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Nutrient and Organic Matter Driven Reorganization of Phytoplankton Communities Downstream of Wastewater Discharge in the Styr River (Ukraine)

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Abstract

The study investigated changes in the phytoplankton community of the Styr River in the area affected by wastewater discharge from a nuclear power plant. The aim was to assess the effects of nutrient loading associated with wastewater discharge on phytoplankton species composition, abundance, biomass, and community structure. Samples were collected in May - August 2024 at two river sites located upstream and downstream of the wastewater discharge point. Water samples were analyzed for dissolved organic carbon (DOC), total nitrogen (TN), and total phosphorus (TP). UV–Vis optical indices indicated a downstream shift toward more aromatic and higher-molecular-weight DOM characteristics, consistent with changes in the composition of the dissolved organic matter pool. Phytoplankton was assessed in terms of abundance, biomass, mean cell biomass, and taxonomic structure. Correlation analysis and principal component analysis (PCA) were used to examine relationships between hydrochemical and biological variables. Downstream of the discharge point, DOC, TN, and TP concentrations increased by 19.4%, 56.5%, and 72.7%, respectively, compared with the reference site. Against this background, phytoplankton abundance decreased from 3.0×10^6 to 0.5×10^6 cells dm^{-3}, while biomass decreased from 0.70 to 0.15 mg dm^{-3}. At the same time, mean cell biomass increased, indicating a shift in the size structure of the community. Bacillariophyta dominated upstream, accounting for 79% of total abundance and 85% of total biomass. Downstream, Chlorophyta and Euglenophyta became predominant, whereas the contribution of diatoms declined markedly. The saprobity index increased from 1.96 to 2.61, indicating increased organic loading. Correlation analysis showed negative relationships between nutrient concentrations and Bacillariophyta, but positive relationships with Chlorophyta and Euglenophyta. PCA confirmed clear spatial separation of the phytoplankton communities along the trophic gradient. Overall, increased organic matter and nutrient concentrations in the discharge-affected area were accompanied by a restructuring of the phytoplankton community, characterized by a reduced contribution of diatoms and an increased contribution of taxa tolerant of organic pollution.

1. Introduction

Phytoplankton is one of the major structural and functional components of aquatic ecosystems because it contributes to primary organic matter production, participates in biogeochemical cycling, and forms the basis of aquatic food webs (Chakraborty, 2021). Owing to their short life cycles and high sensitivity to environmental changes, phytoplankton communities respond rapidly to fluctuations in nutrient concentrations, temperature regimes, and anthropogenic pressure. This makes them widely used as indicators of the ecological status of surface waters (Reynolds, 2006). Over recent decades, the transformation of phytoplankton communities under anthropogenic pressure has remained a major focus of hydroecological research (Naselli-Flores and Padisák, 2023). This is largely associated with the intensive development of industry, energy production, urban areas, and agriculture, which increases the input of carbon, nitrogen, and phosphorus compounds into aquatic systems. These inputs alter the trophic status of water bodies and affect the structure and functioning of aquatic ecosystems (Paerl & Otten, 2013). Elevated concentrations of nutrients can modify the composition of dominant algal

taxonomic groups, reduce biodiversity, and promote the proliferation of species adapted to eutrophic conditions (Dodds & Smith, 2016; Paerl, 2014).

Particular attention should be paid to the ecological effects of local sources of anthropogenic pressure, which can generate pronounced spatial gradients in hydrochemical conditions within river ecosystems. Such sources include wastewater discharges, which may substantially alter the concentrations of dissolved forms of nitrogen, phosphorus, and organic carbon even when discharge volumes are relatively small (Kuznietsov & Biedunkova, 2023). These changes can lead to shifts in the structure of plankton communities, alter the relative contribution of individual algal taxonomic groups, and intensify eutrophication processes, thereby affecting the ecological stability of aquatic ecosystems. Among anthropogenic factors capable of affecting aquatic ecosystem functioning, energy facilities are of particular importance. The operation of nuclear power plants is intrinsically associated with the use of substantial volumes of water resources. Although the environmental aspects of NPP operation have traditionally been considered primarily in the context of radiation safety, recent research indicates the need for a comprehensive assessment of non-radiological impacts. Considerable information is available on the transformation of biological communities in cooling reservoirs; however, the effects of wastewater discharges from nuclear power plant on river ecosystems remain insufficiently studied. Previous studies have shown that changes in phytoplankton community structure are among the most informative indicators of ecological transformation in aquatic ecosystems (Bilous et all, 2024). In particular, the dominance of diatoms is generally associated with well-aerated flowing waters under relatively stable environmental conditions, whereas an increased contribution of green algae, euglenoids, or cyanobacteria often accompanies eutrophication and organic pollution (Archibold, 1995; Dorgham, 2010). Therefore, analysis of changes in phytoplankton taxonomic structure can provide information on community responses to environmental changes and help identify patterns associated with anthropogenic pressure.

The Styr River basin, which belongs to the rivers of Western Polissia in Eastern Europe, performs important ecological and economic functions. However, studies of anthropogenic effects on its phytoplankton communities remain fragmentary. Available research has mainly focused on individual hydrochemical parameters or general water-quality monitoring, whereas changes in phytoplankton communities associated with local sources of anthropogenic pressure have received comparatively little attention. In addition, data on the relationships between dissolved organic carbon (DOC), total nitrogen (TN), and total phosphorus (TP) concentrations and phytoplankton structural characteristics are scarce. Thus, the research problem addressed in this study is the limited understanding of the patterns governing phytoplankton community transformation in river ecosystems affected by wastewater discharges from energy facilities and the role of nutrient loading in these processes. Addressing this issue is important for improving environmental monitoring systems and developing approaches for the bioindication of surface-water status. The novelty of this study lies in characterizing changes in the phytoplankton communities of the Styr River within the zone affected by nuclear power plant wastewater discharge, identifying shifts in dominant algal taxonomic groups along the nutrient-loading gradient, and quantitatively assessing the relationships between DOC, TN, and TP concentrations and phytoplankton

structural characteristics using Pearson correlation analysis (CA) and principal component analysis (PCA). The aim of this study was to assess changes in phytoplankton species composition, abundance, biomass, and community structure associated with wastewater discharge. The following objectives were defined: (i) to determine the species composition of phytoplankton at sampling sites located upstream and downstream of the wastewater discharge point; (ii) to assess patterns in the abundance and biomass of the major algal taxonomic groups; (iii) to characterize structural changes in phytoplankton communities associated with anthropogenic pressure; (iv) to examine relationships between nutrient concentrations and phytoplankton characteristics using CA and PCA; and (v) to determine the ecological significance of the observed changes for river ecosystem functioning within the wastewater discharge zone. The study hypothesis was that a local wastewater discharge creates a mixing zone within the river, where concentrations of dissolved organic compounds and nutrients may change and, consequently, affect the structure of aquatic biocenoses.

2. Materials and Methods

2.1 Study area and sampling design

The study was conducted in May - August 2024 in the Styr River within the zone affected by wastewater discharge from the Rivne Nuclear Power Plant (NPP). The Styr River is one of the largest right-bank tributaries of the Pripyat River and belongs to the Dnipro River basin. Within the study area, the river has a lowland channel, moderate flow velocity, and predominantly mixed water supply. Two sampling sites were established to assess the effects of wastewater discharge. The first site (S1) was located upstream of the discharge point and was used as an upstream reference site because the hydrochemical regime in this section is primarily determined by natural processes and diffuse catchment inputs. The second site (S2) was located downstream of the wastewater discharge and was directly affected by the discharge. The long-term mean river discharge in the NPP monitoring area is 20–33 m^3/s, whereas the volume of wastewater is substantially lower and does not cause significant changes in the hydrological regime of the river (Biedunkova et all, 2024;). NPP wastewater is generated within the technical water-supply system of the power units and includes water used for cooling process equipment as well as water passing through specific components of the water-treatment systems. After completion of the technological cycle, the water is discharged back into the receiving water body in accordance with established water-use regulations (Kuznietsov et all, 2023).

2.2 Analytical methods and measured parameters

Water and phytoplankton samples were collected simultaneously at both sampling sites in accordance with generally accepted hydrobiological and hydrochemical methods (APHA, 2023; ISO 5667-6:2014, 2014). All samples were collected from the surface water layer in the central part of the river channel and subsequently transported to the laboratory for analysis. To assess nutrient loading, DOC, TN, and TP concentrations were determined. DOC was measured after pre-filtration through membrane filters with a pore size of 0.45 μm using high-temperature catalytic oxidation followed by infrared detection of carbon

dioxide (DSTU EN 1484:2003, 2003). TN and TP concentrations were measured after oxidation of all forms of the respective elements to mineral compounds, followed by spectrophotometric determination according to standard procedures for the analysis of natural waters (ISO 6878:2004, 2004; ISO 29441:2010, 2010). Phytoplankton was characterized using abundance (N) and biomass (B), which reflect different aspects of aquatic ecosystem functioning. N characterizes the intensity of population development, whereas B represents standing phytoplankton biomass and integrates differences in cell abundance and cell size. Samples were concentrated by sedimentation and examined using light microscopy. Cell counts were performed in counting chambers according to generally accepted methodological approaches (EN 15204:2006, 2006). Biomass was estimated using a calculation-based method from the mean geometric dimensions of cells, approximating individual cells to simple geometric shapes. Algae were identified to the lowest possible taxonomic level using current identification guides for freshwater algae. The ecological status of the study sites was assessed using the saprobity index, calculated from species composition and the indicator value of the identified phytoplankton taxa (Junqueira et all, 2010). To assess changes in the size structure of phytoplankton communities, the average cell biomass (ACB) was calculated as the ratio between total phytoplankton biomass and total abundance (Hillebrand et all, 2022). The ACB was calculated according to the following Equation (1).

$$ACB = B / N \quad (1)$$

where ACB is the average cell biomass (ng $cell^{-1}$), B is phytoplankton biomass (mg dm^{-3}), and N is phytoplankton abundance (10^6 cells dm^{-3}).

To quantify changes in phytoplankton diversity and community organization between the upstream and downstream river sections, several widely used ecological diversity indices were calculated based on species abundance data. The Shannon–Wiener diversity index (H') was used to characterize species diversity and was calculated according to Eq. (2) (Hosmani et all, 2009). Simpson diversity was expressed as (1 – D), where D represents Simpson's dominance index, and was calculated according to Eq. (3) (Li et all, 2021). Pielou's evenness index (J') was used to assess the uniformity of species distribution within the community and was calculated according to Eq. (4) (Pielou, 1966). Margalef richness index (d) was applied to estimate species richness while accounting for sample size and was calculated according to Eq. (5) (Yanran et all, 2021). All diversity indices were calculated separately for the upstream (S1) and downstream (S2) sampling sites. Higher values of H', 1 – D, J' and d indicate greater community diversity, evenness and species richness, respectively, whereas lower values suggest increasing dominance of a limited number of taxa and progressive simplification of community structure under environmental stress.

$$H' = - \Sigma (pi \times \ln pi) \quad (2)$$

$$D = \Sigma pi^2 \quad (3)$$

$$J' = H' / \ln(S) \quad (4)$$

$$d = (S - 1) / \ln(N) \quad (5)$$

where pi is the proportion of individuals belonging to species i relative to the total phytoplankton abundance; D is Simpson's dominance index; H' is the Shannon–Wiener diversity index; J' is Pielou evenness index; d is Margalef's richness index; S is the total number of species; and N is the total abundance of individuals in the sample. Simpson diversity was expressed as (1 – D), with higher values indicating greater community diversity.

The optical properties of dissolved organic matter (DOM) were characterized using UV–visible absorbance spectroscopy (UV–Vis). Prior to analysis, water samples were filtered through 0.45 μm membrane filters to remove suspended particles. Absorbance spectra were measured between 240 and 600 nm using a UV–Vis spectrophotometer (Helios Gamma, Thermo Spectronic, UK) equipped with 1 cm path-length quartz cuvettes. Ultrapure water was used as the reference blank. Specific ultraviolet absorbance at 254 nm (SUVA254) was calculated as the absorbance coefficient at 254 nm normalized to DOC concentration and expressed in L mg − 1 m − 1 (Zhou et all, 2026; Weishaar et all, 2003). SUVA254 is widely applied as an indicator of DOM aromaticity and hydrophobicity. The E2 ratio was calculated as the ratio of absorbance at 254 nm to absorbance at 365 nm. This parameter is inversely related to the average molecular weight and aromatic condensation of dissolved organic matter (Peuravuori & Pihlaja, 1997). The spectral slope ratio (SR) was determined as the ratio between the spectral slope in the wavelength range 275–295 nm and that in the range 350–400 nm according to (Helms et all, 2008). Lower SR values indicate a predominance of higher-molecular-weight and less photodegraded DOM fractions, whereas higher values are generally associated with lower molecular weight compounds and enhanced photobleaching. The calculated optical indices were used to evaluate changes in DOM composition upstream and downstream of the wastewater discharge and to assess their potential influence on phytoplankton community structure.

Statistical analyses were performed using JASP 0.18.3. For all variables, the mean (M), minimum–maximum (min–max), and standard deviation (SD) were calculated. The resulting data were used to construct graphical relationships and for subsequent multivariate analysis. CA was used to determine relationships between DOC, TN, and TP concentrations and phytoplankton structural characteristics, following (Chen et all, 2018). PCA was applied to reduce the dimensionality of the multivariate dataset and identify the major environmental gradients determining phytoplankton community structure, following (Liu et all, 2023). PCA was based on a matrix of standardized variables that included DOC, TN, and TP concentrations, the abundance and biomass of the major algal taxonomic groups, and sampling sites S1 and S2. Because abundance and biomass characterize different aspects of phytoplankton community organization, CA and PCA were performed separately for N and B. This approach avoids conflating effects associated with cell abundance and cell size and allows a more appropriate interpretation of the observed ecological patterns.

3. Results

For DOC, the upstream section was characterized by M = 10.8 mg dm^{-3}, with values ranging from min-max 7.0 to 15.0 mg dm^{-3} and SD = 3.2 mg dm^{-3} (Fig. 1). Downstream of the discharge, DOC concentrations increased to 12.9 mg dm^{-3}, while the observed range expanded from min-max 9.0 to 17.0 mg dm^{-3}, accompanied by SD = 3.4 mg dm^{-3}. Thus, the downstream site exhibited both higher average concentrations and greater variability, which is consistent with increased organic matter inputs downstream of the discharge point. In the upstream section, TN concentrations varied between min-max 1.5 and 3.5 mg dm^{-3}, M = 2.3 mg dm^{-3} and SD = 0.8 mg dm^{-3}. In contrast, the downstream section displayed substantially elevated concentrations, with M = 3.6 mg dm^{-3}, a min value of 2.3 mg dm^{-3}, a max value of 5.2 mg dm^{-3}, and SD = 0.7 mg dm^{-3}. Consequently, the increase in TN concentrations downstream indicates enhanced nitrogen enrichment associated with the wastewater discharge. Upstream TP concentrations ranged from min-max 0.30 to 1.00 mg dm^{-3}, with M = 0.55 mg dm^{-3} and SD of 0.18 mg dm^{-3}. Downstream, TP concentrations increased to M = 0.95 mg dm^{-3}, while min-max values reached 0.40 and 1.50 mg dm^{-3} and SD = 0.28 mg dm^{-3}.

Показники phytoplankton для N in the upstream section varied from 2.2×10^6 to 4.1×10^6 cells dm^{-3}, with M = 3.0×10^6 cells dm^{-3} and SD = 0.8×10^6 cells dm^{-3}. In the downstream section, N decreased considerably, ranging min 0.2×10^6 and max 0.8×10^6 cells dm^{-3}, M = 0.5×10^6 cells dm^{-3}, SD = 0.2×10^6 cells dm^{-3} (Fig. 2). Therefore, the downstream phytoplankton community was characterized by a sixfold reduction in average cell density relative to the upstream site, indicating a substantial spatial change in community structure downstream of the wastewater discharge. In the upstream section, B values ranged from min 0.42 to max 1.03 mg dm^{-3}, yielding M = 0.70 mg dm^{-3} and SD = 0.23 mg dm^{-3}. Downstream of the wastewater discharge, B varied from man 0.07 to max 0.30 mg dm^{-3}, with a mean value of 0.15 mg dm^{-3} and an SD of 0.08 mg dm^{-3}. Consequently, the average B in the downstream section was more than four times lower than that recorded upstream. Comparison of N and B showed that the decrease in N downstream of the wastewater discharge was more pronounced than the reduction in biomass. N decreased from 3.07×10^6 to 0.55×10^6 cells dm^{-3}, whereas B decreased from 0.705 to 0.168 mg dm^{-3}. Accordingly, the B ratio increased from 0.230 ng $cell^{-1}$ at the upstream site to 0.304 ng $cell^{-1}$ downstream.

Marked differences in the taxonomic composition of phytoplankton communities were observed between the upstream and downstream river sections (Fig. 3), indicating a substantial reorganization of community structure under the influence of зворотних вод. In the upstream section, Bacillariophyta clearly dominated the phytoplankton assemblage, accounting for 79% of total abundance and 85% of total biomass. The community was primarily represented by Asterionella gracillima, Diatoma vulgaris, and Tabellaria fenestrata var. gracilis, which constituted the dominant taxa within the diatom assemblage. The high contribution of diatoms is consistent with the ecological characteristics commonly reported for lotic phytoplankton assemblages. Cyanophyta represented the second most important group, contributing 15% of total abundance and 8% of total biomass. The occurrence of Microcystis aeruginosa indicates a potential sensitivity of the ecosystem to elevated temperatures and increased organic loading. In contrast, Chlorophyta and Dinophyta were represented by a limited number of species and exhibited low abundance and biomass values, collectively accounting for less than 10%

of the phytoplankton community. A markedly different pattern was recorded downstream of the зворотних вод. Species richness declined substantially, whereas total phytoplankton abundance and biomass decreased to 0.552 × 10^6 cells dm^{-3} and 0.168 mg dm^{-3}, respectively. Concurrently, the taxonomic structure shifted from diatom dominance toward communities dominated by Chlorophyta and Euglenophyta. Green algae accounted for 35% of total abundance and 43% of total biomass, thereby becoming the most important group in terms of biomass contribution. Euglenophyta represented 30% of total abundance and 23% of total biomass, while the increasing contribution of the genus Trachelomonas suggests enhanced availability of organic matter within the downstream section. Meanwhile, the contribution of Bacillariophyta decreased sharply to 22% of total abundance and 30% of total biomass, indicating a pronounced reduction in the ecological importance of diatoms. Although diatoms remained present within the community, their dominance was replaced by groups generally associated with nutrient enrichment and elevated organic loading. Therefore, the downstream phytoplankton assemblage was characterized not only by lower abundance and biomass but also by a distinct shift in taxonomic composition. Індекс сапробності зріс від 1,96 у створі 1 до 2,61 у створі 2, що свідчить про перехід від β-мезосапробної до β–α-мезосапробної зони та підвищення рівня органічного забруднення.

The biodiversity indices demonstrated a noticeable decline in phytoplankton diversity downstream of the wastewater discharge (Table 1). The upstream site was characterized by higher species richness (S = 22) and diversity, reflected by H' = 1.87 and 1 − D = 0.36. In contrast, the downstream site exhibited lower diversity metrics, with H' and 1 − D indices decreasing to 1.54 and 0.28, respectively. S also declined substantially downstream, as indicated by the reduction in d index from 2.55 to 1.88. Similarly, J' decreased from 0.61 to 0.57, suggesting that the downstream phytoplankton community became increasingly dominated by a smaller number of tolerant taxa. Overall, the biodiversity metrics indicate that nutrient enrichment and increased organic matter loading downstream of the wastewater discharge were accompanied by a reduction in taxonomic richness, lower diversity, and a simplification of phytoplankton community structure.

Table 1
Phytoplankton biodiversity indices in S1 and S2 sites of the Styr River

Index	S1	S2
S	22	15
H'	1.87	1.54
1 − D	0.36	0.28
J'	0.61	0.57
d	2.55	1.88

The correlation matrices presented in Fig. 4 reveal consistent relationships between nutrient enrichment and phytoplankton community structure, although the strength of these relationships differs slightly depending on whether community development is expressed as A or B.For the abundance-based dataset (Fig. 4a), DOC, TN, and TP were strongly and positively correlated (r = 0.71–0.78), indicating that the downstream site was characterized by a common nutrient-enrichment gradient. N of Bacillariophyta showed moderate negative correlations with DOC (r = − 0.64), TN (r = − 0.71), and TP (r = − 0.69), indicating a progressive decline in abundance with increasing nutrient concentrations. In contrast, N of Chlorophyta was moderately and positively correlated with DOC (r = 0.66), TN (r = 0.69), and TP (r = 0.62), while N of Euglenophyta showed similarly pronounced positive relationships (r = 0.58–0.63). N of Cyanophyta exhibited weak but significant negative correlations with DOC (r = − 0.32, $p < 0.05$), TN (r = − 0.35, $p < 0.05$), and TP (r = − 0.28, $p < 0.05$). The biomass-based correlation matrix (Fig. 4b) revealed generally similar but more pronounced patterns. B of Bacillariophyta was strongly negatively correlated with DOC (r = − 0.80), TN (r = − 0.58), and TP (r = − 0.71), indicating a substantial decline in the contribution of Bacillariophyta to total phytoplankton biomass along the nutrient gradient between S1 and S2. In contrast, B of Chlorophyta showed strong positive correlations with DOC (r = 0.82), TN (r = 0.61), and TP (r = 0.74), whereas B of Euglenophyta was positively correlated with DOC (r = 0.75), TN (r = 0.54), and TP (r = 0.66). These results indicate that nutrient enrichment was associated not only with greater abundance but also with increased biomass accumulation of Chlorophyta and Euglenophyta. Unlike the abundance-based CA, B of Cyanophyta showed positive correlations with DOC (r = 0.41), TN (r = 0.29, $p < 0.05$), and TP (r = 0.35, $p < 0.05$), suggesting that although Cyanophyta remained less abundant, their biomass increased along the nutrient gradient.

Principal component analysis revealed a pronounced separation between the upstream and downstream sites in both abundance-based and biomass-based datasets (Fig. 5). The underlying data used for the PCA are openly available in the Zenodo repository (see the Data availability section). In the abundance-based PCA (Fig. 5a), PC1 explained 56.7% of the total variance, whereas PC2 accounted for an additional 18.2%, together describing 74.9% of the variability within the dataset. The downstream samples were positioned on the positive side of PC1 and showed a clear association with elevated DOC, TN, and TP concentrations. Moreover, the results for S1 samples were grouped on the left side of the diagram and showed closer associations with Bacillariophyta, Cyanophyta, and, to a lesser extent, Chlorophyta. This pattern reflects conditions of relatively lower nutrient loading and the predominance of diatoms within the phytoplankton community. The biomass-based PCA (Fig. 5b) exhibited a somewhat different structure. PC1 explained 42.1% of the total variance, whereas PC2 accounted for 24.7%, resulting in a cumulative contribution of 66.8%. In contrast, the S2 samples were shifted toward the positive sector of the PC1 axis, where the DOC, TN, and TP vectors were concentrated, indicating a major influence of nutrient concentrations on phytoplankton community structure downstream of the discharge point. In the B-based PCA (Fig. 5b), the separation between the S1 and S2 clusters remained distinct. However, the S2 samples were more closely associated with the vectors of Chlorophyta, Euglenophyta, Cyanophyta, DOC, and TN than in the N-based PCA. Consequently, PC1 can be interpreted as a nutrient-enrichment gradient along which the phytoplankton community shifted from diatom dominance toward

assemblages increasingly characterized by green and euglenoid algae. Meanwhile, Cyanophyta was primarily associated with PC2 and oriented in the opposite direction to Euglenophyta, indicating that variation in these groups contributed mainly to secondary community differentiation rather than to the principal nutrient gradient.

The UV–Vis absorption spectra revealed distinct differences in the optical properties of DOM between the upstream (S1) and downstream (S2) sampling sites (Fig. 6). The underlying data used to generate the UV–Vis absorption spectra are openly available in the Zenodo repository (see the Data availability section). Absorbance values at all investigated wavelengths were consistently higher at S2, indicating an increased concentration of DOC downstream of the wastewater discharge. The most pronounced differences were observed in the long-wavelength region of the spectrum. Absorbance at 254 nm increased from 0.41 AU at S1 to 0.63 AU at S2, while absorbance at 465 nm increased from 0.03 to 0.08 AU. Relative increases at wavelengths above 365 nm, suggesting an enrichment of colored and humified organic compounds downstream of the discharge point. Calculated optical indices further confirmed changes in DOM composition. SUVA254 increased from 3.8 L mg − 1 m − 1 at S1 to 4.9 L mg − 1 m − 1 at S2, indicating a higher proportion of aromatic organic compounds in the downstream section. In contrast, the E2 ratio decreased from 5.1 to 3.8, suggesting an increase in the average molecular weight and structural complexity of DOM. Similarly, the slope ratio (SR) declined from 0.94 to 0.77, reflecting a shift toward higher-molecular-weight fractions and reduced photochemical degradation.

4. Discussion

All three nutrient-related parameters showed higher mean values in the downstream section (Fig. 1). Moreover, DOC, TN, and TP exhibited broader concentration ranges downstream of the wastewater discharge, indicating that nutrient enrichment was the principal environmental gradient differentiating the two river sections and likely contributed to the subsequent reorganization of the phytoplankton community observed in the study. Notably, both N and B showed narrower ranges and lower variability downstream, suggesting that under the altered environmental conditions, the phytoplankton community became less variable in its structure and was represented by a smaller number of dominant taxa. Thus, despite the elevated DOC, TN, and TP concentrations observed downstream of the discharge point (Fig. 1), phytoplankton development was suppressed. This finding indicates that nutrient enrichment alone did not promote biomass accumulation and that the altered environmental conditions were accompanied by pronounced reorganization of the phytoplankton community structure. According to Weishaar et all, 2003, SUVA254 (specific ultraviolet absorbance at 254 nm) values exceeding 4 L mg^{-1} m^{-1} are typically associated with highly aromatic, hydrophobic, and terrestrially derived organic matter. The downstream value of 4.9 therefore suggests a substantial contribution of refractory organic compounds to the dissolved organic matter (DOM) pool. The decrease in E2 and SR values further supports this interpretation. Both indices are inversely related to DOM molecular weight and aromatic condensation (Helms et all, 2008; Biedunkova et all, 2026). Consequently, the lower values recorded at S2 indicate the predominance of larger and structurally more complex organic molecules. Such compounds are often associated with wastewater-derived organic matter and secondary microbial

transformation processes occurring within aquatic ecosystems. Comparison of phytoplankton N and B (Fig. 2) showed that the decrease in cell abundance downstream was more pronounced than the reduction in biomass. N decreased from 3.07×10^6 to 0.55×10^6 cells dm^{-3} (82%), whereas B decreased from 0.705 to 0.168 mg dm^{-3} (76%). Consequently, the B ratio increased from 0.230 to 0.304 ng $cell^{-1}$, indicating an increase in mean biomass per phytoplankton cell and a change in community size structure. This pattern suggests that the community affected by the wastewater discharge contained fewer cells but had a greater mean cell size, reflecting structural reorganization of the phytoplankton community. The observed changes demonstrate that wastewater discharge was accompanied by a transition from a diatom-dominated community toward assemblages with greater contributions of Chlorophyta and Euglenophyta. Consequently, the phytoplankton community structure reflected a clear alteration in environmental conditions downstream of the discharge point, consistent with the nutrient-enrichment pattern identified by the hydrochemical analysis.

The observed correlations indicate that increasing DOC, TN, and TP concentrations were among the key factors accompanying structural reorganization of the phytoplankton community within the wastewater discharge zone (Fig. 4). In particular, consistent negative relationships between nutrient-related variables and Bacillariophyta indicate that increasing organic and nutrient loading was associated with environmental conditions less favorable for taxa characteristic of well-aerated river ecosystems with relatively stable hydrochemical regimes. In contrast, the positive correlations of DOC, TN, and TP with Chlorophyta and Euglenophyta indicate that these groups were more strongly associated with the altered environmental conditions downstream of the discharge point. Thus, the increased contribution of green and euglenoid algae can be interpreted as a community response to greater availability of nutrients and organic matter, consistent with the changes in phytoplankton taxonomic structure observed at S2. It is also notable that the correlations were generally stronger for B than for N, suggesting that the observed response involved not only changes in population abundance but also structural reorganization at the level of cell-size composition. The stronger negative correlations between Bacillariophyta and nutrient-related variables in the biomass matrix, together with stronger positive relationships for Chlorophyta and Euglenophyta, suggest that environmental transformation was accompanied by a greater contribution of larger-cell forms of these groups to total phytoplankton biomass. In addition, strong negative relationships between Bacillariophyta and Chlorophyta or Euglenophyta, regardless of whether the community was evaluated using N or B, support the occurrence of taxonomic replacement, whereby the reduced contribution of diatoms was accompanied by an increased contribution of groups more tolerant of elevated organic matter and nutrient concentrations. At the same time, the response of Cyanophyta was less consistent. Whereas N showed weak negative relationships with DOC, TN, and TP, B showed weak positive correlations with these variables. This pattern may indicate an increase in mean cell size or a greater contribution of individual taxa to total biomass without a substantial increase in their abundance. This difference further demonstrates that quantitative characteristics alone may not fully capture changes in the functional organization of a phytoplankton community. The spatial separation of samples along the first principal component further indicates that the trophic gradient was the main factor differentiating the communities between the sampling sites (Fig. 5). In the PCA ordination,

phytoplankton communities upstream and downstream of the discharge formed distinct clusters, reflecting differences in their association with the measured environmental variables.

The reduction in biodiversity indices downstream of the wastewater discharge confirms that the observed changes were not limited to shifts in the relative abundance of major taxonomic groups but also involved a decline in overall community complexity (Table 1). Lower values of H' and 1 – D indicate increasing dominance of a limited number of taxa, whereas the reduction in d suggests a loss of species richness within the phytoplankton assemblage. The decrease in J' further demonstrates that community structure became less balanced under conditions of elevated nutrient and organic matter concentrations. These findings are consistent with the observed replacement of Bacillariophyta-dominated assemblages by communities increasingly characterized by Chlorophyta and Euglenophyta, which are generally considered more tolerant of eutrophic and organically enriched conditions. Consequently, the simultaneous decline in H', 1 – D, J' and d indicates progressive simplification of the phytoplankton community structure and reduced ecological stability in the river section affected by wastewater discharge.

The thermal regime analysis indicates that the return water discharged from the cooling circulation system was substantially warmer than the receiving river water, with mean temperatures reaching approximately 24°C compared to 10–11°C in the Styr River. However, after mixing, the average temperature increase in the river did not exceed 1.07°C, indicating efficient dilution of the thermal load by the river flow (Table 2). Therefore, the pronounced reduction in phytoplankton abundance and biomass observed downstream is unlikely to be explained by direct thermal stress alone. Nevertheless, even moderate temperature increases may alter ecological processes by accelerating microbial decomposition of organic matter, modifying oxygen dynamics, and changing competitive interactions among algal groups. Such mechanisms could contribute to the observed decline of Bacillariophyta and the increasing contribution of Chlorophyta and Euglenophyta in the downstream section. Although nutrient concentrations increased downstream of the discharge, neither hydraulic dilution nor flow acceleration appears sufficient to explain the observed sixfold reduction in phytoplankton abundance and fourfold reduction in biomass. The discharge rate of the cooling circulation system represents only approximately 2.6–7% of the river discharge, depending on hydrological conditions, which is insufficient to generate substantial dilution effects or major alterations in flow velocity. Therefore, the observed decline in phytoplankton development is more likely associated with non-hydraulic factors, including thermal alteration of the receiving water, increased organic matter loading, and potential changes in oxygen conditions. The concurrent reduction in Bacillariophyta and increased contribution of Chlorophyta and Euglenophyta further support this interpretation, as these groups are commonly associated with warmer, more organically enriched environments.

- **Table 2.** Hydrological and thermal characteristics of the cooling-water discharge system (May - August 2024)

Parameter	Value
Mean river discharge	27 m³/s
Range river discharge	20–33 m³/s
Mean water intake	1.68 m³/s
Mean blowdown discharge	0.37 m³/s
Mean return-water temperature	24°C
Mean river temperature upstream	11.01°C
Mean river temperature downstream	12.08°C
Mean ΔT after mixing	1.07°C

Increased inputs of nitrogen and phosphorus compounds generally promote primary production, phytoplankton biomass accumulation, and eutrophication. Similar patterns have been reported for a range of river and lake ecosystems, where increasing trophic status was accompanied by a greater contribution of fast-growing algal taxa and increased phytoplankton biomass [1–4]. However, the results of the present study indicate a different ecological response. Despite the increased concentrations of nutrients downstream of the discharge point, mean phytoplankton abundance decreased. Similar patterns have been reported in studies of the effects of thermal discharges from energy facilities, showing that the input of heated water can suppress planktonic production processes even under sufficient nutrient availability (Pomati et all, 2020). Increased DOC concentrations may promote the development of heterotrophic microorganisms, reduce water transparency, and limit light availability for photosynthesis. Under such conditions, the stimulatory effects of nitrogen and phosphorus may be partly offset by increased competition for resources and reduced light availability. The present results also differ from those of studies in which the effects of power-plant cooling-water discharges were associated with increased abundance of particular algal groups or higher total phytoplankton biomass (Xu et all, 2021). This suggests that the response of phytoplankton communities to anthropogenic disturbance depends not only on nutrient concentrations but also on the combined effects of other environmental factors. The observed shift in taxonomic structure is also generally consistent with previous studies. A reduced contribution of Bacillariophyta and an increased contribution of Chlorophyta and Euglenophyta have often been regarded as characteristic of increased organic loading and deteriorating ecological conditions in aquatic ecosystems (Reinl et all, 2022). Similar changes have been described both in river ecosystems affected by wastewater discharges and in water bodies undergoing eutrophication and saprobization processes (Lv et all, 2020).

Changes in DOM quality may directly affect phytoplankton communities through several pathways. The values of optical indices revealed distinct differences in DOM composition between the studied samples. The higher E_{23} and SR values observed in S1 indicate the predominance of lower-molecular-weight fractions and a stronger influence of photochemical processing. Conversely, the elevated SUVA254 value

and lower E_{23} and SR values in S2 suggest an increased contribution of aromatic and humified organic matter with higher molecular weight. These patterns collectively indicate a transition toward more allochthonous and structurally complex DOM in S2 compared with S1. Increased concentrations of DOM reduce water transparency and attenuate photosynthetically active radiation, thereby altering the underwater light climate. Reduced light penetration can disadvantage diatom assemblages that depend on well-illuminated and hydrodynamically stable conditions. At the same time, elevated organic loading stimulates microbial activity and promotes nutrient regeneration, creating favorable conditions for taxa tolerant to organic enrichment. These mechanisms are consistent with the phytoplankton responses observed in the present study, particularly the reduction in the relative contribution of Bacillariophyta and the increased importance of Euglenophyta downstream of the discharge. Euglenophytes are widely recognized as indicators of organically enriched and eutrophic environments and often proliferate under conditions of elevated dissolved organic matter and nutrient availability. Therefore, the optical characteristics of DOM provide complementary evidence that wastewater discharge alters not only the quantity of organic matter but also its structural composition, thereby influencing ecological conditions and phytoplankton community organization in the Styr River. The study was based on a comparison of two sampling sites located upstream and downstream of the wastewater discharge zone. While this approach enables the detection of hydrochemical and biological changes associated with the discharge, it cannot fully capture the natural spatial variability of river ecosystems. Field observations were conducted during a single sampling campaign. As phytoplankton communities are strongly influenced by seasonal changes in hydrology, temperature, nutrient availability, and biological interactions, the observed patterns represent conditions prevailing during the study period and may not reflect the full range of annual variability. Several factors potentially affecting phytoplankton development were beyond the scope of the present investigation. In particular, dissolved oxygen concentrations, underwater light conditions, suspended solids, zooplankton grazing pressure, and detailed hydrodynamic characteristics were not assessed. Consequently, the extent to which these variables contributed to the observed reduction in phytoplankton abundance and biomass remains uncertain. The study also did not include direct measurements of thermal plume distribution or mixing dynamics within the receiving water body.

Overall, the results indicate that exposure to NPP effluent is associated with changes in phytoplankton community structure and an increased contribution of taxa tolerant of saprobic conditions. The increase in DOC suggests enhanced organic matter loading, which may stimulate microbial respiration and intensify competition for dissolved resources. Such conditions can indirectly influence phytoplankton development through changes in oxygen dynamics and ecosystem metabolism. The simultaneous increase in saprobity indicators and the growing contribution of Euglenophyta further support the role of organic enrichment in shaping downstream phytoplankton assemblages. Additional ecological mechanisms may also contribute to the observed patterns. Elevated nutrient concentrations do not necessarily result in increased phytoplankton biomass when biological regulation becomes stronger. Furthermore, increased concentrations of dissolved organic matter can reduce underwater light penetration, thereby limiting photosynthetic activity and partially offsetting the stimulatory effect of nutrient enrichment. The results suggest that the downstream decline in phytoplankton abundance and

biomass is not a consequence of nutrient limitation but rather reflects the combined influence of thermal modification, organic matter enrichment, altered ecological interactions, and potential light limitation. These factors appear to promote a transition from a diatom-dominated community towards assemblages composed of more environmentally tolerant taxa, resulting in reduced biodiversity and simplified community structure. Future research should focus on expanding both the spatial and temporal scope of observations in order to distinguish discharge-related effects from natural variability within the river ecosystem. Particular attention should be given to the simultaneous assessment of phytoplankton communities, dissolved oxygen, suspended solids, light availability, and zooplankton abundance. Further investigations should also include detailed characterization of thermal plume dynamics and mixing processes within the receiving water body. The observed decline in phytoplankton abundance and biomass despite elevated nutrient concentrations highlights the need for mechanistic studies addressing the interactions among nutrient enrichment, organic matter loading, thermal modification, and biological regulation. In addition, future assessments would benefit from the inclusion of functional and ecological indicators, such as phytoplankton functional groups, diversity metrics, trophic state indices, and molecular approaches for community characterization. These tools could provide a more detailed understanding of ecosystem responses to wastewater discharge and improve ecological risk assessment for river systems.

5. Conclusions

In the area affected by the discharge of NPP effluent, increased concentrations of DOC, TN, and TP were recorded, forming a pronounced nutrient-enrichment gradient between the reference and impacted sections of the Styr River. The increase in organic matter and nutrient concentrations was accompanied by a marked reduction in phytoplankton abundance and biomass. Mean phytoplankton abundance downstream of the discharge point decreased approximately sixfold, while biomass decreased more than fourfold compared with the upstream section. A substantial shift in phytoplankton taxonomic structure was observed. Whereas Bacillariophyta, characteristic of well-aerated river ecosystems, dominated at the reference site, Chlorophyta and Euglenophyta became more prominent downstream of the discharge. The increase in mean cell biomass indicates a shift in the size structure of the community and a relatively greater contribution of larger-cell forms to total phytoplankton biomass. The increase in the saprobity index from 1.96 to 2.61 indicates greater organic loading and a shift from β-mesosaprobic to β–α-mesosaprobic conditions in the discharge-affected area. CA revealed negative relationships between DOC, TN, and TP and Bacillariophyta, whereas these variables were positively associated with Chlorophyta and Euglenophyta, indicating a shift in the taxonomic structure of the phytoplankton community. PCA showed that the trophic gradient was the main factor associated with the spatial differentiation of phytoplankton communities between the upstream and downstream sections of the river. The observed changes in DOM composition further suggest that wastewater discharge affected not only nutrient availability but also the characteristics of the DOM pool. The combined changes in organic matter and nutrient concentrations were associated with a shift in phytoplankton community structure, including a reduced contribution of Bacillariophyta and an increased contribution of

Chlorophyta and Euglenophyta. Overall, the results demonstrate that phytoplankton communities are sensitive indicators of local anthropogenic impacts and can be effectively used for ecological monitoring of river ecosystems affected by energy facilities. Overall, the results demonstrate that phytoplankton communities are sensitive indicators of local anthropogenic impacts and can be effectively used for ecological monitoring of river ecosystems affected by energy facilities.

Declarations

Ethical approval: Not applicable. This study did not involve human participants or animals, and therefore ethical approval was not required.

Consent to participate: Not applicable.

Consent to publish: Not applicable.

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Competing interests: The authors declare no competing interests.

Data availability: The datasets underlying the results presented in this study, including the data used for the PCA and UV–Vis absorption spectra, are openly available in the Zenodo repository at https://doi.org/10.5281/zenodo.23263571. The repository contains the original datasets used for the analyses presented in this manuscript.

Clinical trial number: Not applicable.

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Figures

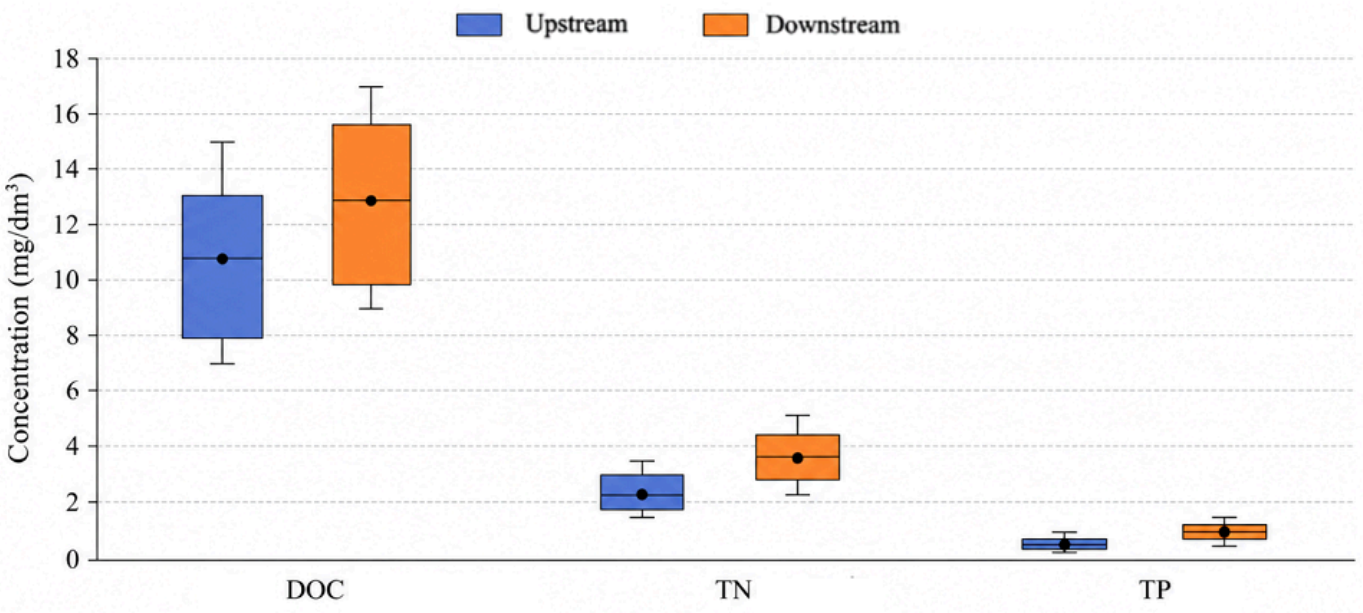


Figure 1

Concentrations of DOC, TN, and TP in river sections upstream (a) and downstream (b) of the wastewater discharge.

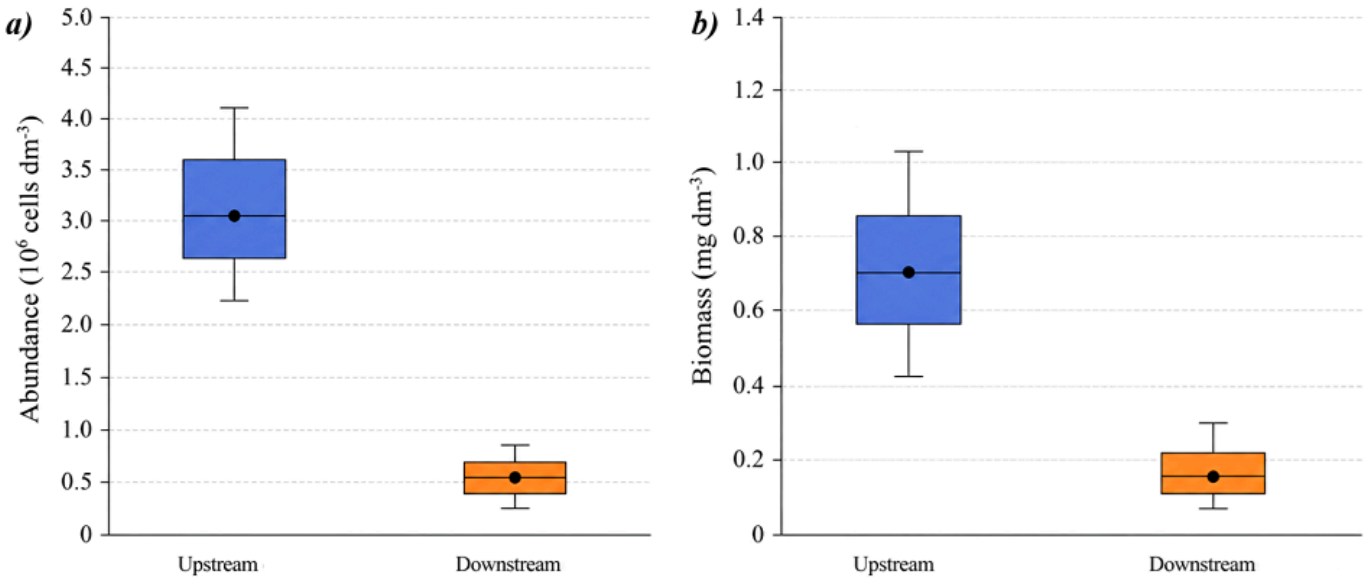


Figure 2

Phytoplankton abundance (a) and biomass (b) in river sections upstream and downstream of the wastewater discharge.

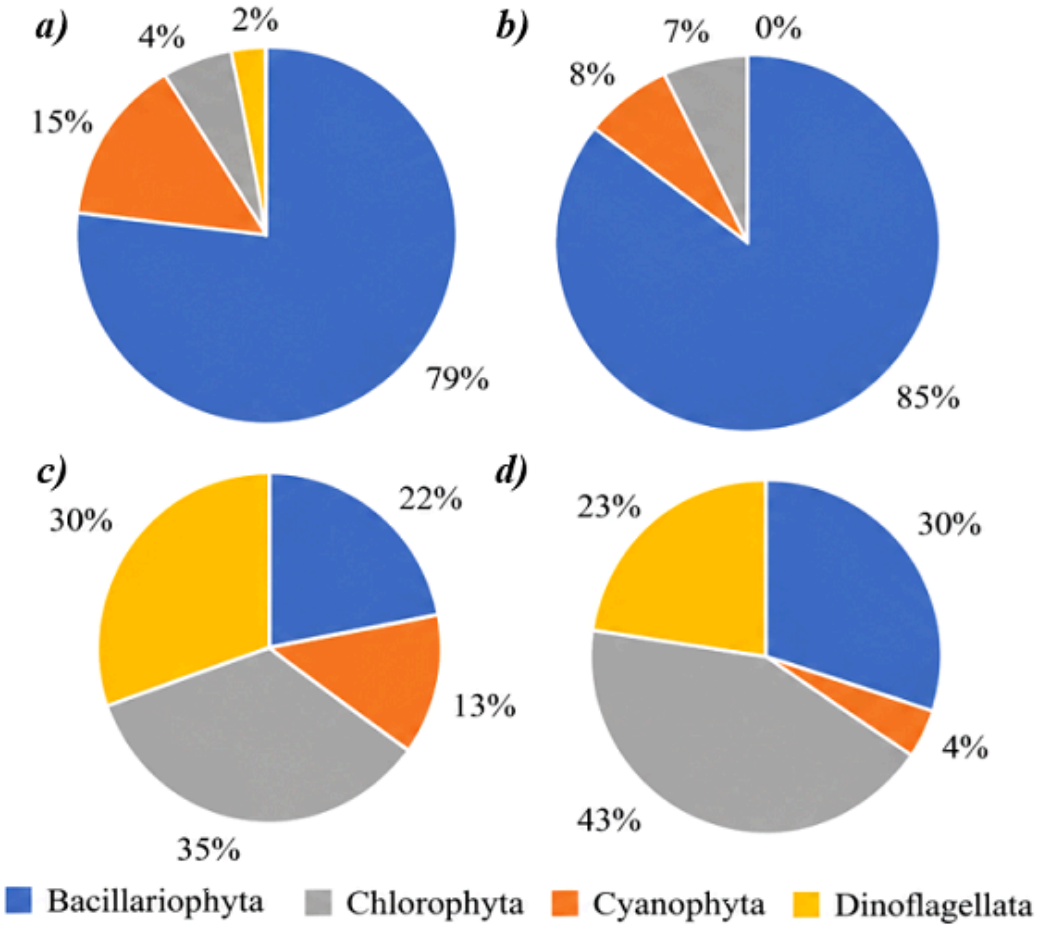


Figure 3

Taxonomic composition of phytoplankton communities based on abundance (a, c) and biomass (b, d) in river sections upstream and downstream of the wastewater discharge.

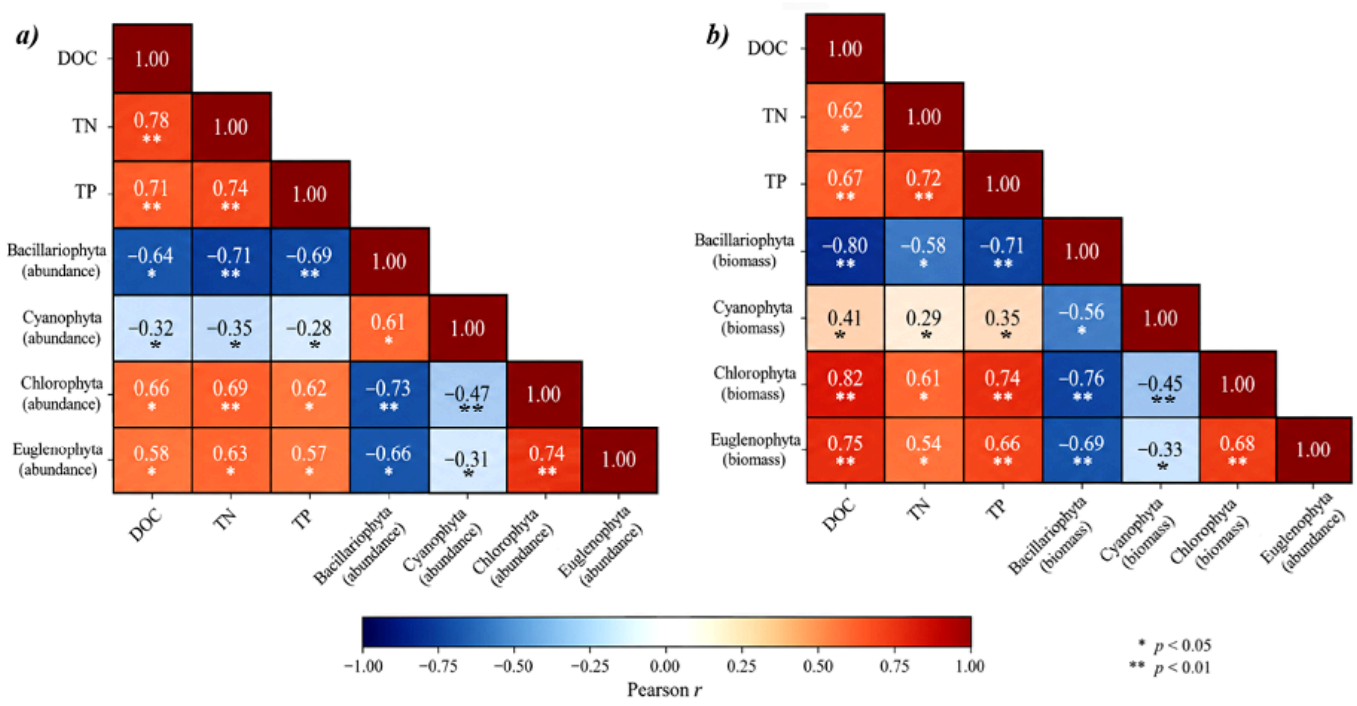


Figure 4

Correlation matrix between nutrient concentrations and phytoplankton community characteristics based on abundance (a) and biomass (b) in river sections upstream and downstream of the wastewater discharge.

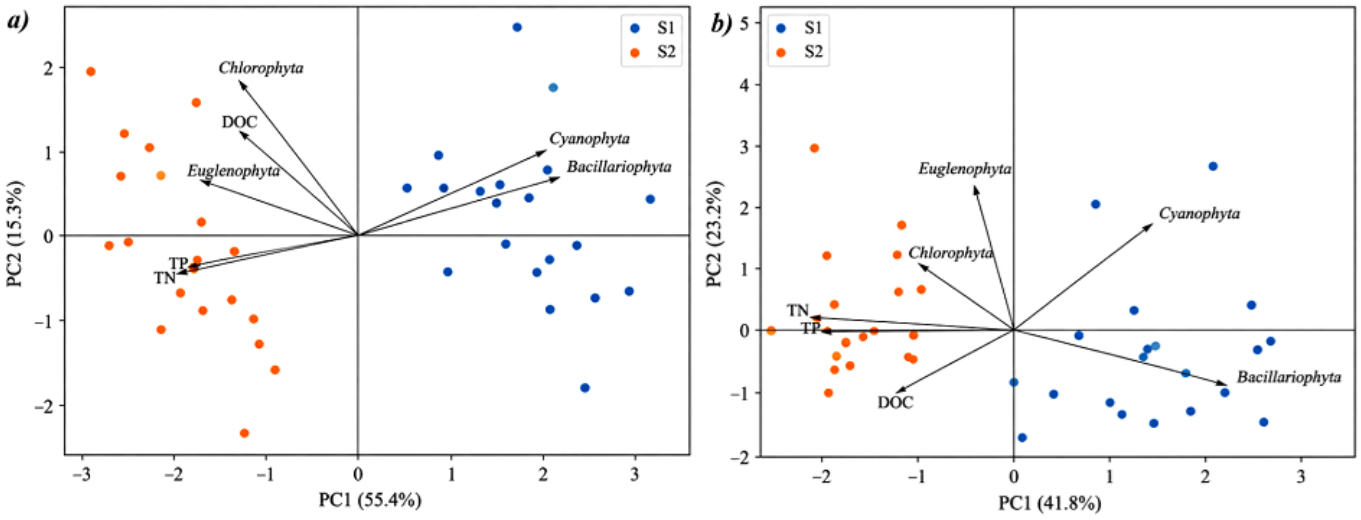


Figure 5

PCA of nutrient variables and phytoplankton community characteristics based on abundance (a) and biomass (b) in river sections upstream and downstream of the wastewater discharge.

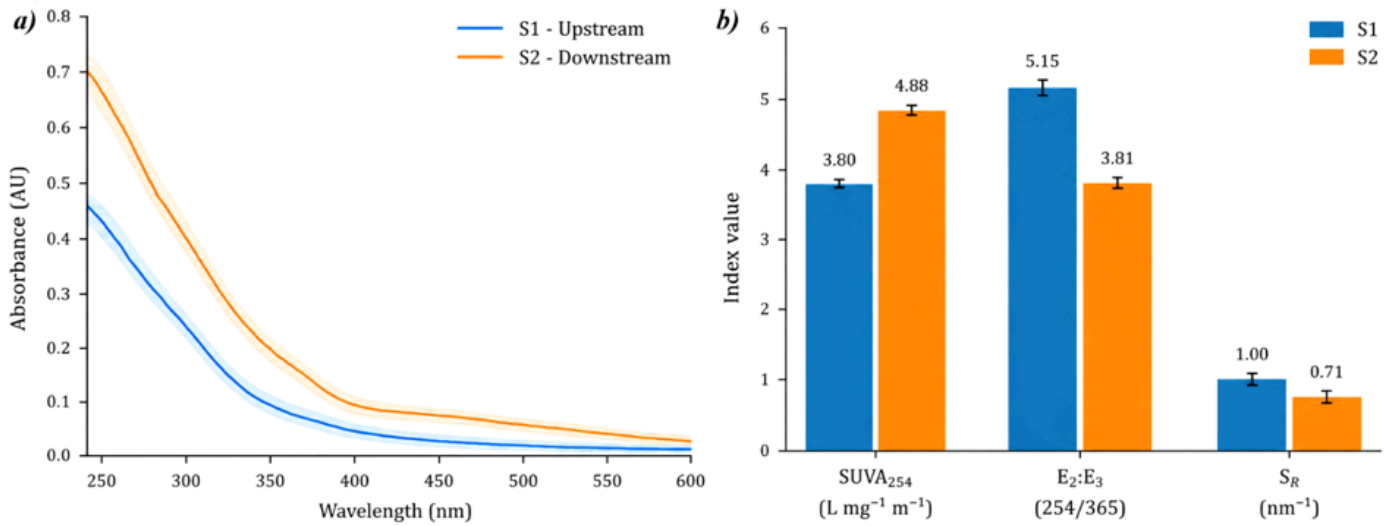


Figure 6

UV–Vis absorption characteristics (a) and derived optical indices of DOM (b) in river sections upstream and downstream of the wastewater discharge.