UNDER PEER REVIEW

Response of the Diatom *Melosira varians* to Domestic Wastewater Effluents

Abstract

This study investigates the physiological and ecological responses of the benthic diatom *Melosira varians* to varying concentrations of domestic wastewater effluents under laboratory conditions. Effluent dilutions of 12.5%, 25%, and 50% were used to assess the species' capacity for nutrient removal, growth, morphological alterations, and pigment composition over a 10-day period. The results demonstrated remarkable phosphate removal efficiencies (≥97%) across all treatments and a progressive nitrate removal response, reaching up to 70.59% in the highest effluent concentration. Morphometric analysis revealed significant increases in cell width and length, particularly under moderate (25%) and high (50%) effluent exposures. Chlorophyll analysis indicated a predominance of chlorophyll c and a reduction of chlorophyll b and c in response to nutrient-rich conditions, suggesting a possible shift towards heterotrophic or mixotrophic metabolism. Multivariate analysis highlighted strong associations between cell morphology and biomass, while chlorophyll content showed no direct correlation with biomass accumulation. These findings demonstrate the metabolic flexibility and nutrient uptake potential of *M. varians*, reinforcing its applicability in sustainable wastewater treatment systems.

Keywords:

Diatoms; *Melosira varians*; Domestic effluents.

1. INTRODUCTION

Diatoms are dynamic microorganisms with rich diversity and detailed membrane design. They are the most dominating phytoplankton with an overall number of around 200,000 species having complex variability in dimensions and shapes [1, 2, 3].

Diatoms' distinctive characteristic compared to the phytoplankton community is their silica cell wall, known as a frustule. The silica shell protects them from negative environmental stress, giving them a competitive advantage [4]. When silicate is not a limiting factor, other microalgae are unable to compete with diatoms.

Patterns of benthic diatom communities are responsive to the nature of the physical and chemical characteristics of lotic systems [5, 6, 7]. They respond rapidly to degradation of water quality, often changing in both taxonomic composition and biomass where even slight contamination occurs [8, 5, 9, 10, 6, 7].

Additionally, diatoms can take up phosphorus faster than other algae, as well as all forms of nitrogen—such as nitrate, nitrite, urea, and ammonia [11].

Phosphorus uptake by benthic diatoms occurs in rapid and continuous episodes (in less than one hour), leading to an increase in the oxygenated layer of sediments and inhibiting phosphorus

release, which contributes to the purification of the water column [12].

In eutrophic lakes, it is not surprising that the most abundant diatoms in the benthic community are species with high nutrient assimilation strategies [13].

Melosira varians is very common in rivers and lakes. This species is considered to be tolerant of poor water quality, although it may occur in more pristine sites as well. This diatom is primarily found in benthic habitats, with cells joined in long filamentous chains. Both individual cells and filaments may become entrained in the plankton.

Melosira had a potential ability to cause the blooms in winter. In this study, the dominant species (or genera) *Melosira varians* is generally considered to be tolerant of low light and ice cover. Nutrient addition, it was shown, can increase the benthic diatom abundance in template rivers [14].

Our objective was to study the ability of the diatom *M. varians* to remove nitrogen and phosphorus from domestic effluents.

2. MATERIALS AND METHODS

2.1. Sampling and Isolation of Microalgae

Samples were collected during the fall-winter period of 2023. For this purpose, sediment was aspirated from different areas of an urban riverbed using a plastic syringe, until a significant amount of sediment was obtained according to standard collection techniques [15].

The samples were filtered on site using a 35 µm mesh sieve and transported in a cooler. One hundred milliliters of each sample were placed in sterile 500 ml bottles and brought to a final volume of 500 ml using sterilized river water. The bottles were kept under natural prevailing conditions at the site for two days, at a temperature of temperature (22 ± 1 ºC), illumination with a 16:8 photoperiod (2,800 lux). Subsequently, two 100 ml samples were taken from each bottle, placed into sterile 500 ml bottles, and brought to a final volume of 500 ml with f/4 culture medium (50% f/2 medium, Guillard, 1975, and 50% sterilized river water) for seven days.

Cell counts were also performed for each sample using a Neubauer haemocytometer (0.1 mm depth) to compare the quantity of algal cells and other microorganisms. The least-contaminated cultures were selected and filtered, 100 ml from each bottle were cultured and brought to a final volume of 500 ml with f/2 culture medium. The culture was purified through serial dilutions.

For taxonomic analysis of the strains, specific literature was consulted: Bellinger & Sigee (2010), Lee (2008), Guiry, M.D. & Guiry, G.M. (2025), *AlgaeBase*. World-wide electronic publication, National University of Ireland, Galway. Available at: http://www.algaebase.org.

The *Melosira varians* inocula were placed in 1-liter photobioreactors containing f/2 medium [16] to obtain sufficient biomass under controlled laboratory conditions: temperature (22 ± 1 ºC), illumination with a 16:8 photoperiod (2,800 lux), and daily provision of carbon dioxide for growth. Once the biomass was obtained, it was stored in a refrigerator and later reactivated to carry out the phycoremediation treatments.

2.2. Wastewater Sampling

The sampling of wastewater effluents was conducted in accordance with the standardized methods for the Examination

of Water and Wastewater [17]. Effluents were collected in 5-liter polypropylene containers. Once collected, samples were refrigerated at 4 °C for subsequent transportation to the laboratory.

The effluent was left to stand in darkness for approximately seven days to induce anaerobic conditions and sedimentation. This process aimed to prevent the proliferation of photoautotrophic organisms and the formation of protozoan cysts and helminth eggs (primary microalgal predators), while simultaneously promoting bacterial concentration [18].

An experimental design was implemented, comprising a control group and increasing concentrations of effluent (12.5%, 25%, and 50%), all inoculated with 1x10^6 cells/mL of the obtained biomass (Fig. 1). Each treatment and its respective controls were performed in triplicate over 10 days, using effluent and culture volumes as proposed by USEPA (1991).

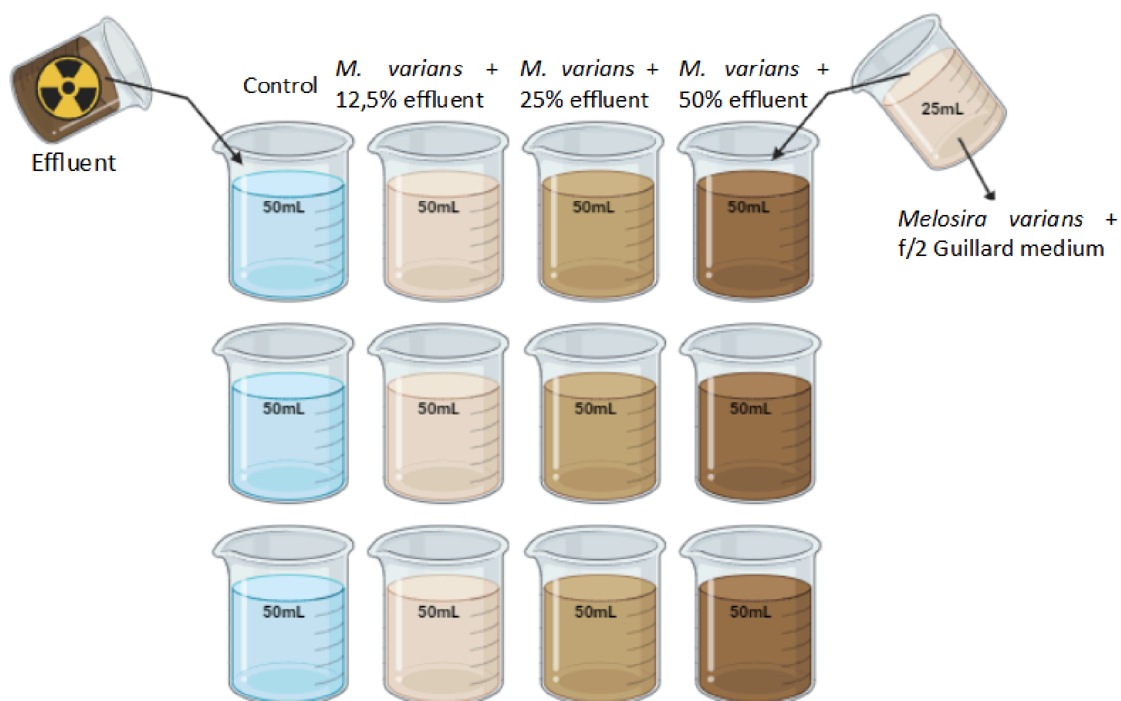

Fig. 1. Graphical Representation of the Phycoremediation Assay.

2.3. Determination of Phosphorus and Nitrogen in Phytoremediation Treatments

Phosphorus and nitrogen concentrations were determined before and after exposure to the various treatments. Considering that microalgae can assimilate nitrogen in the form of nitrates (NO3-), nitrites (NO2-), and ammonium (NH4+), and phosphorus as phosphate (PO43-), the percentage removal of these nutrients was recorded at the start (day 0), after 72 hours (day 3), and at the end (day 10). The following formulas were applied based on Lopez Espinoza (2022), with minor modifications for this experimental setup:

For Nitrates,

$$\left[\left(\frac{Nitrate\ (mg/L)\ \ Day\ 0 - Nitrate\ (mg/L)\ Day\ 10}{Nitrate\ (mg/L)\ Day\ 0}\right) x\ 100\right]$$

For Phosphates,

$$\left[\left(\frac{Phosphate\ (mg/L)\ Day\ 0 - Phosphate\ (mg/L)\ Day\ 10}{Phosphate\ (mg/L)\ Day\ 0}\right) x\ 100\right]$$

2.4. Cell Density Analysis

A 0.1 mL sample was taken from each replicate and placed on a microscope slide for observation under an optical microscope at 40x magnification. A Greek key pattern was used to traverse the slide, and individual cells within each filament were counted across the entire sample [19].

Cellular density was estimated by counting the total number of filaments per treatment (ft) in relation to the total number of filaments across all treatments (fT), calculating the relative frequency (Frt) as follows: [Frt = (ft / fT)] x 100. Similarly, the relative frequency of individual cells per treatment was estimated [20]. This data transformation into continuous variables allowed fulfillment of statistical assumptions [20].

2.5. Morphological Analysis

Photographs of 10 fields per sample were taken under a 40x optical microscope using the preparations described in section 2.4. These images were analyzed using ImageJ software [21], calibrated with a millimeter scale to measure individual cell dimensions. These measurements were later used for statistical analysis to compare between groups.

2.6. Chlorophyll Determination

Chlorophyll values were estimated at wavelengths of 430 nm and 664 nm for chlorophyll a, 460 nm and 647 nm for chlorophyll b, and 442 nm and 630 nm for chlorophyll c, following Larkum (2003).

The following formulas from APHA (2012) were used:

Chlorophyll a:
$[(11.85\ x\ 664\ nm)\ -(1.54\ x\ 647\ nm)\ -(0.08\ x\ 6$

Chlorophyll b:
$[(21.03\ x\ 647\ nm)\ -(5.43\ x\ 664\ nm)\ -(2.66\ x\ 630\ nm)\ ]$

Chlorophyll c:
$[(24.52\ x\ 630\ nm)\ -(7.6\ x\ 647\ nm)\ -(1.67\ x\ 664\ nm)\ ]$

2.7. Statistical Analyses

One-way ANOVA and Tukey's post hoc tests were used to compare nitrogen and phosphorus concentrations across treatments. The same statistical test was employed to evaluate algal growth among treatments. If data violated the assumptions of homogeneity of variances or normality, non-parametric Kruskal-Wallis tests with post hoc analysis were used. Additionally, principal component analysis (PCA) was performed to evaluate associations and responses of each analyzed variable to the treatments. Finally, t-tests were used to compare phosphorus and nitrogen removal between the two types of treated effluents [20].

3. RESULTS

3.1 Effluent Characterization

The following section presents the results of the physicochemical characterization of the effluent (Table 1), showing the initial values of phosphorus and nitrogen prior to the phycoremediation process.

Table 1. Data obtained from the determinations of physicochemical parameters of the urban effluent.

DETERMINATIONS	VALUES	USED METHOD
Turbidity - NTU	63	S.M. 4500-B
Suspended Solids (mg/L)	144.9	S.M. 2510-B
Conductivity (µS/cm)	750	S.M. 2540-B
Total Dissolved Solids – TDS (mg/L)	389	S.M. 2540-B
pH	7.01	S.M. 4500-B
Nitrate (mg/L)	28	S.M-4500-NO_3^- -E

Nitrite (mg/L)	0,16	S.M-4500-NO_2- -B
Ammonium (mg/L)	1.5	S.M- 4500-NH_4 H
Sulfates (mg/L)	45	S.M-4500-SO_4-2 -E
Chlorides (mg/L)	24.9	S.M. 4500-Cl-B
Phosphorus (mg/L)	2.28	S.M- 4500-P-E
Chemical Oxygen Demand – COD (mg/L O_2)	165.2	S.M. -5220-D
Biochemical Oxygen Demand – BOD (mg/L O_2)	143.1	S.M. -5210-B

3.2 Physicochemical Results of the Bioassays with Wastewater Effluents

3.2.1 Phosphate Determination

The results showed a remediation efficiency of 100%, 97.17%, and 99.17% for the treatment with *M. varians* in effluent dilutions of 12.5%, 25%, and 50%, respectively. These results are shown in Fig. 2

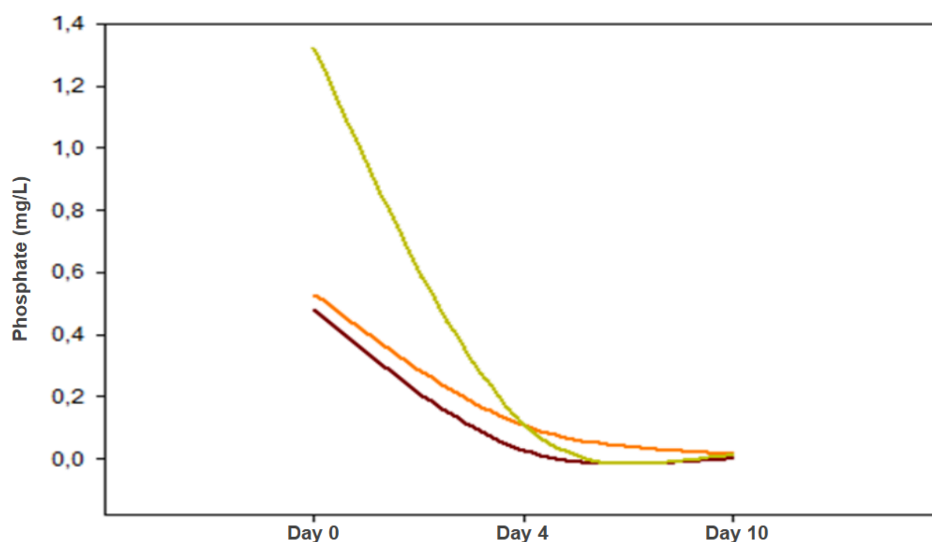

Fig. 2. Phosphorus removal by *Melosira varians* after 10 days. The treatments correspond to 12.5% (brown line), 25% (orange line), and 50% (green line) effluent dilution.

3.2.2 Nitrate Determination

The results indicated nitrate removal efficiencies of 29.41%, 41.18%, and 70.59% for the treatment with the microalga in effluent dilutions of 12.5%, 25%, and 50%, respectively.

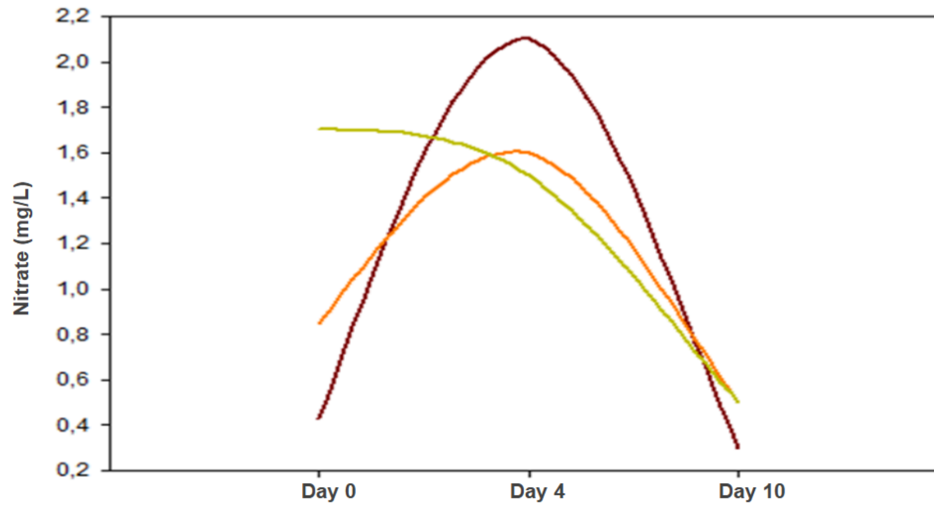

Fig. 3. Nitrogen removal by *Melosira varians* after 10 days. The treatments correspond to 12.5% (brown line), 25% (orange line), and 50% (green line) effluent dilution.

3.3 Cell Density: Determination of Algal Growth

The following results show the growth of *M. varians* after 10 days of treatment. It is worth noting that growth was assessed by measuring the number of cells and the number of filaments per treatment, as represented in Fig. 4. The ANOVA statistical results showed no significant differences between treatments and the control group ($p > 0.05$), despite a marked trend toward increased cell and filament numbers.

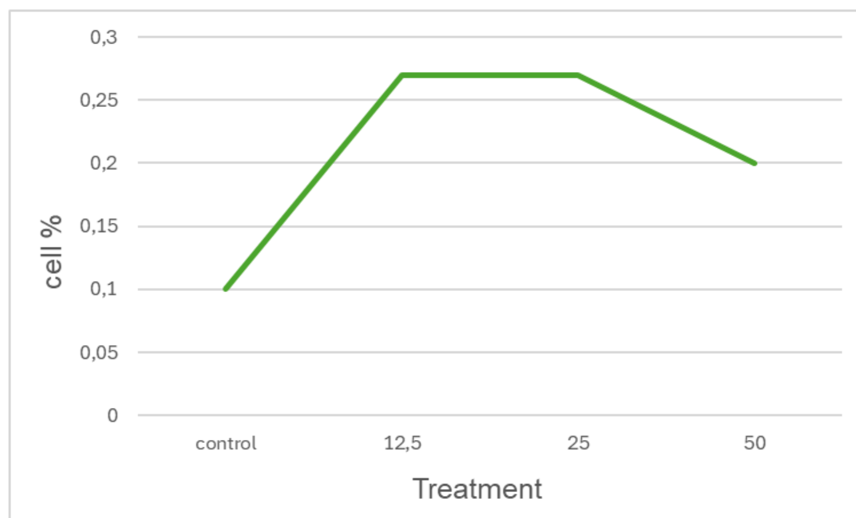

Fig. 4. Percentage of *M. varians* individuals after 10 days of exposure.

3.4 Cell Size: Determination of Cell Width and Length

3.4.1 Cell Width Determination

A continuación, se presentan los resultados del análisis del ancho celular evaluado en los controles y los tratamientos. Los análisis estadísticos demostraron que a los 10 días del tratamiento, el ancho de la microalga aumenta mientras más concentrado está el efluente ($p < 0,05$; Fig. 5).

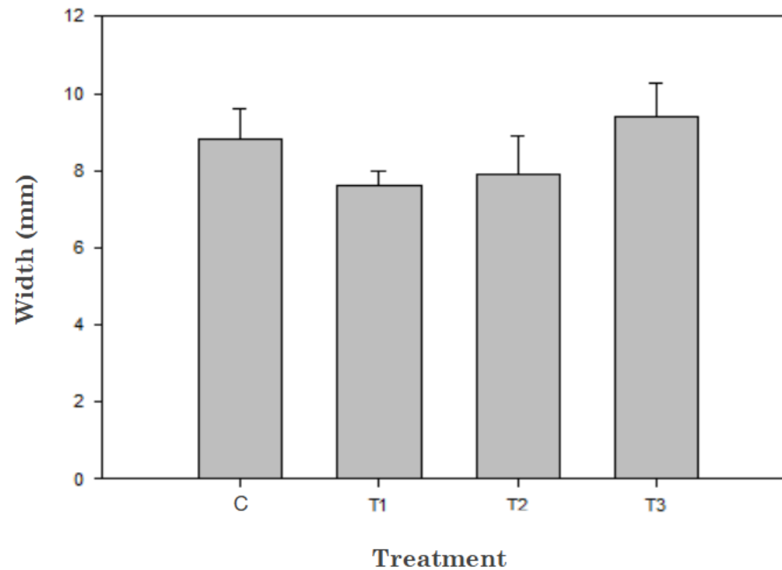

Fig. 5. Cell width of *Melosira varians* measured in the control group and the effluent treatments at 12.5% (T1), 25% (T2), and 50% (T3), obtained on the tenth day of the bioassay with the free-living microalga (A).

3.4.2 Cell Length Determination

The results of the cell length analysis in the controls and treatments are presented below. Cell length values are shown in Fig. 6, while photographs illustrating these cellular changes are shown in Fig. 7. Statistical analysis demonstrated that after 10 days of the "free-living" bioassay, the length of the microalga increased in the 50% and 12.5% effluent treatments and decreased in the 25% effluent treatment ($p < 0.05$; Fig. 6).

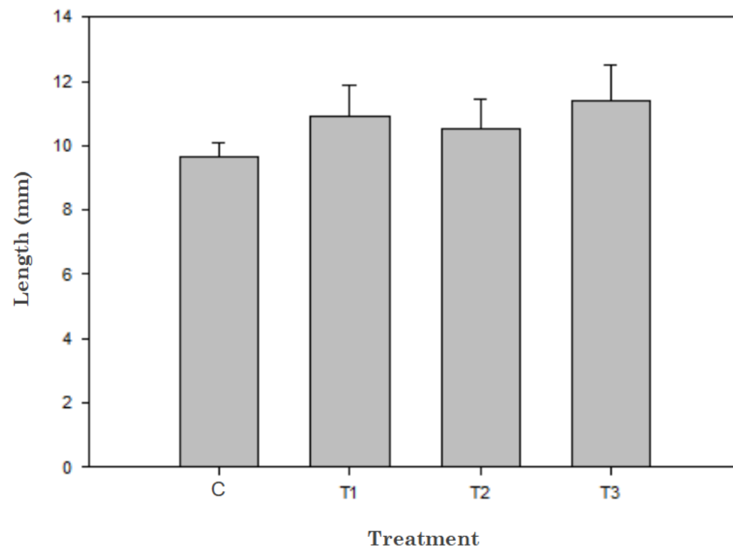

Fig. 6. Optical microscope images (40X) of *Melosira varians* filaments corresponding to the control (A) and effluent treatments at 12.5% (B), 25% (C), and 50% (D) after 10 days of exposure

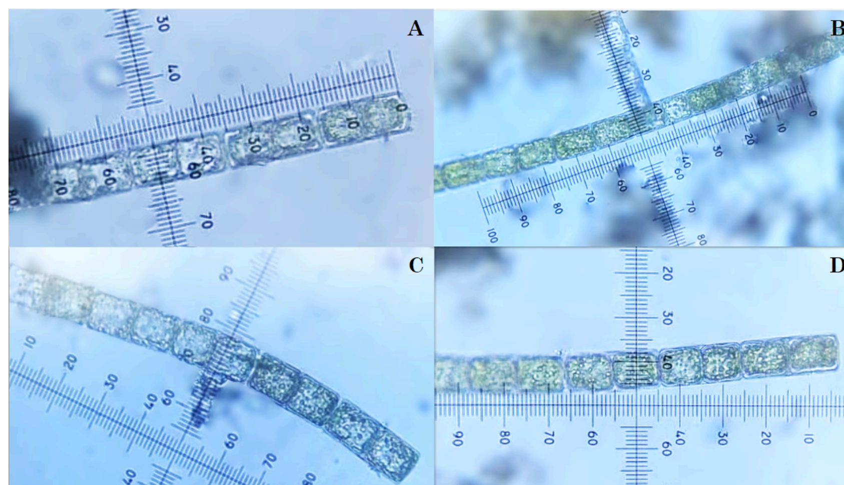

Fig. 7. Cell length of *Melosira varians* measured in the negative control group (CN) and the effluent treatments at 12.5% (T1), 25% (T2), and 50% (T3), obtained on the tenth day of microalga treatment (A).

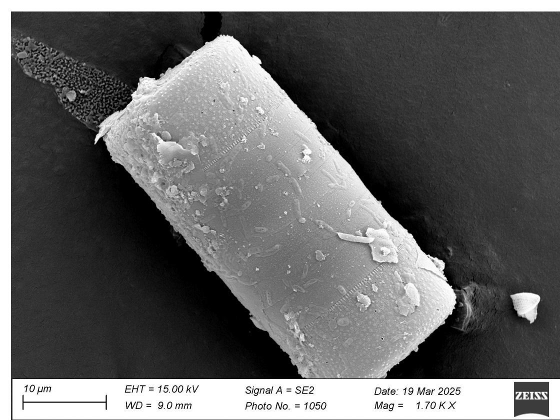

Fig. 8. Scanning electron micrograph (SEM) of *Melosira varians*, showing the typical cylindrical shape of the genus.

3.5 Chlorophyll Determination

The results of the chlorophyll a, b, and c analyses in the controls and phycoremediation treatments are shown in Fig. 9.

After 10 days of phycoremediation, statistical analysis indicated that chlorophyll a levels showed no significant differences between the control and treatment groups ($p > 0.05$; Fig. 9). However, a significant decrease in chlorophyll b was observed in the 25% and 50% effluent treatments compared to the other groups ($p < 0.05$; Fig. 9). Similarly, for chlorophyll c, a significant reduction was detected in the same effluent concentrations compared to the other treatments ($p < 0.05$; Fig. 9). Finally, as observed in Fig. 9, chlorophyll c was the most abundant pigment across the various treatments, compared to chlorophyll a and b.

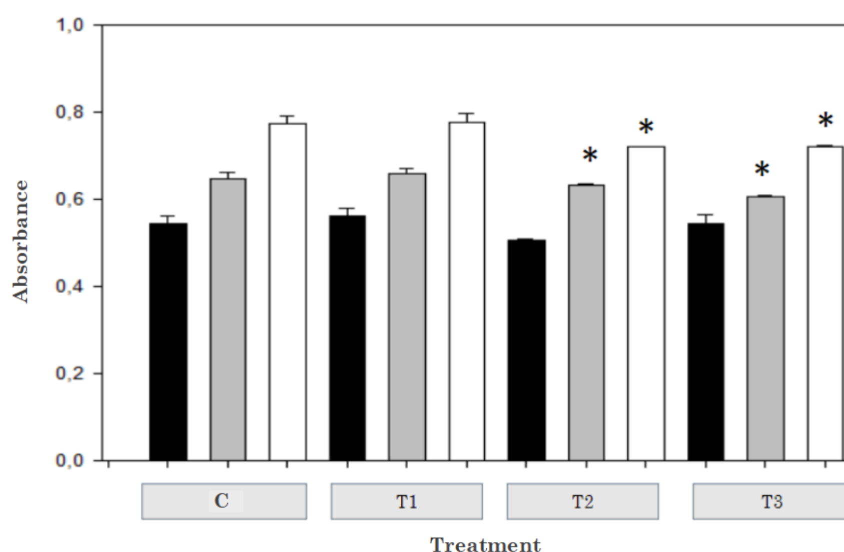

Fig. 9. Chlorophyll *a* (black bars), *b* (gray bars), and *c* (white bars) content in *Melosira varians* from the control (C) and the effluent treatments at 12.5% (T1), 25% (T2), and 50% (T3) after 10 days of exposure. * indicates statistically significant differences compared to the control and other treatments for the respective chlorophyll.

3.6. Multivariate Analysis: Principal Component Analysis

A positive association was observed between chlorophyll a and phosphate levels, as well as with chlorophylls b and c. Additionally, chlorophylls b and c showed a strong positive correlation with each other. Moreover, no correlation was found between chlorophyll content and biomass. This suggests a potential shift of the algae toward heterotrophic metabolism and nutrient uptake, where phosphorus is used for photosynthesis rather than biomass production. Conversely, biomass showed a positive correlation with cell width and length, highlighting the relevance of these measurements for estimating biomass in diatoms, as

opposed to relying solely on chlorophyll content.

Furthermore, nitrate levels showed negative correlations with all analyzed biological parameters, suggesting a possible detrimental effect.

4. DISCUSSION AND CONCLUSION

Under stress conditions, diatoms such as *Melosira varians* demonstrate the ability to assimilate significant amounts of nutrients, particularly nitrogen and phosphorus, which are essential for protein synthesis—accounting for 45–60% of microalgal dry weight [22]. When nutrients are abundant, these species commonly store energy in the form of carbohydrates or lipids, which serve as alternative energy sources during periods of darkness when photosynthesis is not feasible. This metabolic flexibility is critical for survival under fluctuating environmental conditions.

Diatoms are generally considered autotrophic organisms, relying primarily on light within the visible spectrum to conduct photosynthesis. However, recent studies have identified the presence of carbohydrate and amino acid transport mechanisms across several diatom species, suggesting a potential for facultative heterotrophy [23]. In benthic species like *M. varians*, which are often located in microenvironments with limited light but abundant decomposing organic matter, the reliance on heterotrophic metabolism may be more common. This would explain their capacity to persist in dark conditions for extended periods [23, 24].

These characteristics—efficient nutrient assimilation, metabolic flexibility, and resistance to pollutants due to their silica frustules—make diatoms exceptionally well-suited for phycoremediation. *Melosira varians*, demonstrated notable efficacy in removing contaminants from domestic wastewater effluents, reinforcing its utility as a bio-remediator in aquatic systems.

With respect to pigment composition, the results of this study confirmed that *M. varians* possesses a higher proportion of chlorophyll c relative to chlorophylls a and b, consistent with diatom pigment profiles. Furthermore, the significant reduction in chlorophyll b and c in treatments with elevated nutrient loads may indicate a metabolic shift from autotrophy toward heterotrophy. In nutrient-stressed environments—particularly under nitrogen or silica limitation—diatoms rely on stored lipids as energy sources and suppress photosynthetic activity [25, 26]. This also accounts for the observed increase in cell size, which may result from the accumulation of lipid droplets within the cytoplasm.

The high nitrogen removal efficiency observed in the 50% effluent treatment (~70%) likely led to early nutrient depletion, placing the microalgae under physiological stress. This may have triggered a shift in metabolic strategy, consistent with a mixotrophic response. Our findings support the hypothesis that *M. varians* is capable of adjusting its nutritional mode depending on environmental conditions. To our knowledge, this is the first report suggesting potential mixotrophy in *M. varians*.

Moreover, the decline in cell density observed under nutrient-limited conditions may be attributed to the initiation of a resting stage, possibly auxospore formation. This is a common strategy among diatoms to withstand unfavorable environments and may also contribute to increased cell volume [25, 26].

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