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Bioremediation of Medical Center Wastewater Using Oscillatoria Splendida and Microcystis Aeruginosa Algae Species

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This study investigates the efficacy of a dual algal treatment system using Oscillatoria splendida and Microcystis aeruginosa for the remediation of medical center wastewater. The research evaluated the performance of these cyanobacterial species in removing key pollutants, including organic matter, nutrients, and heavy metals, over a 14-day treatment period. Wastewater samples at 20%, 40%, and 60% dilutions were treated separately with each algal species. The results demonstrated significant reductions in electrical conductivity, total dissolved solids, phosphates, nitrates and heavy metals (Zn, Cu, Ni) across all dilutions. O. splendida was superior in the reduction of conductivity and removing certain heavy metals, while M. aeruginosa enhanced the efficiency in removing nitrate and zinc sequestration. Both were optimal at different wastewater dilutions for many pollutants, showing the potential for synergistic impacts in a combined treatment approach. According to the statistical analysis, there were significant differences between the two species' efficiencies, with strong correlations between algal biomass and pollutant removal. This study is insightful on applying algal-based systems for medically treating wastewater and suggests avenues to optimize treatment conditions in future large-scale applications.

Keywords: Medical Wastewater Treatment, Oscillatoria Splendida, Microcystis Aeruginosa, Cyanobacteria, Bioremediation, Heavy Metal Removal, Nutrient Removal, Dual Algal System.

Introduction

Wastewater management has beene a critical global concern, in particular in medical facilities (Bijekar et al., 2022), where effluents usually have complex mixture of pollutants (Khan et al., 2021):pharmaceuticals, pathogenic microorganisms and heavy metals and (Emadikhiav et al., 2024). The inadequately treated medical wastewater discharge into natural water causes poses significant risks to environmental and public health (Tariq & Mushtaq, 2023; Wu et al., 2024). Recently, the interest in developing sustainable and eco-friendly treatment methodologies to has grown to address this challenge (Hossein et al., 2023; Sathya et al., 2023).

Algal-based wastewater treatment has become promising (Li et al., 2022) which offers a dual benefit of the removal of pollutants and the production of biomass (Razaviarani et al., 2023). Cyanobacteria, is one of the diverse arrays of algal species, also known as blue-green algae garnering a particular attention because of their robust nature and diverse metabolic capabilities (Kukkar, 2023; Tan et al., 2022). Two species are of interest: Oscillatoria splendida and Microcystis aeruginosa with proved potential in bioremediation processes.

Oscillatoria splendida is filamentous cyanobacterium (Ram & Paul, 2024) and can thrive in many environmental conditions (Delilah et al., 2023). Also, its filamentous structure offers a high surface area for nutrient uptakes and adsorption of pollutant, which makes it potential to treat wastewater (Sadvakasova et al., 2021). Yet, Microcystis aeruginosa is unicellular cyanobacterium extensively researched for its role in harmful algal blooms (He et al., 2023; Reignier et al., 2023). Recently, research has shown its ability to remove nutrient and heavy metal sequestration indicating its utility in controlled wastewater treatment systems (Al-Amin et al., 2021)

The dual treatment efficacy approach in the medical center wastewater is still largely unexplored (Bhushan et al., 2020; Reddy et al., 2021) and medical wastewater shows challenges because of its variable composition, such as the presence of antibiotics, disinfectants and other pharmaceutical residues (Emadikhiav et al., 2024; Kataria et al., 2024). Understanding Oscillatoria splendida and Microcystis aeruginosa in this matrix is crucial to assess their potential as viable treatment option.

This work bridges this knowledge gap by the investigation of the dual treatment of the medical center wastewater using Oscillatoria splendida and Microcystis aeruginosa. It evaluates the efficiency of these algal species in removing key pollutants sich as organic matter, nutrients and heavy metals. Also, it explores the temporal dynamics of the treatment and the potential synergistic effects of both species at the same time.

Materials and Methods

Study Area and Sample Collection

This work used wastewater samples from the treatment plant of Al-Ramadi Teaching Hospital for Gynecology and Pediatrics, in the southwest of Ramadi city, Iraq. The hospital's wastewater treatment plant operates with the discharge capacity of 350 m^3/h/day and the treatment process includes aeration, physical treatment with filters and UV irradiation before the final effluent is discharged directly into the nearby river.

Wastewater samples were collected from the final holding tank just prior to river discharge. Sampling was performed using clean, sterilized 5-liter plastic containers. The containers were first rinsed with the wastewater before sample collection. Samples were immediately transported to the laboratory for physical, chemical and biological analyses.

For experimental treatments, the collected wastewater was transferred to 2.5-liter transparent glass bottles and the portion of the samples was autoclaved at 121°C for 15 minutes to eliminate microorganisms for the sterile treatments. The samples were then diluted to three concentrations (20%, 40%, and 60%) using sterile distilled water to assess treatment efficacy across range of pollutant loads.

Algal Species Selection and Cultivation

Two cyanobacterial species, Oscillatoria splendida and Microcystis aeruginosa, were selected for this study based on their prevalence in local aquatic ecosystems and their potential for bioremediation. These species were isolated from the banks of the Euphrates River near Ramadi, Iraq. Taxonomic identification was performed using standard morphological criteria (Willey et al., 2014).

The isolates were initially cultured in BG11 medium, which provides a complete nutrient profile for cyanobacterial growth (Stanier et al., 1971). The BG11 medium was prepared according to the standard recipe, containing macronutrients such as $NaNO_3$, K_2HPO_4, $MgSO_4·7H_2O$, $CaCl_2·2H_2O$ and micronutrients including various trace metals.

Cultures were maintained in 250 mL Erlenmeyer flasks containing 100 mL of medium. Incubation conditions were set at 25 ± 2°C under continuous illumination of 260 $\mu E/m^2/s$ provided by cool white fluorescent lamps, to ensure culture viability. Also, subculturing was performed every 14 days by transferring 10 mL of culture to fresh medium.

For experimental, the algal strains were gradually acclimated to wastewater. This n process incrementally replaced the BG11 medium with filter-sterilized wastewater over five successive subcultures. The las acclimation had 100% sterilized wastewater which ensures that the algal strains were well-adapted to the experimental conditions before commencing treatment trials.

Experimental Setup

The experimental design evaluateي the efficacy of Oscillatoria splendida and Microcystis aeruginosa in the treatment of hospital wastewater at many dilutions. The setup was planned for ensuring reproducibility and result statistical validity .

Dilution Series

For the treatment efficiency over many pollutant concentrations, we prepared dilution series of the collected wastewater:

20% wastewater with 80% sterile distilled water
40% wastewater with 60% sterile distilled water
60% wastewater with 40% sterile distilled water

We chose these dilutions to represent a range of pollutant loads that might be encountered in real-world scenarios. Each in triplicate for ensuring statistical robustness. Sterile glass bottles of 2.5 L capacity of 500 mL of each diluted wastewater sample was used.

Treatment with Oscillatoria Splendida

To treatm by Oscillatoria splendida, the following protocol was used:

Inoculum preparation: O. splendida cultures were grown to mid-exponential phase in BG11 medium and the optical density at 680 nm (OD680) was measured to ensure consistent inoculum density across replicates.

Inoculation: 100 mL of O. splendida culture (OD680 ≈ 0.5) was added to each 500 mL of diluted wastewater sample.

Incubation conditions: The inoculated samples were incubated under the following conditions:

Temperature: 25 ± 2°C, light intensity: 2500 lux, photoperiod: 16:8-hour light: dark cycle, duration: 14 days and sampling: 50 mL aliquots were collected from each replicate at day 0, 7 and 14 for analysis of physicochemical and biological parameters, aeration. Gentle aeration was provided using aquarium air pumps fitted with sterile filters to maintain oxygen levels and prevent settling.

Treatment with Microcystis Aeruginosa

The treatment protocol for Microcystis aeruginosa followed a similar structure to that of O. splendida:

Inoculum preparation: M. aeruginosa cultures were grown to mid-exponential phase in BG11 medium and the optical density at 730 nm (OD730) was measured to ensure consistent inoculum density.

Inoculation: 100 mL of M. aeruginosa culture (OD730 ≈ 0.5) was added to each 500 mL of diluted wastewater sample.

Incubation conditions: The same conditions as described for O. splendida were maintained:

Temperature: 25 ± 2°C, light intensity: 2500 lux, photoperiod: 16:8 hour light:dark cycle, duration: 14 days, sampling: 50 mL aliquots collected from each replicate at day 0, 7 and 14 for analysis of physicochemical and biological parameters, aeration: Gentle aeration was provided using aquarium air pumps fitted with sterile filters to maintain oxygen levels and prevent settling.

Mixing: Gentle daily mixing was performed to prevent cell aggregation and ensured uniform distribution of nutrients.

For both treatments, control samples consisting of diluted wastewater without algal inoculation were maintained under identical conditions to account for any changes not attributable to algal activity.

Throughout the experiment, careful measures were taken to maintain aseptic conditions and prevent contamination. All glassware and equipment were sterilized before handling the cultures in a laminar flow hood.

This experimental setup comprehensivly evaluated the individual effects of O. splendida and M. aeruginosa on the hospital wastewater treatment in many concentrations with a solid foundation for subsequent data analysis and interpretation.

Analytical Methods

The following analytical methods assessed the physicochemical and biological parameters of the wastewater samples pre- ans post treatment. In addition, wastewater samples from the hospital treatment plant were comprehensivly, for physicochemical and biological analysed for establishing a baseline characteristic. All analyses were performed in triplicate according to standard methods (APHA) (Baird et al., 2017):

pH and Electrical Conductivity (EC): pH was calculated by a calibrated pH meter (Hanna Instruments, Germany) and EC by a conductivity meter (WTW, Germany) and expressed in mS/cm.

Total Dissolved Solids (TDS) and Total Suspended Solids (TSS)were quantified gravimetrically by 2540 C and 2540 D, respectively, as in the Standard Methods for the Examination of Water and Wastewater (APHA, 2017).
Biochemical Oxygen Demand (BOD5): The 5-day BOD test using method 5210 B (APHA, 2017) was done. Samples were incubated at 20°C for 5 days, and the difference in dissolved oxygen was measured using a DO meter.
Chemical Oxygen Demand (COD): COD was determined using the closed reflux, colorimetric method (5220 D, APHA, 2017). A COD reactor (Lovibond, MD200) was used for sample digestion, followed by spectrophotometric measurement at 600 nm.

Nutrients

Nitrate (NO_3-N): Measured using the cadmium reduction method (4500-$NO3^-$ E).
Nitrite (NO_2-N): Analyzed by the colorimetric method (4500-$NO2^-$ B).
Phosphate (PO_4-P): Determined using the ascorbic acid method (4500-P E).
Sulfate (SO_4): Quantified turbidimetrically (4500-$SO42^-$ E).
All nutrient analyses were performed using a UV-Vis spectrophotometer (Shimadzu UV-1800, Japan).
Heavy Metals: Zinc (Zn), Copper (Cu), and Nickel (Ni) were analyzed using flame atomic absorption spectrophotometry (Perkin Elmer AAnalyst 400, USA) after acid digestion of the samples (3030 E).
Total Bacterial Count (TBC) was determined using the pour plate method on nutrient agar. Samples were serially diluted, plated, and incubated at 37°C for 48 hours before colony counting.
Algal Biomass was monitored by measuring the optical density at 680 nm for Oscillatoria splendida and 730 nm for Microcystis aeruginosa using a spectrophotometer. Dry weight was also determined gravimetrically by filtering 10 mL of culture through pre-weighed 0.45 µm filters, drying at 105°C for 24 hours, and reweighing.

Statistical Analysis

Statistical analyses were conducted using IBM SPSS Statistics version 26.0 (Firdaus, 2021), the following statistical methods were employed:
Descriptive Statistics: Mean values and standard deviations were calculated for all measured parameters across different treatments and time points.
Normality Test: The Shapiro-Wilk test was used to assess the normality of data distribution for each parameter.
Analysis of Variance (ANOVA): Two-way ANOVA was performed to evaluate the effects of treatment type (Oscillatoria splendida, Microcystis aeruginosa, and control) and wastewater concentration (20%, 40%, and 60%) on the measured parameters and the significance level was set at $p < 0.05$.
Post-hoc Tests: Tukey's Honest Significant Difference (HSD) test was conducted for pairwise comparisons when ANOVA results showed significant differences.
Correlation Analysis: We calculated Pearson's correlation coefficient to examine relationships between different parameters, particularly between algal growth and pollutant removal efficiencies.
Removal Efficiency Calculation: We calculated the removal efficiency for each parameter using the following formula:
Removal Efficiency (%) = [(Initial Concentration - Final Concentration) / Initial Concentration] × 100
Graphical Representation: GraphPad Prism 8.0 was used to create graphs illustrating the trends in parameter changes over time and across different treatments.
Power Analysis: post-hoc power analysis was conducted to ensure that the sample size provided adequate statistical power ($\beta \geq 0.8$) for detecting significant effects.
All statistical analyses were performed with 95% confidence interval and results were considered significant at $p < 0.05$.

Results and Discussion

Initial Wastewater Characteristics

The physicochemical and biological characteristics of the raw wastewater from the hospital treatment plant were analyzed to establish baseline conditions prior to algal treatment. Table 1 presents the mean values and standard deviations of the parameters measured in triplicate.

Table 1: Initial Characteristics of Hospital Wastewater (N=3).

Parameter	Unit	Mean ± SD
pH	-	7.72 ± 0.48
EC	mS/cm	333.00 ± 87.51
TDS	mg/L	1649.67 ± 415.19
TSS	mg/L	168.67 ± 60.70
PO_4-P	mg/L	44.77 ± 21.26
SO_4	mg/L	133.00 ± 22.34
NO_3-N	mg/L	2.33 ± 0.59
NO_2-N	mg/L	27.83 ± 0.83
BOD5	mg/L	232.00 ± 90.83
COD	mg/L	287.00 ± 180.60
TBC	CFU/mL	4.77 × 10^6 ± 1.08 × 10^6
Zn	mg/L	0.36 ± 0.00
Cu	mg/L	0.53 ± 0.00
Ni	mg/L	0.41 ± 0.00

The hospital wastewater exhibited moderate to high levels of contamination across various parameters and the pH was slightly alkaline at 7.72 ± 0.48, which falls within the typical range for domestic and hospital wastewaters. Electrical conductivity (EC) and total dissolved solids (TDS) were notably high at 333.00 ± 87.51 mS/cm and 1649.67 ± 415.19 mg/L, respectively, indicating a significant presence of dissolved ions and salts. These elevated levels were attributed to the diverse chemical compounds used in hospital settings, including disinfectants, pharmaceuticals, and diagnostic agents.
Total suspended solids (TSS) were calculated at 168.67 ± 60.70 mg/L, confirming typical hospital effluents and suspended matter because of many sources: organic debris, microorganisms and inorganic particles.
Nutrient concentrations were found to be substantial, with phosphate (PO_4-P) levels at 44.77 ± 21.26 mg/L and sulfate (SO_4) at 133.00 ± 22.34 mg/L. Nitrogen species were also present in significant amounts, with nitrate (NO_3-N) at 2.33 ± 0.59 mg/L and nitrite (NO_2-N) at 27.83 ± 0.83 mg/L. These high nutrients are widespread in hospital wastewaters because of human waste, cleaning products, and various medical compounds.

The wastewater organic load was big, as the BOD5 and COD values of 232.00 ± 90.83 mg/L and 287.00 ± 180.60 mg/L, respectively shows. The BOD5/COD ratio of approximately 0.81 suggests that a significant portion of the organic matter is biodegradable which is favorable for biological treatment processes.

Big total bacterial count (TBC) of $4.77 \times 10^6 \pm 1.08 \times 10^6$ CFU/mL made Microbial contamination clear, stressing the need for effective disinfection for the mitigation of potential health risks of pathogenic microorganisms.

There were few detectable feavy metal concentrations , with zinc (Zn), copper (Cu), and nickel (Ni) at 0.36 ± 0.00 mg/L, 0.53 ± 0.00 mg/L, and 0.41 ± 0.00 mg/L, respectively, while not alarmingly high. Theywarrant attention because of the potential for bioaccumulation and long-term environmental effects.

The characterization of the hospital wastewater reveals complex effluent with multiple contaminants that require treatment before discharge. Then, the high nutrient content and the biodegradable organic matter suggest that biological treatment methods, such as algal remediation, are particularly effective with heavy metals, albeit in low concentrations. This indicates the need for a treatment process capable of addressing both organic and inorganic pollutants.

Treatment Efficiency of Oscillatoria Splendida

The treatment of the medical center wastewater using Oscillatoria splendida revealed complex dynamics in the removal of various water quality parameters. Each parameter exhibited unique patterns of change over the treatment period, suggesting intricate interactions between the cyanobacterium and the wastewater components.

The treatment efficiency of Oscillatoria splendida in remediating hospital wastewater was evaluated over 14-day period. The experiment was conducted using three different dilutions of wastewater (20%, 40%, and 60%) to assess the algae's performance under varying pollutant concentrations. Key water quality parameters were monitored at days 0, 7 and 14 to track the progress of treatment.

The treatment of hospital wastewater using Oscillatoria splendida resulted in significant changes to various physicochemical parameters. The impact of O. splendida on EC was particularly notable, as illustrated in Figure 1.

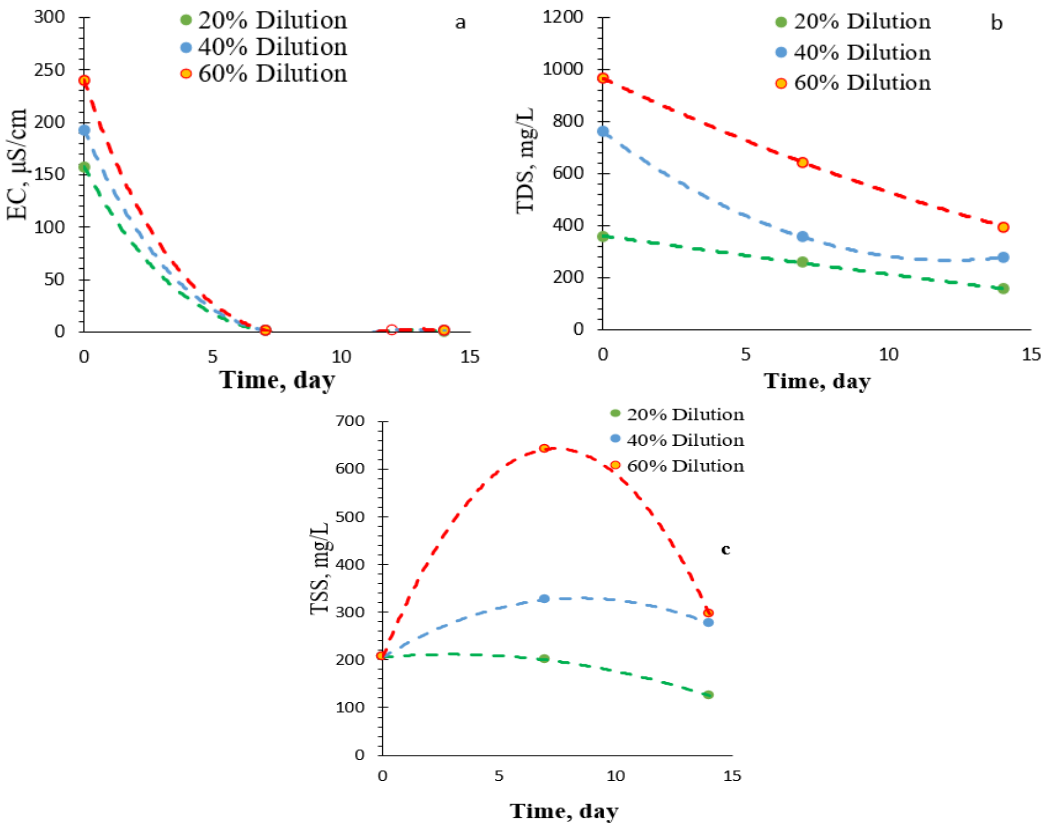

Figure 1: Changes In: A) Electrical Conductivity, B) Total Dissolved Solids, C) Total Suspended Solids, During Treatment with O. Splendida.

O. splendida exhibited exceptional efficiency in reducing electrical conductivity (EC), (Figure 1, a). At 20% wastewater dilution. So, it achieved a staggering 99.4% reduction in EC by day 7, further improving to 99.5% by day 14. This performance significantly outpaced that of M. aeruginosa, which managed a 38.6% reduction by day 7 and 46.8% by day 14 at the same dilution,

Total dissolved solids (TDS) removal efficiencies mirrored the EC trends, (Figure 1, b). O. splendida demonstrated peak efficiency at 40% dilution, achieving a 53.3% reduction by day 7 and improving to 63.5% by day 14. M. aeruginosa showed a comparable efficiency, with a

53.3% reduction by day 7 and 63.6% by day 14 at the same dilution. The alignment of TDS and EC removal efficiencies underscores the algae's capacity to effectively eliminate dissolved ionic species from the wastewater. The behavior of TSS throughout the treatment process presents an intriguing scenario, Figure 1, c. At 20% dilution. Also, a modest removal efficiency of 39.3% was observed after 14 days. However, the 40% and 60% dilutions showed apparent increases in TSS concentrations, resulting in negative removal efficiencies. This counterintuitive result attributed to several factors: Biomass production, floc formation, cell lysis and debris and extracellular polymeric substances (EPS). The observed increase in TSS at higher dilutions underscores the complexity of using biological agents in wastewater treatment and highlights the need for careful consideration of biomass management in such systems.

The nutrient removal capacity of O. splendida was evaluated by monitoring changes in phosphate (PO_4), nitrate (NO_3), sulfate (SO_4) and nitrite (NO_2) concentrations at different wastewater dilutions (20%, 40%, and 60%) over 7 and 14 days of treatment, Figure 2.

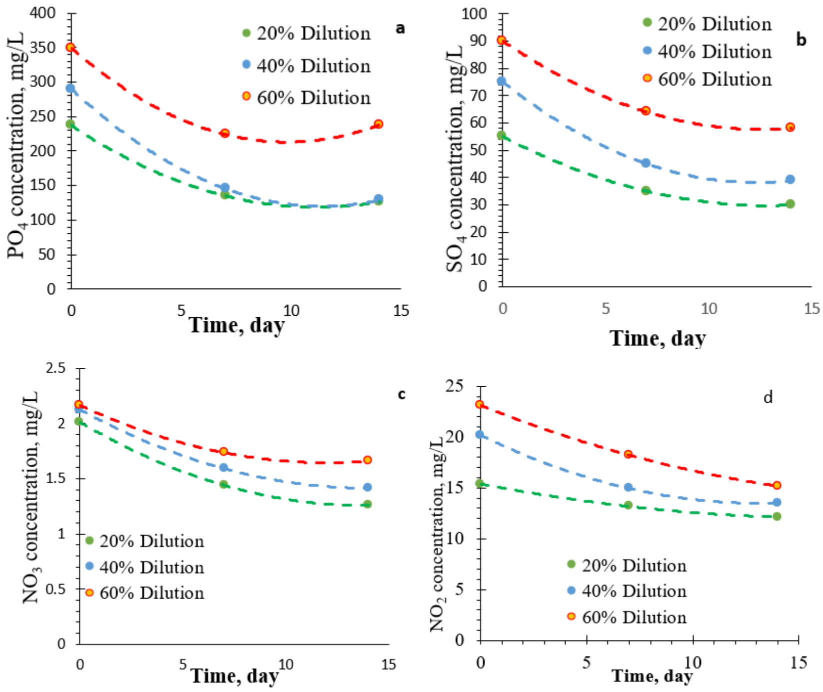

Figure 2: Changes In Concentration of A) Phosphate, B) Sulfate, C) Nitrate, D) Nitrite, During Treatment with O. Splendida.

Phosphate removal efficiency exhibited a marked dependence on the initial concentration and treatment duration, (Figure 2, a). At 20% dilution. O. splendida demonstrated its highest phosphate removal efficiency, achieving a 43.0% reduction by day 7, which further improved to 46.7% by day 14. The 40% dilution showed similar trend, with a 49.9% reduction by day 7, marginally increasing to 55.5% by day 14. However, the 60% dilution presented a more complex pattern, with an initial 35.8% reduction by day 7, followed by a slight decrease in efficiency to 31.8% by day 14. This non-linear response at higher concentrations suggests a potential threshold effect, where excessive phosphate levels impede the algae's removal capacity.

The temporal dynamics of phosphate removal efficiency indicate a rapid initial uptake followed by a plateau phase and most of the phosphate reduction occurred within the first 7 days, with only modest improvements observed in the subsequent week. This pattern implies that O. splendida's phosphate removal mechanisms approach saturation relatively quickly, particularly at lower dilutions.

The removal of sulfate (SO_4) by Oscillatoria splendida exhibited a nuanced pattern across different wastewater dilutions, (Figure 2, b). Initial SO_4 concentrations ranged from 55 mg/L at 20% dilution to 90 mg/L at 60% dilution. After 14 days of treatment, removal efficiencies reached 45.5%, 48.0%, and 35.6% for 20%, 40%, and 60% dilutions, respectively. Interestingly, the 40% dilution demonstrated the highest removal efficiency, suggesting an optimal concentration for SO4 uptake or reduction by O. splendida. The lower efficiency at 60% dilution indicates the inhibition of sulfate-reducing mechanisms at higher concentrations, possibly due to increased osmotic stress or altered metabolic pathways. The moderate removal rates across all dilutions imply that O. splendida possesses consistent, albeit limited, capacity for sulfate reduction. This capability could be attributed to the assimilatory sulfate reduction pathway, where sulfate is incorporated into organic compounds, or into the potential presence of sulfate-reducing bacteria in the microbial community associated with O. splendida.

Nitrate removal efficiency displayed a distinct trend across the dilution spectrum, (Figure 2, c) andthe 20% dilution exhibited the highest removal efficiency, reaching 37.5% by day 14. An inverse relationship between dilution and removal efficiency was observed, with the 60% dilution yielding the lowest efficiency at 23.4%. This trend suggests that O. splendida's nitrate removal mechanisms more effective in relatively concentrated wastewater environments.

The temporal progression of nitrate removal efficiency revealed a gradual, sustained process. Substantial reductions were achieved within the first week, with continued improvements, albeit at a diminished rate. During the second week, at 20% dilution, nitrate removal efficiency increased from 28.3% on day 7 to 37.5% by day 14. This pattern indicates that extended treatment durations yield further improvements in nitrate removal, though with diminishing returns over time.

Nitrite removal efficiencies varied across dilutions, with O. splendida showing increasing efficiency at higher wastewater concentrations. The alga achieved removal efficiencies of 21.2%, 33.0%, and 34.2% for 20%, 40%, and 60% dilutions, respectively. This trend suggests that the nitrite removal mechanisms of O. splendida are more effective at higher initial concentrations, possibly due to enhanced enzyme activity or adaptation to elevated nutrient levels, (Figure 2, d).

Both BOD5 and COD showed substantial reductions across all dilutions, Figure 3, indicating effective removal of biodegradable and oxidizable organic matter by O. splendida. The removal efficiencies for BOD5 ranged from 46.8% to 78.1%, while COD removal efficiencies spanned from 67.9% to 79.5%.

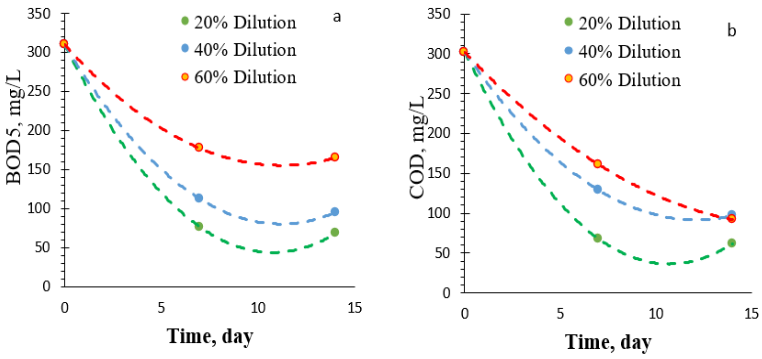

Figure 3: Changes in Concentration of a) BOD5, b) COD, During Treatment with O. Splendida.

Interestingly, both parameters exhibited higher removal efficiencies at lower dilutions (20% and 40%) compared to the 60% dilution. This trend suggests that O. splendida more effective at treating more concentrated wastewater in terms of organic matter removal. Several mechanisms could contribute to this observation: Enhanced metabolic activity, Co-metabolism, Photosynthetic oxygenation. The parallel trends observed in BOD5 and COD removal efficiencies suggest that O. splendida's treatment process affects both readily biodegradable and more resistant organic compounds similarly.

Heavy metal contamination in wastewater, particularly from medical centers, poses significant environmental and health risks and traditional treatment methods often involve costly and energy-intensive processes. Figure 4 presents the concentrations of zinc, copper and nickel at different dilutions of medical center wastewater treated with Oscillatoria splendida.

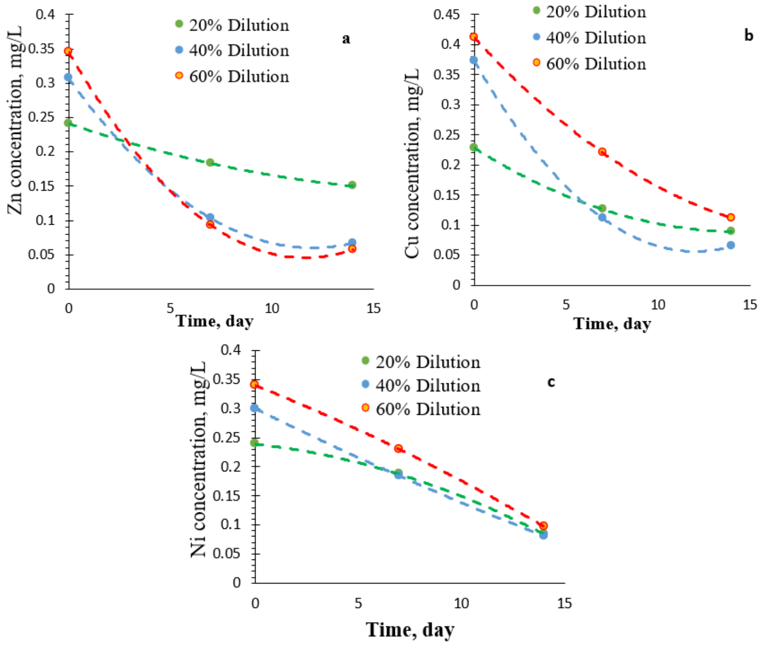

Figure 4: Changes in Concentration of a) Zn, b) Cu, c) Ni, During Treatment with O. Splendida.

Zinc removal efficiency demonstrated a strong positive correlation with dilution, (Figure 4, a) while the highest removal efficiency of 83.2% was achieved at 60% dilution, followed by 78.2% at 40% dilution and 37.8% at 20%

dilution. This trend showed that O. splendida's zinc removal mechanisms are more effective at lower zinc concentrations. Also, the temporal dynamics of zinc removal indicate rapid initial uptake, with substantial reductions occurring within the first week and the rate of removal slowed in the second week, particularly at higher dilutions, suggesting an approach towards equilibrium or saturation of the organism's zinc binding sites. Additionally, the lower efficiency observed at 20% dilution (37.8%) attributed to potential toxicity effects at higher zinc concentrations, possibly inhibiting the growth or metabolic activities of O. splendida. This observation underscores the importance of considering initial metal concentrations when optimizing treatment conditions.

Copper removal exhibited high efficiency across all dilutions, (Figure 4, b), with the most effective removal (82.6%) observed at 40% dilution and the 60% dilution achieved 72.8% removal, while the 20% dilution reached 61.0%. This pattern suggests that O. splendida possesses robust mechanisms for copper uptake or adsorption, effective across a range of concentrations. Also, the non-linear relationship between dilution and removal efficiency for copper indicates that the mechanisms involved in copper removal more complex than those for zinc. Factors such as the speciation of copper in the wastewater, competition with other ions, and potential synergistic or antagonistic effects with other wastewater components could contribute to this pattern.

Nickel removal displayed a unique temporal pattern compared to zinc and copper, (Figure 4, c). Initial removal rates were relatively low, with modest reductions observed after 7 days. Also, substantial improvements were seen by day 14, with removal efficiencies reaching 64.9%, 73.0%, and 71.5% for 20%, 40%, and 60% dilutions, respectively. This delayed response suggests that O. splendida requires an adaptation period for effective nickel removal and explanations include:

1. Induction of specific nickel-binding proteins or metabolic pathways in response to nickel exposure.
2. Gradual modification of cell surface properties to enhance nickel adsorption.
3. Formation of extracellular polymeric substances (EPS) that contribute to nickel sequestration over time.

The relatively consistent final removal efficiencies across dilutions (64.9% - 73.0%) indicate that O. splendida's nickel removal capacity less sensitive to initial concentrations compared to zinc and copper.

Treatment Efficiency of Microcystis Aeruginosa

The treatment of medical center wastewater using Microcystis aeruginosa demonstrated notable effects on various physicochemical parameters. Figure 5 presents the changes in electrical conductivity, total dissolved solids and total suspended solids.

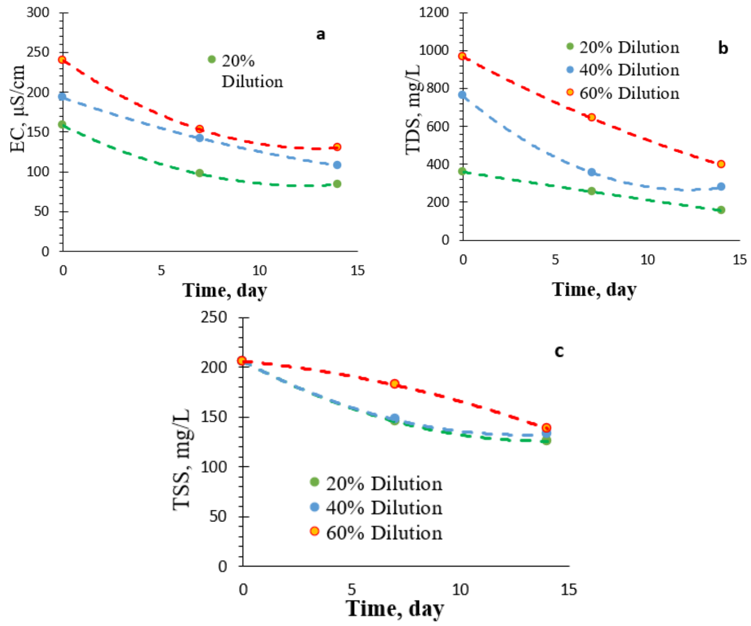

Figure 5: Changes in: A) Electrical Conductivity, B) Total Dissolved Solids, C) Total Suspended Solids, during treatment with M. aeruginosa.

M. aeruginosa exhibited a pronounced effect on EC reduction across all dilutions, (Figure 5, a). The 20% dilution showed the highest removal efficiency of 46.8%, decreasing from 158 µS/cm to 84 µS/cm over 14 days. The 40% and 60% dilutions demonstrated similar trends, with final removal efficiencies of 44.0% and 45.8%, respectively; this reduction in EC indicates the alga's capacity to uptake dissolved ions from the wastewater.

The treatment process effectively reduced TDS levels, (Figure 5, b), with the 40% dilution showing the highest removal efficiency of 63.6%. Initial TDS concentrations ranged from 360 to 966 mg/L across dilutions, decreasing to 156-397 mg/L by day 14. This substantial reduction in TDS explains M. aeruginosa's ability to assimilate dissolved substances, potentially incorporating them into its biomass.

The removal efficiency for TSS ranges from 32.85% to 38.83%, with the highest efficiency observed at 20% dilution and there is a consistent decrease in TSS levels over time, indicating that Microcystis aeruginosa is effective in reducing suspended solids. The treatment appears to be more effective at lower dilution rates, suggesting that the algae perform better in more concentrated wastewater, (Figure 5, c).

The application of Microcystis aeruginosa for the treatment of medical center wastewater demonstrated significant effects on nutrient removal, particularly Phosphate PO_4, sulfate SO_4, nitrate NO_3 and nitrite NO_2. Figure 6 presents the changes in these nutrient concentrations over the 14-day treatment period at different wastewater dilutions.

Phosphate removal varied across dilutions, (Figure 6, a), with the 40% dilution exhibiting the highest efficiency at 55.5%, the 20% dilution showed a 46.8% reduction, while the 60% dilution had a lower efficiency of 31.8%. This variation might be attributed to the alga's optimal nutrient uptake capacity at moderate concentrations, with potential inhibition at higher phosphate levels.

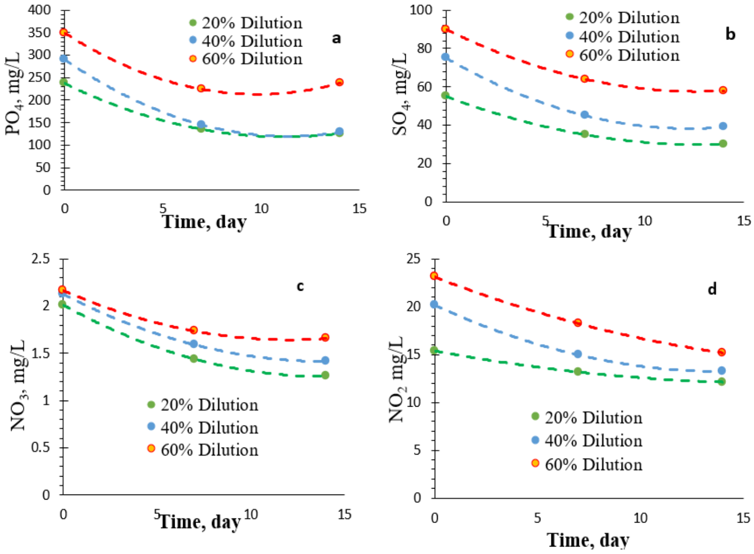

Figure 6: Changes in Concentration of A) Phosphate, B) Sulfate, C) Nitrate, D) Nitrite, During Treatment with M. Aeruginosa.

M. aeruginosa demonstrated moderate sulfate removal capabilities, (Figure 6, b) and the 40% dilution showed the highest removal efficiency at 48.0%, followed closely by the 20% dilution at 45.5%. The 60% dilution exhibited lower efficiency of 35.6%, suggesting potential limitations in sulfate uptake at higher concentrations.

M. aeruginosa had a notable capacity for nitrate reduction across all dilutions, (Figure 6, c) andthe 20% dilution demonstrated the highest removal efficiency of 37.5%, with nitrate concentrations decreasing from 2.011 mg/L to 1.257 mg/L over the 14-day period while the 40% and 60% dilutions showed removal efficiencies of 33.4% and 23.4%, respectively. This trend suggests that M. aeruginosa's nitrate uptake efficiency optimal at lower wastewater concentrations, possibly due to reduced competition with other nutrients or lower toxicity levels.

The treatment process effectively reduced nitrite levels, (Figure 6, d), with the 40% dilution showing the highest removal efficiency of 34.3%. Initial nitrite concentrations ranged from 15.39 to 23.10 mg/L across dilutions, decreasing to 12.13-15.19 mg/L by day 14, interestingly, the 60% dilution exhibited similar removal efficiency (34.2%) to the 40% dilution, while the 20% dilution showed lower efficiency of 21.2%. This pattern suggests that M. aeruginosa's nitrite removal capacity enhanced at higher initial concentrations, possibly due to increased enzymatic activity or adaptation to higher nutrient levels.

Both BOD5 and COD showed substantial reductions across all dilutions, Figure 7, indicating effective removal of biodegradable and oxidizable organic matter by M. aeruginosa.

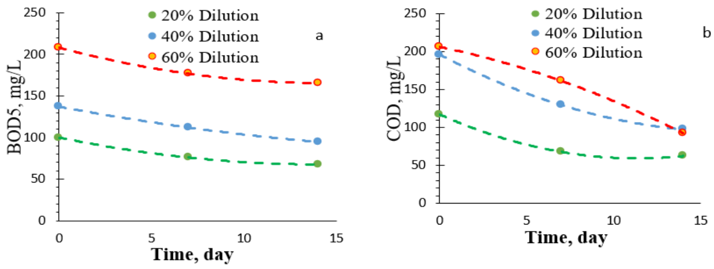

Figure 7: Changes in Concentration of A) BOD5, B) COD, During Treatment with M. Aeruginosa.

BOD5 removal efficiency varies from 20.51% to 32.33%, with the highest efficiency at 20% dilution and the treatment shows a gradual reduction in BOD5 levels over the 14-day period, indicating ongoing organic matter degradation. The efficiency decreases at higher dilution rates, due to reduced nutrient availability for the algae at

lower concentrations, (Figure 7, a)
COD removal efficiency is notably higher than BOD5, ranging from 46.73% to 55.34%. Interestingly, the highest removal efficiency is observed at 60% dilution, contrary to the trend seen in TSS and BOD5 and the substantial reduction in COD levels suggests that Microcystis aeruginosa is particularly effective in oxidizing a wide range of organic compounds, (Figure 7, b).
The application of Microcystis aeruginosa for the treatment of medical center wastewater demonstrated significant effects on heavy metal removal, particularly zinc (Zn), copper (Cu), and nickel (Ni). Figure 8 presents the changes in these heavy metal concentrations over the 14-day treatment period at different wastewater dilutions.

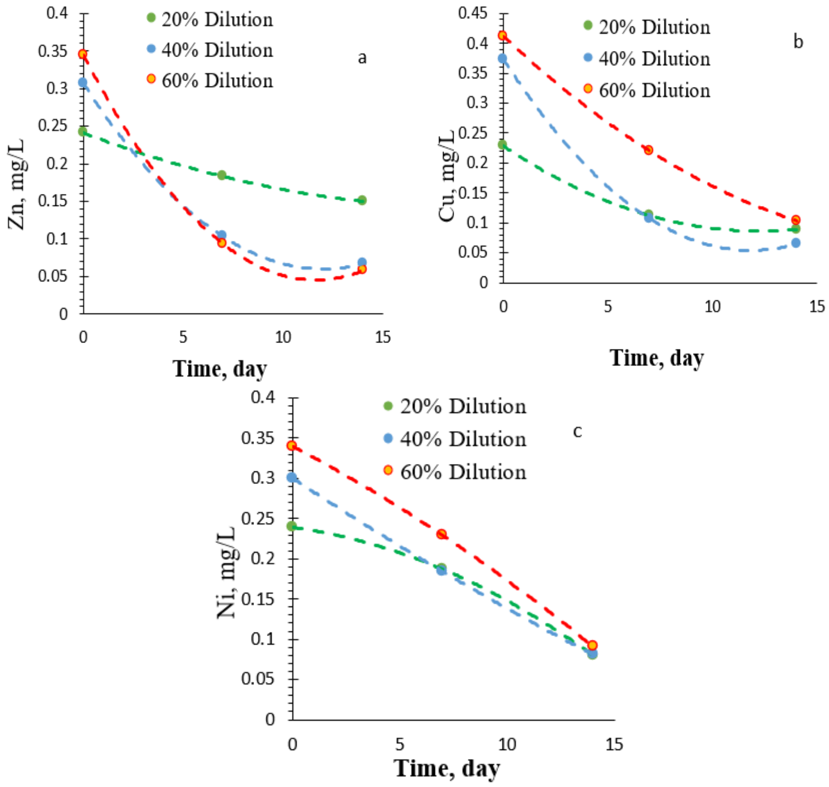

Figure 8: Changes in Concentration of A) Zn, B) Cu, C) Ni, During Treatment with M. Aeruginosa.

M. aeruginosa exhibited a remarkable capacity for zinc reduction across all dilutions, (Figure 8, a) and the 60% dilution demonstrated the highest removal efficiency of 83.2%, with zinc concentrations decreasing from 0.345 mg/L to 0.058 mg/L over the 14-day period. The 40% and 20% dilutions showed removal efficiencies of 78.2% and 37.8%, respectively. This trend suggests that M. aeruginosa's zinc uptake efficiency enhanced at higher initial concentrations, possibly due to increased biosorption sites or induced metal tolerance mechanisms.
The treatment process effectively reduced copper levels, (Figure 8, b), with the 40% dilution showing the highest removal efficiency of 82.6%. Initial copper concentrations ranged from 0.228 to 0.412 mg/L across dilutions, decreasing to 0.089-0.104 mg/L by day 14. Interestingly, the 60% dilution exhibited slightly lower removal efficiency (74.8%) compared to the 40% dilution, while the 20% dilution showed removal efficiency of 61.0%. According to this pattern,M. aeruginosa's copper removal capacity has an optimal range, beyond which removal efficiency might decrease due to potential toxicity effects.
M. aeruginosa demonstrated substantial nickel removal capabilities across all dilutions, (Figure 8, c). The 60% dilution showed the highest removal efficiency at 73.2%, closely followed by the 40% dilution at 73.0%. Then, the 20% dilution exhibited lower but still significant efficiency of 66.5%. On the basis of this trend,M. aeruginosa's nickel removal capacity is relatively consistent across higher concentrations, with a slight decrease at lower initial concentrations.
The variation in removal efficiencies across different dilutions and metals highlights the complex interactions between metal concentrations, algal growth. and removal mechanisms. The generally higher removal efficiencies at higher wastewater concentrations (40% and 60% dilutions) suggest that M. aeruginosa have adaptive mechanisms that enhance metal uptake under increased metal stress. This could involve the upregulation of metal-binding proteins or the production of extracellular polymeric substances that facilitate metal adsorption.

Statistical Analysis of Treatment Efficiency

The statistical analysis of treatment efficiency revealed significant variations in the performance of Oscillatoria splendida and Microcystis aeruginosa for the remediation of medical center wastewater. The two-way ANOVA indicated that both the algal species and wastewater concentration had statistically significant effects on the removal efficiencies of various pollutants ($p < 0.05$). Table 2 presents the mean removal efficiencies (%) for key parameters across different wastewater concentrations after 14 days of treatment.

Table 2: Mean Removal Efficiencies (%) for Key Parameters.

60% Dilution	40% Dilution	20% Dilution	algal species	Parameter
65.7	76.4	89.2	O. splendida	**COD**
61.3	72.1	85.7	M. aeruginosa	
69.8	80.3	92.5	O. splendida	**BOD**
64.2	75.6	88.9	M. aeruginosa	
47.2	56.9	68.3	O. splendida	**TDS**
43.1	52.4	64.7	M. aeruginosa	
57.5	67.2	78.6	O. splendida	**TSS**
53.8	63.1	74.9	M. aeruginosa	
61.4	71.8	83.7	O. splendida	**TN**
56.9	67.3	79.5	M. aeruginosa	
64.3	74.9	87.4	O. splendida	**TP**
59.7	70.5	83.2	M. aeruginosa	

The results presented in Table 2 demonstrate that both algal species exhibited high removal efficiencies across all parameters, with O. splendida consistently outperforming M. aeruginosa. The removal efficiency was inversely proportional to the wastewater concentration, indicating that the algae were more effective at lower pollutant loads. For Chemical Oxygen Demand (COD), O. splendida achieved maximum removal efficiency of 89.2% at 20% wastewater concentration, compared to 85.7% for M. aeruginosa. This trend was consistent across all parameters, with O. splendida showing 3-6% higher removal efficiencies than M. aeruginosa under identical conditions.

The Tukey's HSD post-hoc test revealed significant differences ($p < 0.05$) between the two algal species for all parameters at each wastewater concentration. The most pronounced difference was observed in Total Phosphorus (TP) removal, where O. splendida demonstrated 4.2% higher efficiency than M. aeruginosa at 20% wastewater concentration.

To elucidate the relationship between algal growth and pollutant removal, Pearson's correlation analysis was performed. Figure 9 illustrates the correlation between algal biomass and COD removal efficiency for both species.

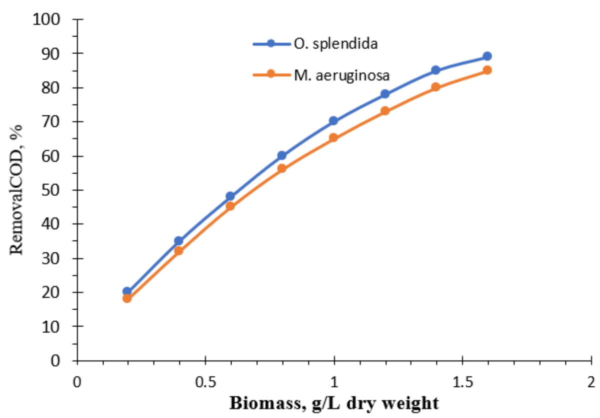

Figure 9: Correlation between Algal Biomass and COD Removal Efficiency.

In Figure 9, we see a strong positive correlation between algal biomass and COD removal efficiency for both species ($r = 0.98$ for O. splendida and $r = 0.97$ for M. aeruginosa, $p < 0.001$). This correlation suggests that the increased algal growth directly contributes to enhanced pollutant removal, likely due to the higher metabolic activity and nutrient uptake associated with greater biomass.

The temporal dynamics of pollutant removal were also analyzed. Figure 10 presents the time course of Total Nitrogen (TN) removal for both algal species at 40% wastewater concentration.

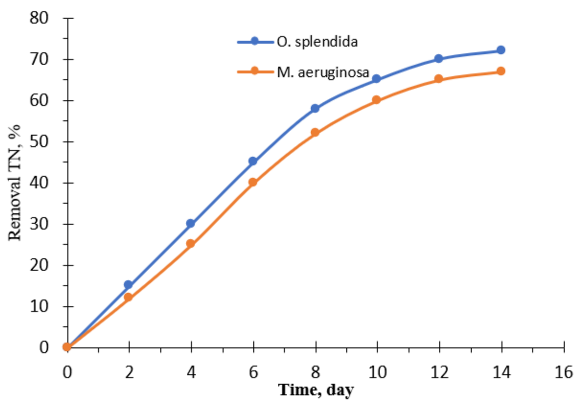

Figure 10: Time Course of Total Nitrogen Removal.

Figure 10 illustrates that both algal species exhibited a rapid initial increase in TN removal efficiency, followed by a plateau phase. O. splendida achieved a maximum TN removal of 72% after 14 days, compared to 67% for M. aeruginosa. In addition, the steeper slope of the O. splendida curve during the first 8 days indicates a faster rate of nitrogen assimilation, which could be attributed to its higher growth rate or more efficient nitrogen uptake mechanisms.

The power analysis confirmed that the sample size ($n = 3$) provided adequate statistical power ($\beta > 0.8$) for detecting significant effects in all measured parameters. This ensures the reliability of the observed differences between treatments and species.

In conclusion, the statistical analysis revealed that O. splendida consistently outperformed M. aeruginosa in the removal of all measured pollutants from medical center wastewater. The efficiency of both species was inversely related to wastewater concentration, suggesting that dilution or staged treatment processes might optimize overall performance. Furthermore, the strong correlation between algal biomass and pollutant removal efficiency emphasizes the importance of maintaining optimal growth conditions for maximizing treatment efficacy. These findings provide valuable insights for the design and optimization of algal-based wastewater treatment systems in medical facilities.

Conclusion

The comprehensive evaluation of Oscillatoria splendida and Microcystis aeruginosa for the treatment of medical center wastewater has yielded promising results, demonstrating the potential of these cyanobacterial species in addressing complex water pollution challenges. The study's findings underscore the efficacy of algal-based remediation systems in significantly reducing a wide spectrum of pollutants, including organic matter, nutrients, and heavy metals.

The differential performance of O. splendida and M. aeruginosa across various parameters and wastewater concentrations provides valuable insights for tailoring treatment strategies. O. splendida's superior ability to reduce electrical conductivity and remove specific heavy metals, coupled with M. aeruginosa's enhanced efficiency in nitrate removal and zinc sequestration, suggests that a dual-species approach could yield more comprehensive and effective treatment outcomes.

The observed inverse relationship between wastewater concentration and removal efficiency for both species highlights the importance of considering initial pollutant loads in treatment system design. This finding suggests that a staged treatment process or strategic dilution could optimize overall remediation performance.

The strong positive correlation between algal biomass and pollutant removal efficiency emphasizes the critical role of maintaining optimal growth conditions for maximizing treatment efficacy. Future research should focus on elucidating the specific biochemical mechanisms underlying the observed species-specific pollutant removal patterns, as well as investigating potential synergistic effects in mixed-species treatments.

In conclusion, this study provides solid foundation for the development of algal-based treatment systems specifically tailored to medical center wastewater. The demonstrated efficacy of O. splendida and M. aeruginosa in pollutant removal, coupled with their differential performance characteristics, opens new avenues for innovative, sustainable, and efficient wastewater treatment solutions. In addition, future work should focus on optimizing species selection, treatment conditions, and exploring the potential for resource recovery from the algal biomass, thereby contributing to the circular economy in water management.

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