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# Response of phytoplankton to heavy pollution of water bodies

by

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**Abstract**

Phytoplankton structure was studied in five ponds (located in the Oleksandriya Nature Park, Ukraine), which significantly differed in the level of their contamination. The concentration of ammonium ($NH_4^+$-N) in the studied water bodies was 0.02–74.00 mg $l^{-1}$, of nitrite ($NO_2^-$-N) – 0.002–1.750 mg $l^{-1}$, nitrate ($NO_3^-$-N) – 0.13–58.00 mg $l^{-1}$, inorganic compounds of phosphorus ($P_{inorg}$) – 0.041–0.160 mg $l^{-1}$, chloride ($Cl^-$) – 35.4–560.5 mg $l^{-1}$, whereas the content of organic matter (PO and DO) – 4.4–10.4 and 18.0–81.0 mg O $l^{-1}$, respectively. It has been found that the response of phytoplankton to heavy pollution consisted in changes in its species richness, taxonomic structure, species composition, quantitative indices, numbers and biomass structure, dimensional structure, pigment index, dominant complex, as well as changes in its ecological spectrum. Individual divisions of algae differed in their response to heavy pollution of water bodies, which was conditioned by the specificity of algae metabolism. Bacillariophyta and Cyanoprokaryota proved to be more sensitive to contamination, whereas Chlorophyta and Euglenophyta – more tolerant. The obtained data can be used to monitor the status of water bodies and their biota and to determine the type and intensity of contamination.

**Key words:** pollution, nitrogen, phosphorus, chloride, organic matter, phytoplankton structure

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## Introduction

At present, an increase in the anthropogenic load has a significant impact on the status of water bodies. The influx of large amounts of pollutants results in dramatic transformations, even in water bodies located within conservation areas (Klochenko et al. 2018; Shevchenko et al. 2018).

Phytoplankton along with other communities of hydrobionts is used to monitor the ecological status of water bodies (Directive 2000/60/EP; Afanasyev 2019). It is very sensitive to various ecological factors (Barinova et al. 2006; 2015). Due to the capability of algae to respond to the impact of anthropogenic load, they are used in the bioindication of the status of different types of water bodies: reservoirs (Klochenko et al. 2014; Klochenko & Shevchenko 2019; Shevchenko et al. 2019), cooling ponds of thermal and nuclear power stations (Protasov et al. 2017; Shevchenko 2007), rivers (Bilous et al. 2014; Khuram et al. 2017), lakes (Korneva 2012), and ponds (Amengual-Morro et al. 2012; Klochenko et al. 2018; Shevchenko et al. 2018).

Anthropogenic eutrophication, accompanied in most cases by an increase in the concentration of nitrogen and phosphorus compounds in water, and technogenic pollution are among the main factors having an adverse effect on the status of water bodies.

Individual algal divisions differ in their response to an increase in the level of anthropogenic eutrophication. It has been found that the contribution of Cyanoprokaryota, Chlorophyta, Euglenophyta, and Dinophyta to the total number of species and to the phytoplankton biomass increases with the trophic level of water bodies. For example, the total biomass of phytoplankton and the contribution of Cyanoprokaryota were higher at the elevated trophic level of lakes (Trifonova & Afanasyeva 2017). On the other hand, the lowest diversity of Cyanoprokaryota was observed in oligotrophic and dystrophic lakes. A relatively high trophic level was responsible for an increase in the abundance of green algae (Trifonova 1998). The largest number of Euglenophyta species was observed in hypertrophic lakes with well-developed macrophyte beds, whereas the smallest number – in oligotrophic and slightly-mesotrophic water bodies (Trifonova & Afanasyeva 2017). Dinophyta dominated in deep eutrophic and mesotrophic lakes in terms of biomass. Their mass development along with Cyanoprokaryota is indicative of the eutrophication process (Trifonova & Afanasyeva 2017). In addition, the relationship was established between the total biomass of phytoplankton and Cyanoprokaryota biomass and the total concentration of phosphorus (Trifonova 1998).

Technogenic pollution has been shown to reduce species diversity and abundance of various algal divisions (Khaibullina et al. 2011; Shilova 2014). Thus, the species richness of blue-green algae from the orders Chroococcales, Oscillatoriales, and Nostocales, and green algae from the orders Sphaeropleales and Chlorellales decreased in lakes polluted by heavy metals. Chrysophyta proved to be sensitive even to low concentrations of heavy metals (Snit'ko & Snit'ko 2019).

An increase in acidification of lakes has been found to reduce the species richness of green algae (primarily those belonging to the orders Sphaeropleales and Chlorellales) and to increase the biomass of Streptophyta from the orders Desmidiales and Zygnematales (Korneva 2012).

The objective of the present work was to determine the response of various algal divisions to heavy inorganic and organic pollution based on a comparison of phytoplankton structure and its ecological spectrum in water bodies with different levels of pollution.

## Materials and methods

### Description of the study site

The research was carried out in the Oleksandriya Nature Park (the town of Bila Tserkva, Ukraine) in July 2016 and covered three cascades of decorative

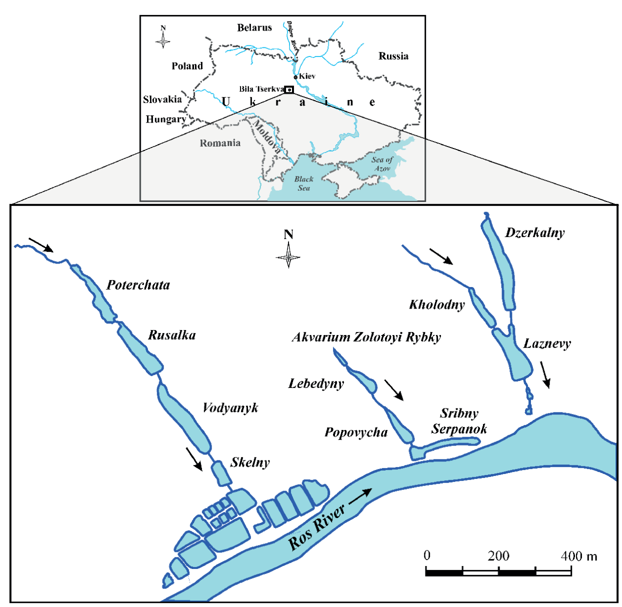


**Figure 1**

Schematic map of the ponds in the Oleksandriya Nature Park ponds of anthropogenic origin (Fig. 1). Samples were collected from five water bodies, including Rusalka – 49°48′9″N; 30°3′25″E, Laznevy – 49°48′38″N; 30°4′19″E, Dzerkalny – 49°48′46″N; 30°4′17″E, Lebedyny – 49°48′36″N; 30°3′58″E, and Sribny Serpanok – 49°48′30″N; 30°4′11″E.

In the 1990s, the western part of the park, including its soil and surface waters, was heavily polluted. The maximum content of ammonium ($NH_4^+$-N) in the Rusalka pond was 660.0 mg $l^{-1}$, of nitrite ($NO_2^-$-N) – 9.13 mg $l^{-1}$, and nitrate ($NO_3^-$-N) – 123.41 mg $l^{-1}$ (Pleskach 2004). The content of pollutants in water continued to increase over time. For example, the maximum content of ammonium ($NH_4^+$-N) in the Rusalka pond in 2003–2004 was 862.2 mg $l^{-1}$, nitrite ($NO_2^-$-N) – 6.06 mg $l^{-1}$, nitrate ($NO_3^-$-N) – 115.40 mg $l^{-1}$, and chloride ($Cl^-$) – 684 mg $l^{-1}$ (Krot et al. 2005; Kyryziy 2014). The geodesic research carried out in the park has shown that fertilizers stored in the "Agrokhimobyednannya" storehouse and the selection station located outside the park can be a possible source of pollution (Kulyk 2003). Despite the fact that a considerable amount of time elapsed, soil water that flows currently into the ponds of the western ravine still contains high levels of pollutants.

### Sampling and laboratory studies

In July 2016, water samples for hydrochemical analysis along with phytoplankton samples were collected from the littoral zone of the studied ponds in the sections free of vegetation. In all the studied ponds, samples were collected from the surface layer of water using a Ruttner water sampler (Arsan et al. 2006).

Algal samples of 0.5 l volume were fixed with 40% solution of formaldehyde (final concentration of 4%) and concentrated by the method of sedimentation. Some samples were analyzed in the live state. The algal cell numbers (the number of algae cells) were determined by the direct count method in a Nageotte counting chamber of 0.02 ml volume. Biomass was calculated by the count cell-volume method (Arsan et al. 2006). The algal numbers were expressed in thousand cells $l^{-1}$, whereas biomass – in mg $l^{-1}$. Species whose contribution to the total numbers and/or biomass of phytoplankton in a sample was ≥ 10% were assigned to dominants. Algal samples were analyzed at a magnification of 200×, 400×, and 1000× using a Zeiss Axio Imager-A1 microscope. Bacillariophyta were identified using permanent preparations. Diatoms were treated with hydrogen peroxide and mounted in Naphrax high-refractive index medium. The following manuals were used to identify algal taxa (Ettl 1978; 1983; 1988; Komarek & Anagnostidis 1999; 2005; Rieth 1980; Starmach 1985; Vetrova 2004; Popovský & Pfiester 2008; Kulikovskiy et al. 2016). The Latin names and the volume of algal taxa are given in accordance with the classification systems (Tsarenko et al. 2006; 2009; 2011). Ecological characteristics of algae indicators are given according to Van Dam et al. 1994; Barinova et al. 2006.

The species composition of algae found in various water bodies was compared using the Sorensen coefficient of community similarity (Arsan et al. 2006). Cluster analysis of plankton communities was carried out using the Paleontological Statistics Software (PAST). The Pantle-Buck saprobic index (*S*) as modified by Sládeček (Pantle & Buck 1955, Sládeček 1973, Wasser et al. 1989) and the Shannon index of species diversity (*H′*) (Odum 1969) were also calculated.

All samples collected from five ponds in July 2016 were analyzed for ammonium ($NH_4^+$-N), nitrite ($NO_2^-$-N), nitrate ($NO_3^-$-N), inorganic phosphorus ($P_{inorg}$), chloride ($Cl^-$), and organic matter. The content of inorganic compounds of nitrogen and phosphorus was determined by the colorimetric method using the KFK-2 photoelectric colorimeter. The chloride ion concentration was determined by titration with silver nitrate (Mohr's method). The indicator used was a diluted potassium chromate solution. The concentration of dissolved organic matter was determined in terms of permanganate (PO) and dichromate (DO) oxidizability (Arsan et al. 2006). Water transparency was measured using the Secchi disk. The content of chlorophyll *a* and the total content of carotenoids were determined by the method of spectrophotometry in acetone extracts and calculated using relevant formulas (Jeffrey & Humphrey 1975; Parsons & Strickland 1963). The obtained data were processed using the MS Excel 2010.

## Results

### General characteristics of the studied ponds

As Table 1 shows, the studied ponds are very similar in their morphometric characteristics. Their depth ranges from 1.0 to 2.5 m, whereas their transparency – from 0.4 to 0.7 m. All ponds are artificial water bodies, which are supplied by water from springs. The cascades of the ponds are arranged 350–400 m apart. Thus, the light intensity at their water surface is very similar.

The surface area of water occupied by macrophyte beds was insignificant in the surveyed ponds. Higher aquatic plants were represented by nine species.

**Table 1**

Characteristics of the ponds in the Oleksandriya Nature Park

| Characteristics | Unit | Ponds | | | | |
|---|---|---|---|---|---|---|
| | | Rusalka (1) | Laznevy (2) | Dzerkalny (3) | Lebedyny (4) | Sribny Serpanok (5) |
| Area | ha | 0.53 | 0.72 | 0.74 | 0.19 | 0.42 |
| Average depth | m | 1.5 | 2.5 | 2.0 | 1.0 | 1.5 |
| Transparency | m | 0.4 | 0.7 | 0.7 | 0.4 | 0.5 |

No macrophytes were found in the Lebedyny pond, while in the other water bodies they were represented by one to three species.

## Hydrochemical analysis

The content of nutrients, including nitrogen and phosphorus, is one of the main characteristics of the hydrochemical regime of water bodies. Their high content results in the deterioration of the aquatic environment.

The hydrochemical analysis has shown that the studied water bodies significantly differed in the content of nutrients, primarily in the content of inorganic compounds of nitrogen, which was due to the difference in the anthropogenic load. As shown in Table 2, the Rusalka pond located in the western ravine was characterized by the highest concentration of ammonium, nitrite, nitrate, and chloride. In addition, the highest (compared to other studied water bodies) concentration of dissolved organic matter was also recorded in the Rusalka pond.

## Phytoplankton

In total, 93 algal species represented by 96 infraspecific taxa were found in the water column of the studied ponds during the study period, including those containing the nomenclatural types of species from seven divisions. Chlorophyta, Bacillariophyta, and Euglenophyta (91.4% of the total number of species) were highly diverse in their species composition (Table 3).

The classes comprising the largest number of species were Chlorophyceae (36), Bacillariophyceae (22), and Euglenophyceae (13), whereas the orders represented by the largest number of species were Sphaeropleales (28), Euglenales (13), Cymbellales (6), Fragilariales (4), Bacillariales (3), and Naviculales (3). The major families (57.0% of the total number of species) included: Scenedesmaceae (17), Euglenaceae (13), Hydrodictyaceae (5), Selenastraceae (5), Fragilariaceae (4), Cymbellaceae (3), Gomphonemataceae (3), and Bacillariaceae (3), whereas the main genera (41.9%) were: *Desmodesmus* (Chodat) An, Friedl et E. Hegew. (9), *Euglena* Ehrenb. (6), *Phacus* Dujard. (5), *Monoraphidium* Komárk.-Legn. (4), *Acutodesmus* (E. Hegew.) P. Tsarenko (3), *Coelastrum* Nägeli (3), *Cyclotella* Kütz. (3), *Ulnaria* (Kütz.) Compère (3), *Cymbella* C. Agardh (3), and *Gomphonema* (C. Agardh) Ehrenb. (3).

The distribution of plankton algae in the ponds differing in the level of anthropogenic load was not uniform. Their lowest number (21) was found in the most contaminated Rusalka pond. The number of algal species in the other ponds varied from 33 to 44 (Table 3).

**Table 2**

Average values of physicochemical variables in the ponds of the Oleksandriya Nature Park in July 2016

| Variables | Unit | Ponds | | | | |
|---|---|---|---|---|---|---|
| | | Rusalka (1) | Laznevy (2) | Dzerkalny (3) | Lebedyny (4) | Sribny Serpanok (5) |
| $NH_4^+$-N | mg l$^{-1}$ | 74.0 | 0.04 | 0.02 | 0.18 | 0.08 |
| $NO_2^-$-N | mg l$^{-1}$ | 1.750 | 0.036 | 0.035 | 0.065 | 0.002 |
| $NO_3^-$-N | mg l$^{-1}$ | 58.0 | 3.08 | 5.62 | 0.65 | 0.13 |
| $P_{inorg.}$ | mg l$^{-1}$ | 0.120 | 0.088 | 0.090 | 0.160 | 0.041 |
| $Cl^-$ | mg l$^{-1}$ | 560.5 | 78.0 | 70.4 | 37.7 | 35.4 |
| Permanganate oxidizability (PO) | mg O l$^{-1}$ | 10.4 | 6.1 | 4.4 | 5.8 | 5.8 |
| Dichromate oxidizability (DO) | mg O l$^{-1}$ | 81.0 | 54.0 | 22.0 | 20.0 | 18.0 |
| pH | | 8.21 | 8.13 | 7.98 | 8.47 | 8.49 |
| T | °C | 24.5 | 22.0 | 19.0 | 21.0 | 22.5 |

**Table 3**

The number of plankton algae species (infraspecific taxa) in the ponds of the Oleksandriya Nature Park in July 2016

| Divisions | Ponds | | | | | Total |
|---|---|---|---|---|---|---|
| | Rusalka (1) | Laznevy (2) | Dzerkalny (3) | Lebedyny (4) | Sribny Serpanok (5) | |
| Cyanoprokaryota | | | 1<br>2.3 | 1<br>3.0 | 2<br>4.9 | 3<br>3.2 |
| Euglenophyta | 7<br>33.3 | 5<br>13.8 | 4<br>9.1 | 4<br>12.2 | 1<br>2.4 | 13<br>13.9 |
| Chrysophyta | | 1<br>2.8 | 1<br>2.3 | 1<br>3.0 | | 1<br>1.1 |
| Xanthophyta | | | | 1<br>3.0 | 1<br>2.4 | 2<br>2.2 |
| Bacillariophyta | 5<br>23.8 | 6<br>16.7 | 14<br>31.8 | 8<br>24.2 | 9<br>22.0 | 28<br>30.1 |
| Dinophyta | | | | 2<br>6.1 | 2<br>4.9 | 2<br>2.2 |
| Chlorophyta | 9<br>42.9 | 24 (26)<br>66.7 | 24 (26)<br>54.5 | 16<br>48.5 | 26 (27)<br>63.4 | 44 (47)<br>47.3 |
| Total | 21<br>100 | 36 (38)<br>100 | 44 (46)<br>100 | 33<br>100 | 41 (42)<br>100 | 93 (96)<br>100 |

Note: The absolute number of species is given above the bar, their number in percentage terms – below the bar. The number of species with infraspecific taxa is given in parentheses.

Taxonomic spectra of phytoplankton varied in the studied ponds. In almost all ponds, Chlorophyta (42.9–66.7%) and Bacillariophyta (16.7–31.8%) were represented by the largest number of species. Euglenophyta (33.3%) and Bacillariophyta (23.8%) ranked second and third in the Rusalka pond characterized by the highest level of contamination. Euglenophyta (16.7–31.8%) ranged third in the Laznevy, Dzerkalny, and Lebedyny ponds, whereas Cyanoprokaryota (4.9%) and Dinophyta (4.9%) – in the Sribny Serpanok pond (Table 3).

The species composition of plankton algae varied considerably in the ponds that differed in the level of contamination. The dendrogram of phytoplankton species composition similarity built based on the Sorensen coefficient produced four clusters: the first cluster included the heavily polluted Rusalka pond, the second cluster – the Lebedyny pond, the third cluster – the moderately contaminated Dzerkalny and Laznevy ponds, whereas the fourth cluster – the Sribny Serpanok pond characterized by the lowest concentration of pollutants (Fig. 2). The lowest similarity (less than 30%) was observed between the cluster representing the Rusalka pond and other clusters, which indicates significant transformations of phytoplankton species composition in this water body under the influence of heavy pollution.

The distribution of quantitative phytoplankton development indices was highly heterogeneous. The algal numbers varied from 17 828 thousand cells $l^{-1}$ to 88 569 thousand cells $l^{-1}$, whereas their biomass ranged from 8.235 to 34.225 mg $l^{-1}$. The highest numbers (88 569 thousand cells $l^{-1}$) and the lowest biomass of phytoplankton (8.235 mg $l^{-1}$) were observed in the heavily polluted Rusalka pond. The structure of the algal numbers and biomass also differed significantly (Tables 4 and 5).

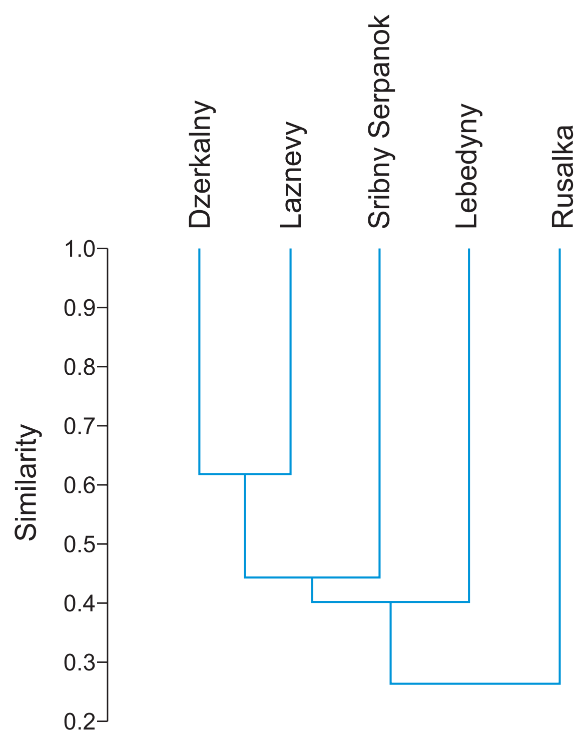


**Figure 2**

Dendrogram of similarity of phytoplankton species composition in the ponds of the Oleksandriya Nature Park

**Table 4**

Phytoplankton cell numbers in the ponds of the Oleksandriya Nature Park in July 2016

| Divisions | Ponds | | | | |
|---|---|---|---|---|---|
| | Rusalka (1) | Laznevy (2) | Dzerkalny (3) | Lebedyny (4) | Sribny Serpanok (5) |
| Cyanoprokaryota | | | 1824<br>4.6 | 180<br>1.0 | 1856<br>4.2 |
| Euglenophyta | 466<br>0.5 | 56<br>0.1 | 178<br>0.5 | 54<br>0.3 | 16<br>0.1 |
| Chrysophyta | | 256<br>0.4 | 3556<br>9.0 | 90<br>0.5 | |
| Xanthophyta | | | | 48<br>0.3 | 16<br>0.1 |
| Bacillariophyta | 158<br>0.2 | 424<br>0.7 | 3372<br>8.5 | 8112<br>45.5 | 5880<br>13.3 |
| Dinophyta | | | | 18<br>0.1 | 32<br>0.1 |
| Chlorophyta | 87 945<br>99.3 | 59 368<br>98.8 | 30 580<br>77.4 | 9326<br>52.3 | 36 274<br>82.2 |
| On the whole | 88 569<br>100 | 60 104<br>100 | 39 510<br>100 | 17 828<br>100 | 44 074<br>100 |

Note: The absolute values of algal numbers (thousand cells $l^{-1}$) are given above the bar, their numbers in percentage terms – below the bar.

**Table 5**

Phytoplankton biomass in the ponds of the Oleksandriya Nature Park in July 2016

| Divisions | Ponds | | | | |
|---|---|---|---|---|---|
| | Rusalka (1) | Laznevy (2) | Dzerkalny (3) | Lebedyny (4) | Sribny Serpanok (5) |
| Cyanoprokaryota | | | 0.036<br>0.2 | 0.004<br>0.1 | 0.052<br>0.2 |
| Euglenophyta | 3.160<br>38.4 | 0.373<br>2.1 | 0.838<br>5.6 | 0.512<br>4.7 | 0.045<br>0.1 |
| Chrysophyta | | 0.384<br>2.2 | 3.897<br>26.2 | 0.135<br>1.2 | |
| Xanthophyta | | | | 0.062<br>0.6 | 0.008<br>0.1 |
| Bacillariophyta | 0.064<br>0.8 | 0.519<br>2.9 | 2.239<br>15.0 | 7.870<br>72.5 | 21.978<br>64.2 |
| Dinophyta | | | | 0.234<br>2.2 | 0.555<br>1.6 |
| Chlorophyta | 5.011<br>60.8 | 16.563<br>92.8 | 7.897<br>53.0 | 2.029<br>18.7 | 11.587<br>33.8 |
| On the whole | 8.235<br>100 | 17.839<br>100 | 14.907<br>100 | 10.846<br>100 | 34.225<br>100 |

Note: The absolute values of algal biomass (mg $l^{-1}$) are given above the bar, their biomass in percentage terms – below the bar.

In the Rusalka pond with a high concentration of pollutants, the distribution of phytoplankton numbers and biomass among individual species was not uniform, which is supported by very low values of the Shannon index (0.38 in terms of the numbers and 1.96 in terms of the biomass), which is accounted for by a high contribution of *Chlorella vulgaris* Beijer. to the total numbers (92.1%) and biomass (32.7%) of phytoplankton. Its higher values (2.54 in terms of the numbers and 2.27 in terms of the biomass) were recorded in the Sribny Serpanok pond with the lowest concentration of pollutants. In the other ponds, the Shannon index was 1.88–2.51 in terms of the numbers and 1.42–2.39 in terms of the biomass (Table 6). The obtained results correlate well with the literature data presented below. For example, phytoplankton in water bodies with the minimum level of anthropogenic load was characterized by high species diversity (Shannon index was 2.26–4.38), while water bodies with a high level of anthropogenic load were generally characterized by lower values of the Shannon index (1.81–1.84; Shchur et al. 2011).

In the studied ponds, phytoplankton dimensional structure was varied. In the heavily polluted pond, cells of plankton algae were smaller compared to those occurring in other ponds. Thus, the relationship

**Table 6**

Phytoplankton structural characteristics, the saprobic index, and the $C_{car}/C_{chl}$ ratio in the ponds of the Oleksandriya Nature Park

| Indices | Ponds | | | | |
|---|---|---|---|---|---|
| | Rusalka (1) | Laznevy (2) | Dzerkalny (3) | Lebedyny (4) | Sribny Serpanok (5) |
| Shannon index (*H'*) | | | | | |
| in terms of numbers | 0.38 | 1.88 | 2.51 | 1.94 | 2.54 |
| in terms of biomass | 1.96 | 1.42 | 2.39 | 2.02 | 2.27 |
| Dimensional structure (B/N) | 0.09 | 0.30 | 0.38 | 0.61 | 0.78 |
| Saprobic index (*S*) | 3.11 | 2.31 | 2.10 | 1.91 | 1.91 |
| $C_{car}/C_{chl}$ ratio | 0.70 | 0.37 | 0.40 | 0.49 | 0.32 |

Note: B – biomass; N – numbers; $C_{car}$ – the total content of carotenoids; $C_{chl}$ – chlorophyll *a* content.

between the average biomass and the average numbers of phytoplankton in the Rusalka pond was only 0.09. In the other studied water bodies, the value of this index varied from 0.30 to 0.78. In the Sribny Serpanok pond with the lowest concentration of pollutants, the size of algal cells was one order of magnitude larger (0.78) than that in the heavily polluted Rusalka pond (Table 6).

In the studied water bodies, values of the saprobic index varied over a wide range – from 1.91 to 3.11. The highest values were determined for the Rusalka pond – 3.11 (α-mesosaprobic zone), which indicates intensive contamination of this water body by organic matter and correlates with the data from hydrochemical analysis (Table 2). In the other ponds, the saprobic index values were lower – 1.91–2.31 (β-mesosaprobic zone; Table 6).

In the pond with the maximum concentration of pollutants, the relationship between the sum of carotenoids and chlorophyll *a* content – indicating the physiological state of phytoplankton and its functioning (Yelizarova 1973; Mineyeva 2004; Sidelev & Babanazarova 2008) – was almost twice as high (0.70) as in the pond with the minimum level of contamination (0.32), which was conditioned by chlorophyll *a* decomposition and by the increase in the total content of carotenoids. Thus, the chlorophyll *a* content in the heavily polluted Rusalka pond was 1.4 times lower compared to the Sribny Serpanok pond with the minimum concentration of pollutants, whereas the content of carotenoids was higher by a factor of 1.5. The $C_{car}/C_{chl}$ ratio decreased with the decreasing level of contamination (Table 6). The obtained data correlate well with the results of previous research (Klochenko et al. 2019). The literature data (Belaya 2011) suggest that the accumulation of carotenoids and chlorophyll decomposition (high values of the $C_{car}/C_{chl}$ ratio) are due to the impact of adverse factors.

The conducted research showed that different algal species dominated in the studied ponds, which is evidenced by very low Sorensen coefficient values (0–25%). Only *Chlorella vulgaris* dominated in four heavily and moderately polluted ponds, whereas *Ulnaria acus* (Kütz.) Aboal and *Aulacoseira granulata* (Ehrenb.) Simonsen dominated in two water bodies – the Lebedyny and Sribny Serpanok ponds. *Chlorella vulgaris* was not found in the pond with the minimum concentration of pollutants. Other algal species dominated only in one of the studied water bodies (Table 7).

It is significant that algal species that are indicators of organic contamination dominated in the Rusalka pond with a high content of organic matter. Those were *Chlorella vulgaris* and *Euglena viridis* Ehrenb. that are poly-α-mesosaprobic organisms. The algal species that are indicators of water salinity were also found in the Rusalka pond with a high content of chloride, i.e. *Chlorella vulgaris* – a halophile, and *Euglena viridis* – a mesohalobe occurring at relatively high concentrations of chloride, which correlates with data obtained from hydrochemical analysis (Table 2). The pond with the minimum concentration of pollutants was dominated by algal species that are indicators of moderate organic contamination – *Desmodesmus armatus* (Chodat) E. Hegew. (o-α–mesosaprobiont), *Desmodesmus brasiliensis* (Bohlin) E. Hegew. (β–mesosaprobiont), and *Aulacoseira granulata* (β–mesosaprobiont) indifferent to water salinity.

It should be noted that the studied water bodies differed not only in the ecological structure of the dominant complex, but also in the ecological spectra of phytoplankton in general.

In the Rusalka pond with a high content of chloride (560.5 mg $l^{-1}$), the contribution of halophiles and mesohalobes (indicators of water salinity) to the total numbers of species was higher (99.0%) compared to the other water bodies (3.2–87.2%; Fig. 3). The lowest

**Table 7**

Algal species dominating in the water column of the ponds in the Oleksandriya Nature Park and their ecological characteristics

| Taxa | Ecological characteristics | | | Ponds | | | | |
|---|---|---|---|---|---|---|---|---|
| | hal | S | *S* | 1 | 2 | 3 | 4 | 5 |
| **Chlorophyta** | | | | | | | | |
| *Chlorella vulgaris* Beijer. | hl | p-α | 3.6 | d | d | d | d | – |
| *Desmodesmus armatus* (Chodat) E. Hegew. | | o-α | | + | + | + | + | d |
| *D. armatus* var. *bicaudatus* (Guglielm.) E. Hegew. | | β | | – | + | + | – | d |
| *Desmodesmus brasiliensis* (Bohlin) E. Hegew. | | β | 2.0 | – | + | + | – | d |
| *Monoraphidium komarkovae* Nygaard | | | | d | + | + | + | – |
| *Monoraphidium minutum* (Nägeli) Komárk.-Legn. | | β-α | | – | + | + | d | + |
| *Mychonastes homosphaera* (Skuja) Kalina et Punčoch. | | β | | – | d | + | – | – |
| *Oocystis marsonii* Lemmerm. | | β-o | | + | d | – | – | + |
| *Pandorina morum* (O.F. Müll.) Bory | i | β | 2.0 | + | + | d | – | – |
| *Pteromonas armata* Korschikov | | | | – | – | – | d | – |
| **Euglenophyta** | | | | | | | | |
| *Euglena viridis* (O.F. Müll.) Ehrenb. | mh | p-α | 4.5 | d | – | – | – | – |
| **Bacillariophyta** | | | | | | | | |
| *Aulacoseira granulata* (Ehrenb.) Simonsen | i | β | 1.8 | – | – | + | d | d |
| *Ulnaria acus* (Kütz.) Aboal | i | β | 1.85 | – | + | + | d | – |
| **Chrysophyta** | | | | | | | | |
| *Synura petersenii* Korschikov | hb | β | 2.25 | – | + | d | + | – |

Note: hal – relation to water salinity (halobity); S – saprobity zone; *S* – saprobic index; "d" – dominant species; "+" – species did not belong to dominants; "–" – species was not found.

contribution of these organisms (3.2%) was observed in the Sribny Serpanok pond characterized by the lowest content of chloride (Table 2).

In the Rusalka pond with the highest content of organic matter (permanganate oxidizability = 10.4 mg O $l^{-1}$, dichromate oxidizability = 81.0 mg O $l^{-1}$), the contribution of polysaprobic organisms (S = 3.5–4.0) – indicators of organic contamination – to the total numbers of algae accounted for 99.3%. In the other ponds studied, the contribution of polysaprobic organisms gradually decreased from 78.5 to 23.4% with decreasing concentration of organic matter (Table 2). No polysaprobic organisms were found in the Sribny Serpanok pond (Fig. 4). On the other hand, the contribution of β–mesosaprobionts increased with decreasing organic matter content from 0.6 to 99.6%. Its highest values were recorded in the Sribny Serpanok pond (Fig. 5).

It can be concluded that, in terms of contamination, the studied ponds can be arranged in the following order: Rusalka > Laznevy > Dzerkalny > Lebedyny > Sribny Serpanok.

The correlation analysis showed that in most cases significant negative correlations were found between the content of inorganic compounds of nitrogen, phosphorus, chloride, and organic matter and the total number of plankton algae species, the number of Cyanoprokaryota, Bacillariophyta, and Chlorophyta

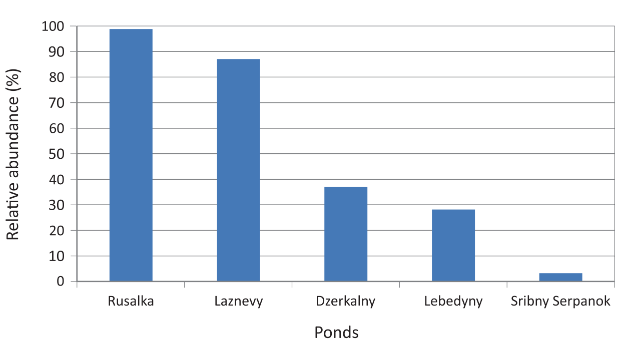


**Figure 3**

Contribution of halophiles and mesohalobes to the numbers of algae – indicators of water salinity

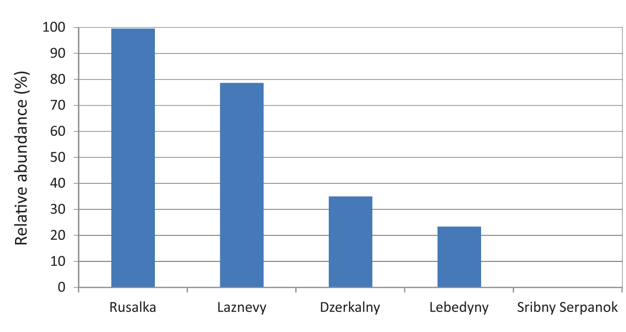


**Figure 4**

Contribution of poly-saprobionts to the numbers of algae – indicators of organic contamination

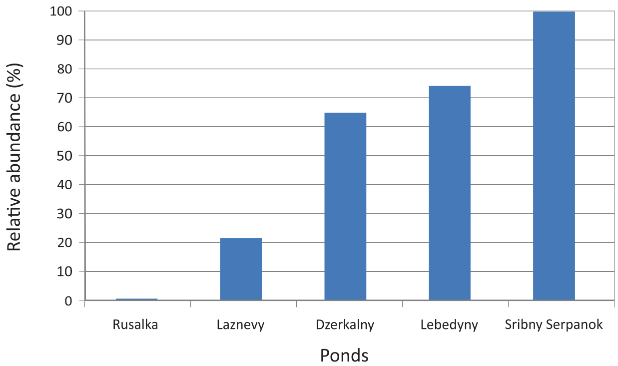


**Figure 5**

Contribution of β-mesosaprobionts to the numbers of algae – indicators of organic contamination

species and the numbers of Bacillariophyta. At the same time, significant positive correlations were found between the content of inorganic compounds of nitrogen, phosphorus, chloride, and organic matter and the number of Euglenophyta species, as well as between the content of inorganic compounds of nitrogen, chloride, and organic matter and the total numbers of phytoplankton, the numbers and biomass of Euglenophyta, the numbers and biomass of *Chlorella vulgaris*, and the numbers of Chlorophyta. In addition, significant positive correlations were found between pH values and the number of Cyanoprokaryota species, between the numbers and biomass of Bacillariophyta, and between water temperature and the total numbers of phytoplankton, the numbers of Chlorophyta, the biomass of Euglenophyta, and the numbers and biomass of *Chlorella vulgaris*. At the same time, significant negative correlations were found between water temperature and the total number of phytoplankton species and the number of Bacillariophyta and Chlorophyta species (Tables 8, 9, 10).

## Discussion

Chemical factors, including the content of inorganic compounds of nitrogen, phosphorus, chloride, and organic matter were the main drivers of the modification of algal community structure in the studied ponds (Tables 8, 9, 10). In general, the above-mentioned chemical factors had, in most cases, an adverse effect on the total number of phytoplankton species and their biomass. At the same time, high concentrations of inorganic nitrogen, chloride, and organic matter favored phytoplankton numbers.

**Table 8**

Pearson correlations of physicochemical variables and the number of phytoplankton species in the ponds of the Oleksandriya Nature Park

| Variables | Number of species | | | | |
|---|---|---|---|---|---|
| | total | Cyan | Eugl | Bac | Chl |
| $NH_4^+$-N | **−0.88** | **−0.53** | **0.72** | **−0.54** | **−0.84** |
| $NO_2^-$-N | **−0.89** | **−0.55** | **0.74** | **−0.54** | **−0.86** |
| $NO_3^-$-N | **−0.85** | **−0.57** | **0.75** | −0.49 | **−0.83** |
| $P_{inorg.}$ | **−0.52** | −0.46 | **0.59** | −0.25 | **−0.72** |
| $Cl^-$ | **−0.87** | **−0.59** | **0.76** | **−0.53** | **−0.83** |
| PO | **−0.95** | **−0.55** | **0.67** | **−0.76** | **−0.83** |
| DO | **−0.82** | **−0.84** | **0.85** | **−0.72** | **−0.65** |
| pH | −0.16 | **0.55** | −0.50 | −0.33 | −0.09 |
| T (°C) | **−0.79** | −0.32 | 0.35 | **−0.87** | **−0.56** |

Note: Cyan – Cyanoprokaryota, Eugl – Euglenophyta, Bac – Bacillariophyta, Chl – Chlorophyta. n = 15. Correlations marked in bold are significant at $p \leq 0.05$.

**Table 9**

Pearson correlations of physicochemical variables and phytoplankton numbers in the ponds of the Oleksandriya Nature Park

| Variables | Numbers | | | | | |
|---|---|---|---|---|---|---|
| | total | Cyan | Eugl | Bac | Chl | *Chlorella vulgaris* |
| $NH_4^+$-N | **0.82** | −0.44 | **0.94** | **−0.56** | **0.80** | **0.99** |
| $NO_2^-$-N | **0.81** | −0.46 | **0.95** | **−0.55** | **0.80** | **0.99** |
| $NO_3^-$-N | **0.83** | −0.42 | **0.97** | **−0.60** | **0.82** | **0.99** |
| $P_{inorg.}$ | −0.18 | **−0.68** | 0.29 | −0.18 | −0.15 | 0.27 |
| $Cl^-$ | **0.85** | −0.45 | **0.96** | **−0.62** | **0.84** | **0.99** |
| PO | **0.82** | **−0.59** | **0.81** | **−0.52** | **0.82** | **0.93** |
| DO | **0.92** | **−0.68** | **0.78** | **−0.84** | **0.94** | **0.87** |
| pH | −0.36 | −0.04 | −0.38 | **0.70** | −0.36 | −0.22 |
| T (°C) | **0.74** | −0.50 | 0.49 | −0.41 | **0.75** | **0.69** |

Note: Cyan – Cyanoprokaryota, Eugl – Euglenophyta, Bac – Bacillariophyta, Chl – Chlorophyta. n = 15. Correlations marked in bold are significant at $p \leq 0.05$.

**Table 10**

Pearson correlations of physicochemical variables and phytoplankton biomass in the ponds of the Oleksandriya Nature Park

| Variables | Biomass | | | | | |
|---|---|---|---|---|---|---|
| | total | Cyan | Eugl | Bac | Chl | *Chlorella vulgaris* |
| $NH_4^+$-N | −0.49 | −0.43 | **0.97** | −0.39 | −0.36 | **0.99** |
| $NO_2^-$-N | **−0.52** | −0.45 | **0.98** | −0.41 | −0.37 | **0.99** |
| $NO_3^-$-N | **−0.52** | −0.43 | **0.99** | −0.45 | −0.34 | **0.99** |
| $P_{inorg.}$ | **−0.86** | **−0.75** | 0.36 | **−0.52** | **−0.73** | 0.27 |
| $Cl^-$ | **−0.52** | −0.45 | **0.98** | −0.46 | −0.31 | **0.99** |
| PO | −0.41 | **−0.52** | **0.88** | −0.31 | −0.26 | **0.96** |
| DO | −0.50 | **−0.67** | **0.82** | **−0.63** | 0.07 | **0.88** |
| pH | 0.40 | 0.15 | −0.28 | **0.67** | −0.27 | −0.17 |
| T (°C) | −0.01 | −0.35 | **0.58** | 0.04 | 0.03 | **0.73** |

Note: Cyan – Cyanoprokaryota, Eugl – Euglenophyta, Bac – Bacillariophyta, Chl – Chlorophyta. n = 15. Correlations marked in bold are significant at $p \leq 0.05$.

It is likely that water temperature and pH in the heavily polluted Rusalka pond only indirectly affected the formation of phytoplankton structure. It has been known (Collos & Harrison 2014) that the relative concentration of unionized ammonia ($NH_3$) and ionized ammonium ($NH_4^+$) is strongly correlated with pH and, to a lesser extent, with temperature. As pH and water temperature increase, so does the concentration of ammonia, which is considered to be the most toxic form (Collos & Harrison 2014).

It has been found that individual algal divisions differed in their response to heavy pollution. Thus, Bacillariophyta proved to be sensitive to the effect of inorganic compounds of nitrogen, chloride, and organic matter, resulting in their reduced species richness and numbers (Tables 8, 9). It is known that many genera of diatoms are sensitive to pollutants, while fewer are tolerant to their adverse effect (Van Dam et al. 1994). In particular, many species of Bacillariophyta are sensitive to high concentrations of ammonium (Collos & Harrison 2014).

Although the process of eutrophication favors the development of Cyanoprokaryota, often resulting in the formation of water bloom (Kirpenko et al. 2019), their species richness was inhibited by high concentrations of inorganic compounds of nitrogen, chloride, and organic matter, whereas their numbers and biomass – by high concentrations of inorganic compounds of phosphorus and organic matter (Tables 8, 9, 10). Literature data suggest that Cyanoprokaryota are also rather sensitive to high concentrations of ammonium (Collos & Harrison 2014).

The species richness of Chlorophyta also decreased in high concentrations of pollutants. However, their numbers were favored by high concentrations of inorganic compounds of nitrogen, chloride, and organic matter (Tables 8, 9). Chlorophyta, which are often found in water bodies with a high content of nutrients and organic matter, have been proven capable of using these substances in large amounts (Tsarenko 1990). In addition, it has been shown that Chlorophyta are significantly more tolerant to high concentrations of ammonium compared to other algal divisions (Collos & Harrison 2014).

The development of Euglenophyta was favored by high concentrations of inorganic compounds of nitrogen, phosphorus, chloride, and organic matter (Tables 8, 9, 10). According to Vetrova (2004), representatives of this algal division are characterized by rather high demands for nutrients, especially ammonium. In addition, intensive development of species from the genera *Trachelomonas*, *Euglena*, and *Phacus* is observed in water bodies with a high content of allochthonous organic matter. Euglenophyta include auto-, meso-, and heterotrophic organisms. In addition, representatives of Euglenophyta tolerate increased water salinity.

One of the reasons for the difference in the response of individual algal divisions to heavy pollution of water bodies may be the specificity of their enzymatic systems involved in ammonium assimilation. Thus, representatives of Chlorophyta (*Chlorella sorokiniana* and *Chlorella vulgaris*) and Euglenophyta (*Euglena gracilis*) are characterized by high ammonium incorporation activity of not only glutamine synthetase (GS) and glutamate synthase, (GOGAT), but also glutamate dehydrogenase (GDH) (Tischner 1984; Everest & Syrett 1983; Fayyaz-Chaudhary et al. 1984; 1985).

At the same time, the activity of GDH in representatives of Cyanoprokaryota was almost 20 times lower compared to representatives of Chlorophyta (Klochenko et al. 2003). Thus, the increase in the concentration of ammonium in water may result in death of Cyanoprokaryota species due to a low rate of ammonia detoxification.

In *Navicula atomus* (= *Mayamaea atomus* (Kütz.) Lange-Bert.) belonging to Bacillariophyta and having high GS activity, the activity of GDH was not detected (Bodnar 2009; Vasylenko 2013). It is likely that this phenomenon is responsible for high sensitivity of diatoms to high concentrations of ammonium (Collos & Harrison 2014).

Nitrate reductase is also one of the main enzymes in algal nitrogen metabolism. It has been found (Medved' 2009) that Cyanoprokaryota plankton (in particular *Microcystis aeruginosa*) are not competitive with Chlorophyta in the process of nitrate reduction due to low activity of this enzyme. This phenomenon may be responsible for the absence of mass development of Cyanoprokaryota at high concentrations of nitrate in water.

The development of *Chlorella vulgaris*, dominant in the heavily polluted Rusalka pond, was favored by high concentrations of inorganic compounds of nitrogen, chloride, and organic matter (Tables 9, 10). It is known that this algal species is particularly tolerant to ammonium. It contributes to the removal of ammonium from wastewater effluent as it grows well at concentrations ranging up to 20 000 μM (Kim et al. 2010; Collos & Harrison 2014).

Changes in phytoplankton dimensional structure and dominance of *Chlorella vulgaris* – a small, unicellular and physiologically active algal species – in the heavily polluted Rusalka pond can be considered as a response of phytoplankton to heavy pollution, which is supported by literature data. Thus, the increase in the trophic level of water bodies was accompanied by changes in phytoplankton dimensional structure and by the increase in the contribution of small forms (Korneva 2011). The presence of small algae from the genus *Chlamydomonas* in Lake Baikal was conditioned by the increase in the content of readily available organic matter (Bondarenko & Logacheva 2017). The increase in the content of nutrients in the water resulted in changes in the complex of dominant species and in the dominance of small, unicellular and physiologically active algal species (Ivanova & Avdeyeva 1987; Lavrentyeva 1987). In addition, it is known that the photosynthetic activity of small algal cells is higher compared to large cells (Gutelmakher 1980).

The dominance of *Euglena viridis* in the Rusalka pond was also favored by high concentration of pollutants. For example, more intensive development of Euglenophyta was observed in the sections of the Dniester River (Moldova) characterized by higher concentrations of ammonium and organic matter (Unguryanu et al. 2012). In the Lysvenskoye Reservoir (the Kama River basin), mass development of Euglenophyta, represented mainly by species of the genera *Trachelomonas* and *Phacus*, was recorded at a relatively high concentration of nitrate (Belyayeva et al. 2008).

Thus, it has been found that individual algal divisions differed in their response to heavy pollution of water bodies, which resulted from the specificity of their metabolism.

To summarize, the response of phytoplankton to high concentrations of inorganic compounds of nitrogen, phosphorus, chloride, and organic matter (when comparing the ponds with the maximum and minimum level of contamination) involved: reduced species richness; changes in the phytoplankton taxonomic structure; significant difference in the species composition of plankton algae; increase in phytoplankton numbers and decrease in phytoplankton biomass; changes in the structure of phytoplankton numbers and biomass; changes in the dimensional structure of phytoplankton (increase in the contribution of small unicellular algal species); increase in the total content of carotenoids and chlorophyll *a* decomposition (increase in the $C_{car}/C_{chl}$ ratio); decrease in Shannon's species diversity index; increase in the saprobic index; changes in the complex of dominant species (replacement of sensitive species by organisms tolerant to contamination); changes in the ecological structure of the dominant species complex and in the ecological spectra of phytoplankton as a whole.

The response of phytoplankton to heavy pollution of the studied water bodies proved to be rather similar to that of phytoepiphyton, which was studied in the same ponds in July 2016 (Shevchenko et al., 2018). The response of both phytoplankton and phytoepiphyton to contamination involved in particular: reduction in species richness; changes in taxonomic structure; significant difference in the species composition of algae; changes in the structure of algal numbers and biomass; decrease in Shannon's species diversity index; increase in the saprobic index; changes in the complex of dominant species; changes in the ecological structure of the dominant species complex and in the ecological spectra as a whole.

The obtained data can be used to monitor the status of water bodies and their biota, and to determine the type and intensity of contamination.

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