001 0,033 0,103 0,048 1,46 |           |           |
| 3   | NH$_4^+$ (mg N-NH$_4$.dm$^{-3}$) | 0,03 0,09 0,13 0,04 0,50 0,01 0,12 0,34 0,15 1,34 |           |           |
| 4   | Temperature (ºC) | 6,0 13,0 21,0 6,5 0,50 -2,0 11,5 23,3 11,9 1,04 |           |           |
| 5   | pH (pH units) | 7,70 7,98 8,29 0,26 0,03 7,86 8,07 8,31 0,21 0,03 |           |           |
| 6   | COD-Mn (mg O$_2$.dm$^{-3}$) | 8,0 9,4 10,4 1,1 0,11 7,9 10,5 14,2 2,7 0,25 |           |           |
| 7   | O$_{2,dis}$ (mg O$_2$.dm$^{-3}$) | 4,20 6,08 8,30 1,95 0,32 2,4 9,66 17,00 6,48 0,67 |           |           |

Table 3. Significant level of Pearson correlation between investigated water quality indices

| Water quality indices (units) | Year 2008 | Year 2013 |
|------------------------------|-----------|-----------|
|                              | NO$_3^-$ (mg N-NO$_3$.dm$^{-3}$) | NO$_2^-$ (mg N-NO$_2$.dm$^{-3}$) | NH$_4^+$ (mg N-NH$_4$.dm$^{-3}$) | NO$_3^-$ (mg N-NO$_3$.dm$^{-3}$) | NO$_2^-$ (mg N-NO$_2$.dm$^{-3}$) | NH$_4^+$ (mg N-NH$_4$.dm$^{-3}$) |
| Temperature (ºC) | -0,851** | -0,516 | 0,520 | -0,304 | -0,127 | -0,550 |
| pH (pH units) | -0,861** | -0,959*** | 0,320 | 0,389 | -0,259 | -0,693 |
| COD-Mn (mg O$_2$.dm$^{-3}$) | -0,970*** | -0,671 | 0,652 | -0,153 | 0,025 | -0,768* |
| O$_{2,dis}$ (mg O$_2$.dm$^{-3}$) | 0,906*** | 0,735* | -0,457 | 0,034 | 0,209 | 0,633 |
| Temperature (ºC) | -0,890** | -0,718* | 0,453 | -0,055 | -0,215 | -0,617 |
| pH (pH units) | -0,388 | 0,351 | 0,720* | -0,944*** | 0,524 | -0,310 |
| COD-Mn (mg O$_2$.dm$^{-3}$) | 0,932*** | 0,821** | -0,680 | -0,219 | -0,242 | 0,953*** |
| O$_{2,dis}$ (mg O$_2$.dm$^{-3}$) | 0,552 | 0,527 | -0,004 | 0,041 | 0,618 | 0,133 |

Significant level: *** α≤0,001, ** 0,001<α≤0,01, * 0,01<α≤0,05
On the basis of the data presented in Tables 1 and 2, it was observed that the concentrations of NO$_3^-$ in 2008 showed a downward trend during the research period, while in 2013 they showed periodic cyclical changes. In 2008 the concentration of NO$_3^-$ was significantly higher than in 2013. In turn, the concentrations of NO$_2^-$ and NH$_4^+$ in both years (2008 and 2013) showed regular cyclical changes, and their values in 2008 were significantly lower than in 2013.

With regard to additionally indicated water quality indices in this paper, the pH values of the surface waters of Lake Warnowo in both years (2008 and 2013) were very close to each other and amounted to about 7.70-8.30 pH units. On the other hand, water temperature in March and April 2008 was higher on average by about 6.5ºC than in the same investigated period in 2013, while in May and June the water temperature was higher on average by about 4.0ºC in 2013 than in 2008. This shows a significantly greater variation in values of water temperature in 2013. The concentration values of O$_2$(diss.) and COD-Mn in the investigated season of the year 2008 showed an upward trend, and in 2013 the situation was reversed.

For a more complete description of the experimental data presented in Tables 1 and 2, box-whisker diagrams were prepared to graphically illustrate some [28] selected statistical characteristics.

After [29], Pearson correlations, discriminant analysis and principal component analysis (PCA) were applied to assess the temporal variations in water quality indices and estimation of latent pollution factors in surface water of Lake Warnowo.

The experimental data were analyzed by principal components analysis (PCA) (Tables 3 and 4, Fig. 3). Also, dendrograms of the surface water quality indices of Lake Warnowo were performed by means of cluster analysis and Ward’s method (Fig. 4). On the basis of results presented in Tables 3 and 4 and Figs. 3 and 4, an attempt was made to determine which of the additional water quality indices investigated in this work have the greatest effect on concentrations of NO$_3^-$, NO$_2^-$ and NH$_4^+$. 

![Fig. 2. Selected statistical characteristics of data set of investigated surface water quality indices of Lake Warnowo.](image-url)
PCA that represents the matrix correlation of water quality indices investigated in this work in 2008 and 2013 was used to identify independent factors explaining the spatial variability of concentrations of NO$_3^-$, NO$_2^-$ and NH$_4^+$. PCA was performed on the basis of normalized results of hydrochemical analyses. A similar approach to hydrochemical interpretation of water was presented by [30]. Table 3 presents the Pearson correlation coefficients describing the degree of dependence of individual water quality indices from each other in 2008 and 2013. Investigated water quality indices showed both positive and negative correlations. The greater the absolute value, the greater the correlation between the individual indicators.

The analysis of the eigenvalues main components $>1$ PCA shows that the plane of the first and the second principal component (Tab. 4, Fig. 3) describes 98.75% and 90.03% of the variance of the primary (original) data in years 2008 and 2013. According to the above criteria, only principal components (PCs) with values higher than the values of the principal components were considered [1, 31, 32].

The percentage explained by the first two dimensions is in both cases above 90%, and as according to [33] it is very high. In literature it is assumed that if the percentage explained by the first two dimensions is above 70%, it means that it is statistically significant [34, 35]. Some of the authors say that if the percentage explained by the first two dimensions is 64%, then it is also very significant statistically [e.g. 36].

The author of this work claims that the amount of data used in the analysis should be taken into consideration when giving the statistical significance of the percentage value of the variance of the original data. The smaller the set of data, the percentage explained by the first two dimensions should be higher.

Fig. 3 clearly shows which of the investigated water quality indices have a similar nature of change and which are clearly different from each other. Those of the water quality indices that had higher intrinsic values had the corresponding self-correlated vector values less correlated with the other parameters [37].
From the data presented in Table 4 and Fig. 3, it is clearly shown that in 2008 there were statistically significant correlations between NO₃⁻ and COD-Mn (strong negative correlation), NO₂⁻ and O₂ (strong positive correlation), and NO₃⁻ and pH (strong negative correlation), and also worse positive correlations between NH₄⁺ and COD-Mn as well as between NH₄⁺ and temperature and negative correlations between NO₃⁻ and temperature, and between NO₂⁻ and pH. In 2013 there were statistically significant correlations between NO₃⁻ and pH (strong negative correlation), and between NH₄⁺ and COD-Mn (strong positive correlation). On the other hand, NO₂⁻ showed worse positive correlations with O₂ and pH of water.

The correlations presented graphically in Fig. 3 are very similar to the correlations between investigated water quality indices shown numerically in Table 3. A similar satisfactory optimization of results was also received by [38] in his research on the hydrological parameter classification. Ren et al. [38] modeled in his research on the studies of [39-42] showing that the values of parameters on PCA diagrams can be presented as correlation tables between investigated parameters.

Then, using the cluster analysis, hierarchical dendrograms of the surface water quality indices of Lake Warnowo were presented in Fig. 4. Water quality indices were grouped into indicators that provide the most and least information about the interactions between the examined indicators [28, 43]. The investigated results were grouped into two groups. The first group consisted of indices: NO₃⁻, NO₂⁻ and NH₄⁺, while the other group had water temperature, pH, COD-Mn and O₂ (diss.). In 2008 the highest influence on the concentration of nitrogen compounds and the nature of surface water in Lake Warnowo was pH of water and COD-Mn, followed by O₂ (diss.) and water temperature. In turn, in 2013 there wasn’t a direct influence of temperature on the concentration of nitrogen compounds (NO₃⁻, NO₂⁻ and NH₄⁺) in water. This shows that parameters that can be significant in contributing to water quality in one season may be less significant or not in the other season [44].

Generally, the analysis of the dendrograms presented in Fig. 4 shows that the pH of the tested waters and COD-Mn had the strongest influence on the presence of nitrogen compounds as ions of NO₃⁻, NO₂⁻ and NH₄⁺, while the lowest influence was seen by water temperature.

The results of the investigations (Fig. 4) presented as the hierarchical dendrograms, confirm and complement the results obtained by using the PCA method (Fig. 3, Tables 3 and 4). Similar results have been reported by [45] in his research on surface waters of the Ganjiang River (China), who used identical statistical methods for interpreting experimental data.

The methods presented in this work are well-developed and often used by researchers as statistical techniques to identify and pre-characterize the different groups of elements in waters that exhibit similar seasonal variability and correlations [14, 46-50]. In addition, this study demonstrates the usefulness of multivariate statistical techniques for the analysis and interpretation of experimental data, and also for a better understanding of seasonal variations (changes) in water quality [1, 51, 52].

The content and seasonal variability of nitrogen compounds in the surface waters of Lake Warnowo undoubtedly depended on many factors [30]. The most important factors are the location of the lake, meteorological conditions [53] and hydrological conditions.

The highest concentrations of NO₃⁻, NO₂⁻ and NH₄⁺ were noted in both 2008 and 2013 in early spring (March), and almost immediately after the end of freezing. Probably this was due to the period of winter stagnation of waters, the flow of melting waters from the area around the lake [21, 30], as well as reduced vegetation of aquatic organisms [54]. Between April and June, lower concentrations of NO₃⁻, NO₂⁻ and NH₄⁺ were noted, which was more likely related to the absorption of these biogenic compounds by aquatic organisms [14, 54, 55]. Nevertheless, the concentrations of NO₃⁻ in 2008 were significantly higher than in 2013, while the concentrations of NO₂⁻ and NH₄⁺ in 2008 were much lower than in 2013. Higher concentrations of NH₄⁺ in water samples taken for investigation, especially in 2013, at relatively lower NO₃⁻ concentrations may indicate increased human activity [13, 56], and especially the “fresh” effluent of communal sewage [7, 8] coming from residential buildings adjacent to the lake near Warnowo. According to [12], the concentrations of investigated biogenic substances classified the waters of Lake Warnowo due to the concentration of NO₃⁻ as non-class waters in 2008 and water class II in 2013, and due to the concentration of NH₄⁺ as water class I in 2008 and as non-class waters in 2013. Excess amounts of NO₃⁻, NO₂⁻ and NH₄⁺ compounds in water led to a high fertility of the lake’s waters and caused a rapid and uncontrolled development of aquatic vegetation. This is confirmed by the pH values of the investigated waters as well as the low concentrations of oxygen in May and June in the lake ecosystem. It also confirms the high trophy of Lake Warnowo.

The high abundance of biogenic compounds in the lake is probably related to the location of the lake on earlier agricultural areas [57], now a large part of which is built up with households in Warnowo [6] and allotment gardens, which is probably the cause of additional continuous supply of the lake water with nitrogen compounds [9] in the form of various illegal pollutants discharged directly into the lake. Such additional “injection” of pollutants rich in NO₃⁻, NO₂⁻ and NH₄⁺ significantly accelerate eutrophication [10, 11, 58].

Such a load of water with biogenic substances is unfavorable because of the increase in water temperature. Water saturation by oxygen is lower and therefore the water self-purification capacity is lower in
the summer than in the winter, as well as the increase in water stagnation meaning less dilution of pollutants in water [8].

The biological tests, especially short-term bioassays on bacteria or higher plants, is the best way to detect and estimate the pollution load in any matrices approach [15]. The tests in plants are relatively inexpensive, easy to use, and most importantly they are a rapid and sensitive method for environmental monitoring. Other short-term tests for monitoring water toxicity are the widely used vibrio fischeri bioluminescence inhibition bioassay (VFBI) test [59].

Statistical analysis indicated in this paper showed statistically significant correlations between concentrations of biogenic substances and other investigated water quality indices, which were dependent among other things on lake location, meteorological and hydrological conditions [60, 61]. The above results undoubtedly show the importance of these factors in shaping the abundance of lake waters biogenic substances. As [61] suggests, the trophic state analysis of a lake should be repeated based on the collected data taking into account the local variation (changes) in water quality and external factors that disrupt the uniformity of hydrochemical conditions.

Conclusions

Based on the studies conducted in 2008 and 2013, it was found that in the waters of Lake Warnowo (Wolin National Park) in 2013 (as compared to 2008) saw an increase of NO\textsubscript{2} and NH\textsubscript{4} concentrations observed during the research period. This shows that, despite the advancement of civilization, there are still built-up areas with insufficient communal infrastructure in the 21st century, as it was in the case of the investigated area. This resulted in an uncontrolled inflow of sewage to the waters of Lake Warnowo (most probably from households in Warnowo Village), which were very rich in nitrogen compounds and organic matter.

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Conflict of Interest

The authors declare no conflict of interest.

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