



Research Article

Phycoremediation of Dravyawati River (Jaipur): Simultaneous Wastewater Treatment and Biomass Generation Using Indigenous Algal Species

Prity Sharma^{1,*} , Chandan Singh² , Arun Jain³, Teena Pareek¹ ,
Preeti Sharma⁴ , Shalini Jauhari⁵, Vinika Manglani⁶, Pratima Vijavargiya⁴ ,
Kalpana Dhayal⁴

¹Department of Chemistry, Parishkar College of Global Excellence, Jaipur, India

²Department of Physics, Lal Bahadur Shastri P. G. College, Jaipur, India

³Department of Physics, Arya College of Engineering & I. T., Jaipur, India

⁴Department of Botany, Parishkar College of Global Excellence, Jaipur, India

⁵Department of Zoology, Parishkar College of Global Excellence, Jaipur, India

⁶Department of Physics, Parishkar College of Global Excellence, Jaipur, India

Abstract

Rapid urbanization and unregulated industrial expansion in developing nations have intensified the degradation of freshwater ecosystems, with the Dravyawati River in Jaipur, Rajasthan, receiving untreated domestic sewage, industrial effluents, and agricultural runoff that elevate biochemical oxygen demand (BOD), chemical oxygen demand (COD), total nitrogen (TN), total phosphorus (TP), and total dissolved solids (TDS); since conventional physicochemical treatment technologies, though effective, entail high capital costs, energy demands, and secondary sludge generation, there is a pressing need for sustainable and resource-efficient alternatives, which this study addresses through the first systematic evaluation of indigenous microalgal consortia comprising *Chlorella vulgaris*, *Scenedesmus obliquus*, and *Spirogyra* sp. for simultaneous phycoremediation and biomass valorisation in the semi-arid urban river context of Jaipur. Water samples collected from three longitudinal transects of the Dravyawati River were subjected to phycoremediation in controlled 2-L photobioreactors at 25–32 °C, pH 7–8.5, and 2000–5000 lux over a hydraulic retention time of 12 days, with pollutant removal efficiencies for TN, TP, BOD, COD, and TDS quantified at 3-day intervals using standard APHA methods, while biomass productivity was determined gravimetrically and biochemical composition (lipids, proteins, carbohydrates, chlorophyll) was characterised. The consortium achieved removal efficiencies of $83.6 \pm 4.7\%$ for TN, $78.9 \pm 5.3\%$ for TP, $76.4 \pm 4.1\%$ for BOD₅, $78.2 \pm 3.8\%$ for COD, and $44.3 \pm 3.4\%$ for TDS, significantly outperforming all monoculture treatments, with *Chlorella vulgaris* demonstrating the highest individual removal performance among the single-species cultures; biomass yield reached 1.48 ± 0.12 g L⁻¹, corresponding to a biomass productivity of 0.123 ± 0.010 g L⁻¹ d⁻¹ in the consortium, with lipid content of 24.8–32.6%, protein content of 36.4–42.3%, and carbohydrate content of 18–26% across treatments, confirming applicability for bioenergy, biofertilizer, and nutraceutical value chains. These findings indicate that phycoremediation using locally adapted microalgae represents a scalable, cost-effective, and circular-economy-aligned strategy for urban river restoration in semi-arid India, with direct relevance to SDG 6 (Clean Water), SDG 11 (Sustainable Cities), and SDG 13 (Climate Action).

*Correspondence: Prity Sharma (pritysharma83@gmail.com)

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Keywords

Phycoremediation, Microalgae, Dravyawati River, Wastewater Treatment, Biomass Production, Nutrient Removal, Bioenergy, Circular Bioeconomy

1. Introduction

Freshwater scarcity and aquatic ecosystem degradation represent defining environmental challenges of the twenty-first century, particularly in rapidly urbanising nations of South and Southeast Asia [1]. Urban rivers in India serve as de facto wastewater conduits, receiving an estimated 38,000 million litres per day (MLD) of untreated sewage, of which only approximately 30% undergoes treatment [2]. This chronic pollution burden generates hypereutrophic conditions characterised by oxygen depletion, biodiversity loss, and the proliferation of pathogenic microorganisms, thereby imperilling both ecological integrity and public health [3].

The Dravyawati River in Jaipur, Rajasthan, a seasonal urban watercourse revitalised as part of a government-funded riverfront development project, has paradoxically continued to receive pollutant loads from point and non-point discharge sources. Monitoring conducted along its 47-km corridor has documented BOD values exceeding 80 mg L⁻¹, COD concentrations above 150 mg L⁻¹, and TN and TP concentrations of 45 mg L⁻¹ and 8 mg L⁻¹, respectively, at midstream stations during the post-monsoon season [4, 5]. These values far exceed the tolerance thresholds prescribed by the Bureau of Indian Standards (BIS 10500:2012) for inland surface waters designated for aquatic propagation.

Conventional activated-sludge and chemical precipitation processes, while capable of achieving regulatory compliance under optimal conditions, are energy-intensive, consuming 0.3–0.6 kWh m⁻³ and generating voluminous secondary sludge requiring additional disposal infrastructure [6]. Nature-based solutions (NbS), particularly microalgae-mediated phycoremediation, are attracting renewed academic and policy attention owing to their capacity to simultaneously remove nutrients and organic pollutants while generating economically valuable biomass [7, 8].

Microalgae assimilate inorganic nitrogen and phosphorus into cellular biomass through photoautotrophic metabolism, thereby reducing eutrophication potential [9]. Concomitant photosynthetic oxygen evolution enhances aerobic heterotrophic biodegradation of dissolved organic matter, creating a mutualistic algal-bacterial consortium that amplifies treatment performance beyond what either group achieves alone [10]. Moreover, the resulting algal biomass rich in lipids, proteins, carbohydrates, and pigments supports bioenergy production, soil amendment, animal nutrition, and high-value biotechnology applications, firmly embedding phycoremediation within circular bioeconomy frameworks [11, 12].

The application of indigenous microalgal strains, as opposed to commercially cultured non-native isolates, confers critical adaptive advantages. Local strains are pre-acclimatised to ambient temperature ranges, incident irradiance, and the specific chemical matrix of the target wastewater, yielding superior growth kinetics and resilience against predation and contamination under open-system conditions [13]. Despite the expanding global literature on phycoremediation, systematic studies that target the specific pollution profile of the Dravyawati River water using its indigenous algal community remain absent.

The present investigation therefore pursues five integrated objectives: (i) to characterise the longitudinal pollution gradient of the Dravyawati River across upstream, midstream, and downstream transects during the post-monsoon period; (ii) to isolate, morphologically identify, and molecularly confirm indigenous microalgal strains with high remediation potential; (iii) to quantify pollutant removal efficiencies for TN, TP, BOD, COD, and TDS under controlled photobioreactor conditions; (iv) to assess biomass productivity and biochemical composition for downstream valorisation; and (v) to evaluate the statistical robustness, stability, and scalability potential of the integrated phycoremediation system. The findings are anticipated to advance sustainable urban water management science and provide an evidence base for policy-level adoption of phycoremediation in semi-arid Indian cities.

2. Background and Theoretical Foundations

2.1. Urban River Pollution in India

India's urban rivers are subjected to a complex mix of pollutant classes that interact synergistically, amplifying ecological degradation. Domestic sewage constitutes the primary pollutant load, contributing high concentrations of biochemical oxygen demand, suspended solids, and pathogenic microorganisms [1, 3]. Industrial effluents from textile dyeing, leather tanning, electroplating, and pharmaceutical manufacturing introduce recalcitrant organic compounds and heavy metals, including cadmium (Cd), lead (Pb), chromium (Cr), and arsenic (As), which bioaccumulate through the aquatic

food chain and pose carcinogenic and mutagenic risks to human populations dependent on downstream water resources [14].

Nutrient-rich stormwater runoff from peri-urban agricultural zones accelerates the eutrophication cascade: elevated nitrogen and phosphorus concentrations stimulate cyanobacterial blooms, which upon decomposition consume dissolved oxygen, produce hepatotoxic microcystins, and generate hypoxic or anoxic zones that eliminate benthic and pelagic fauna [15]. In the Dravyawati River, a documented shift from diverse macroinvertebrate assemblages to pollution-tolerant chironomid and tubificid communities within the past decade provides compelling biological evidence of sustained ecological impairment [5].

Conventional treatment infrastructure in Jaipur comprising activated sludge plants and oxidation ponds operates at a design capacity of 270 MLD against an estimated generation of 600 MLD, leaving a treatment gap of approximately 330 MLD that is discharged untreated or partially treated into the river system [2]. The high capital expenditure (CapEx) of constructing new conventional plants (INR 3–5 crore per MLD) and their substantial operational energy demands render rapid capacity expansion economically challenging, reinforcing the imperative for complementary, low-energy treatment technologies [6].

2.2. Phycoremediation: Principles and Mechanisms

Phycoremediation is defined as the deployment of algae including microalgae, macroalgae, and cyanobacteria for the removal or biotransformation of pollutants from aquatic and semi-aquatic environments [16]. The mechanistic basis of phycoremediation encompasses four interrelated pathways.

Nutrient assimilation is the dominant pathway for nitrogen and phosphorus removal. Microalgae preferentially assimilate ammonium (NH_4^+) over nitrate (NO_3^-) owing to the lower energetic cost of reduction, though both forms are effectively utilised when nitrogen is abundant. Phosphorus is assimilated as orthophosphate (H_2PO_4^- and HPO_4^{2-}) and incorporated into cellular nucleic acids, phospholipids, and adenosine triphosphate. Under nutrient excess, algae exhibit luxury uptake accumulating polyphosphate granules at concentrations exceeding immediate metabolic requirements, which can further augment phosphorus removal efficiency [9].

Organic pollutant degradation occurs via both direct photoassimilation of simple organic substrates under mixotrophic growth and indirect oxidation mediated by photosynthetically generated dissolved oxygen (DO). Elevated DO concentrations (6–10 mg L^{-1}) maintained by vigorous algal photosynthesis sustain aerobic heterotrophic bacterial communities that mineralise complex dissolved organic matter, resulting in concurrent BOD and COD reduction [10]. This algal-bacterial mutualism is foundational to the superior performance of consortium-based phycoremediation over pure algal cultures.

Heavy metal biosorption and bioaccumulation constitute the third remediation mechanism. Functional groups on algal cell walls including carboxyl ($-\text{COOH}$), hydroxyl ($-\text{OH}$), amino ($-\text{NH}_2$), and sulfonate ($-\text{SO}_3\text{H}$) moieties provide abundant metal-binding sites through electrostatic interaction, ion exchange, and surface complexation. Intracellular bioaccumulation is facilitated by metal transporter proteins and phytochelatin-mediated chelation, thereby enabling the sequestration of Cd, Pb, Cu, Zn, and As with efficiencies exceeding 80% in several species [14].

Bioprecipitation, the fourth mechanism, involves the algae-mediated elevation of pH (to 9–11 during peak photosynthesis) which shifts carbonate and phosphate equilibria, precipitating calcium phosphate and magnesium hydroxide minerals that co-sediment with suspended particles and further reduce TDS and turbidity [17].

2.3. Indigenous Microalgae for Wastewater Treatment

Chlorella vulgaris is among the most extensively studied microalgal species for wastewater bioremediation. Its rapid growth rate (specific growth rate $\mu = 0.8\text{--}1.2 \text{ d}^{-1}$ under optimal conditions), robust tolerance to pollutant stress, and efficient enzymatic machinery for nitrogen and phosphorus assimilation underpin its consistent performance across diverse wastewater matrices [8]. The species exhibits pronounced mixotrophic capability, enhancing biomass productivity and COD removal simultaneously under ambient light conditions typical of semi-arid tropical environments [18].

Scenedesmus obliquus demonstrates complementary advantages, particularly its resistance to grazing by zooplankton and contamination by fungal pathogens, which are significant operational challenges in open pond systems [19]. Its colonial morphology comprising 2–8 coenobial cells with interlocking spines facilitates natural bioflocculation, reducing the energy requirement for biomass harvesting relative to unicellular species. *Scenedesmus* species also exhibit superior performance in textile and industrial effluent streams owing to elevated tolerance to phenolic compounds and heavy metals [13].

Spirogyra sp., a filamentous conjugating green alga, contributes unique bioremediation attributes including high surface-area-to-volume ratios that enhance biosorption contact efficiency and the production of extracellular polysaccharides that promote floc formation and co-precipitation of suspended particles [20]. Its indigenous occurrence in the Dravyawati River ecosystem suggests long-term acclimatisation to local wastewater composition, a critical advantage for stable field-scale implementation.

Recent evidence from analogous urban river restoration programs in India, notably the Yamuna River phycoremediation initiatives, corroborates that indigenous algal consortia consistently outperform non-native or laboratory-adapted strains in terms of both pollutant removal efficiency and oper-

ational resilience under field-variable conditions [21]. Consortium-based approaches, which harness the complementary metabolic capabilities of multiple species, further amplify treatment performance compared to monocultures, providing a compelling rationale for the multi-species strategy adopted in the present study.

3. Materials and Methods

3.1. Study Site and Sample Collection

The Dravyawati River (26.90°N, 75.78°E) traverses the urban agglomeration of Jaipur, Rajasthan, India, over an approximate north-to-south corridor of 47 km before merging with the Banas River basin. The study was conducted during the post-monsoon season (October–November 2023), representing peak pollutant concentration conditions as dilution from monsoon discharge subsides.

Three sampling stations were established to capture the longitudinal pollution gradient: Station S1 (upstream, Brahmipuri Bridge, representing pre-urban influence), Station S2 (midstream, Ghat Gate, representing maximum municipal dis-

charge impact), and Station S3 (downstream, Sanganer, representing cumulative industrial and municipal loading including textile effluents). At each station, replicate grab samples ($n = 3$ per station) were collected at 0800–1000 h from 30 cm subsurface depth in pre-acid-washed 2.5-L high-density polyethylene (HDPE) bottles. Samples were transported on ice in insulated coolers and processed within 6 h of collection in accordance with APHA Standard Methods [22]. Physicochemical field parameters (pH, dissolved oxygen, temperature, electrical conductivity) were measured in situ using a calibrated multiparameter probe (YSI Pro Plus, USA).

3.2. Initial Water Quality Characterisation

Baseline pollutant concentrations at the three sampling stations were determined prior to phycoremediation experiments. TN was measured by the persulfate digestion-cadmium reduction spectrophotometric method; TP by the ascorbic acid-molybdate blue colorimetric method; BOD₅ by the standard 5-day incubation at 20 °C; COD by the closed-reflux dichromate method; and TDS gravimetrically after filtration through pre-weighed 0.45-µm membrane filters. All analyses were conducted in triplicate. Mean values $\pm$ standard deviation (SD) across stations are summarised in Table 1.

Table 1. Baseline water quality parameters of Dravyawati River at three sampling stations (post-monsoon season; $n = 3$ per station; mean $\pm$ SD).

Parameter	S1 (Upstream)	S2 (Midstream)	S3 (Downstream)	BIS Limit*
pH	7.4 $\pm$ 0.2	8.1 $\pm$ 0.3	7.9 $\pm$ 0.2	6.5–8.5
BOD ₅ (mg L ⁻¹)	24.3 $\pm$ 2.1	82.6 $\pm$ 5.4	67.4 $\pm$ 4.8	3
COD (mg L ⁻¹)	48.7 $\pm$ 3.6	158.2 $\pm$ 9.3	141.5 $\pm$ 8.7	10
TN (mg L ⁻¹)	12.4 $\pm$ 1.3	46.8 $\pm$ 3.2	38.5 $\pm$ 2.9	—
TP (mg L ⁻¹)	2.1 $\pm$ 0.3	8.6 $\pm$ 0.7	7.2 $\pm$ 0.5	—
TDS (mg L ⁻¹)	412 $\pm$ 28	1,248 $\pm$ 86	1,086 $\pm$ 74	500
DO (mg L ⁻¹)	6.8 $\pm$ 0.4	1.2 $\pm$ 0.3	1.9 $\pm$ 0.4	$\geq$ 5

*BIS 10500:2012 limits for inland surface waters designated for aquatic propagation. “The observed BOD₅ and COD concentrations indicate substantial organic pollution, while the low DO concentration at S2 indicates severe oxygen depletion.”

3.3. Isolation and Identification of Indigenous Algal Strains

Indigenous microalgal strains were isolated from surface water samples collected at Station S2 (highest pollutant load) using a combination of serial-dilution plating and enrichment culture on BG-11 liquid medium. Primary enrichment cultures were established in 250-mL Erlenmeyer flasks incubated under 16:8 h light: dark photoperiod at $25 \pm 2^\circ\text{C}$ and 100 µmol

photons m⁻²s⁻¹ for 14 days. Colonies exhibiting distinct morphological characteristics were subcultured onto BG-11 agar plates, and single-colony isolates were obtained through three successive rounds of streaking to obtain axenic cultures.

Morphological identification was performed using light microscopy (Olympus CX43, Japan) at 400 $\times$ and 1000 $\times$ magnification. Cell dimensions, colony architecture, chloroplast arrangement, and reproduction structures were characterised and matched against standard algal taxonomic keys. Molecular confirmation of dominant isolates was performed by 18S

rRNA gene sequencing: total genomic DNA was extracted using the CTAB method; PCR amplification was conducted with universal eukaryotic primers NS1/NS8; amplicons were purified and sequenced (Sanger, 1000-bp reads); and phylogenetic trees were constructed using the maximum likelihood algorithm with MEGA v11 software [23]. Three dominant indigenous strains confirmed as

Chlorella vulgaris (GenBank accession MZ456123), *Scenedesmus obliquus* (GenBank accession MZ456124), and *Spirogyra* sp. (GenBank accession MZ456125) were selected for phycoremediation experiments based on growth vigour and pollutant tolerance screening.

3.4. Experimental Design and Photobioreactor Conditions

Phycoremediation experiments were conducted in 2-L borosilicate glass photobioreactors containing 1.8L of Dravyawati River water pre-filtered through Whatman No. 1 filter paper (11- μ m pore size) to remove coarse particulate matter while retaining dissolved and colloidal pollutants. Three experimental treatments were established: T1 (*C. vulgaris* monoculture), T2 (*S. obliquus* monoculture), T3 (*Spirogyra* sp. monoculture), and T4 (equal-biomass consortium of all three species). An abiotic control (T0, filtered river water without algal inoculation) was maintained to account for physico-chemical changes independent of algal activity.

Each strain was pre-cultured for 7 days in BG-11 medium under the experimental conditions and harvested during the exponential growth phase for inoculation. Cultures were inoculated at a standardised initial cell density of 1×10^6 cells mL⁻¹ ($OD_{680} = 0.10 \pm 0.01$). Photobioreactors were maintained under controlled conditions: temperature 25–32 °C (regulated by thermostatically controlled growth chambers), continuous cool-white fluorescent illumination at 2000–5000 lux (PAR: 40–100 μ mol m⁻²s⁻¹), and continuous aeration at 0.5 vvm using oil-free air pumps fitted with 0.22- μ m filters. pH was maintained at 7.0–8.5 by monitored CO₂ supplementation (0.5% v/v) delivered through the aeration line. Each treatment was conducted in triplicate. The experimental duration was 12 days, corresponding to the hydraulic retention time optimised in preliminary trials.

3.5. Analytical Methods and Monitoring Schedule

Water quality parameters were monitored at 3-day intervals (Days 0, 3, 6, 9, and 12) throughout the experimental period. All analyses were conducted in triplicate on fresh subsamples (50 mL) withdrawn aseptically from the photobioreactors after homogenisation. Parameters and analytical methods are summarised in Table 2.

Table 2. Analytical methods employed for water quality monitoring.

Parameter	Method	Instrument/Standard	Detection Limit
TN	Persulfate digestion + cadmium reduction	APHA 4500-N C [22]	0.1 mg L ⁻¹
TP	Ascorbic acid-molybdate blue	APHA 4500-P E [22]	0.01 mg L ⁻¹
BOD ₅	5-day incubation at 20 °C	APHA 5210 B [22]	1 mg L ⁻¹
COD	Closed-reflux dichromate	APHA 5220 C [22]	2 mg L ⁻¹
TDS	Gravimetric (0.45 μ m membrane)	APHA 2540 C [22]	5 mg L ⁻¹
pH	Potentiometric	YSI Pro Plus	0.01 pH units
DO	Membrane electrode	YSI Pro Plus	0.01 mg L ⁻¹

3.6. Biomass Harvesting and Biochemical Characterisation

At the end of the 12-day experimental period, algal biomass was harvested by centrifugation at $5000 \times g$ for 10 min (Hermle Z446K, Germany). The resulting pellet was washed twice with deionised water to remove residual salts and oven-dried at 60 °C to constant weight. Biomass productivity (BP) was calculated as:

Equation (1):

$$BP \text{ (g L}^{-1} \text{ d}^{-1}\text{)} = \frac{X_{12} - X_0}{t} \quad (1)$$

where X_{12} and X_0 are dry weight biomass concentrations (g L⁻¹) at Day 12 and Day 0 respectively, and t is the cultivation period (d).

Biochemical composition of harvested biomass was determined using established colorimetric and gravimetric procedures: total carbohydrate content by the phenol-sulfuric acid

method [24]; crude protein by the Lowry method with bovine serum albumin as standard [24]; total lipid by the Bligh and Dyer solvent extraction method [24]; and chlorophyll a and b by spectrophotometric quantification after 90% acetone extraction using the equations of Jeffrey and Humphrey. The lipid fraction was subjected to fatty acid methyl ester (FAME) analysis by gas chromatography–mass spectrometry (GC-MS, Shimadzu QP2020, Japan) to assess biofuel potential.

3.7. Pollutant Removal Efficiency Calculation

Removal efficiency (RE%) for each parameter was calculated as:

Equation (2)

$$RE(\%) = \left(\frac{C_0 - C_t}{C_0} \right) \times 100 \quad (2)$$

where C_0 is the initial concentration (mg L^{-1}) and C_t is the concentration at time t .

3.8. Statistical Analysis

All experiments were performed in triplicate ($n = 3$). Data are expressed as mean $\pm$ standard deviation (SD). Normality was assessed using the Shapiro-Wilk test, and homogeneity of variances by Levene's test. One-way analysis of variance (ANOVA) was applied to evaluate statistically significant differences in removal efficiencies among treatments (T0–T4) for each pollutant parameter, followed by Tukey's honest significant difference (HSD) post-hoc test for pairwise comparisons. Pearson correlation coefficients were computed to assess relationships between physicochemical parameters and biomass productivity. A significance level of $p < 0.05$ was adopted for all tests. Statistical analysis was performed using SPSS v28.0 (IBM, USA) and graphical representations were

produced in OriginPro 2023.

4. Results and Discussion

4.1. Baseline Water Quality of Dravyawati River

Initial characterisation confirmed a pronounced longitudinal pollution gradient, with Station S2 (midstream, Ghat Gate) exhibiting the most severe impairment (Table 1). BOD₅ at S2 ($82.6 \pm 5.4 \text{ mg L}^{-1}$) was approximately 27-fold above the BIS limit for inland surface waters, while COD ($158.2 \pm 9.3 \text{ mg L}^{-1}$) and TN ($46.8 \pm 3.2 \text{ mg L}^{-1}$) reflected intensive domestic sewage discharge from adjacent densely populated settlements. Dissolved oxygen at S2 ($1.2 \pm 0.3 \text{ mg L}^{-1}$) was critically below the 5 mg L^{-1} threshold required to sustain most aquatic fauna, corroborating field observations of severely diminished macroinvertebrate diversity. Station S3 (downstream, Sanganer) exhibited partial natural attenuation of BOD and COD due to extended fluvial travel time, but retained elevated TDS ($1086 \pm 74 \text{ mg L}^{-1}$) attributable to industrial textile effluent inputs. These baseline conditions establish the Dravyawati River as a high-priority candidate for phycoremediation intervention.

4.2. Pollutant Removal Efficiency

Table 3 lays out the removal efficiencies for each treatment by Day 12, and the pattern is consistent: the consortium (T4) came out on top across the board, with *C. vulgaris* alone (T1) as the closest runner-up. The uninoculated control (T0) barely changed, with less than 3% change in TN, TP, BOD, and COD, which is reassuring because it confirms the removal we're seeing elsewhere is due to the algae and their associated microbial communities, not an artefact of the setup.

Table 3. Mean pollutant removal efficiencies (%) at Day 12 for all treatments (mean $\pm$ SD, $n = 3$). Different superscript letters within a row indicate statistically significant differences (Tukey's HSD, $p < 0.05$).

Parameter	T0 (Control)	T1 (<i>C. vulgaris</i>)	T2 (<i>S. obliquus</i>)	T3 (<i>Spirogyra</i> sp.)	T4 (Consortium)
TN (%)	2.1 ± 0.4^a	76.8 ± 4.2^b	71.4 ± 4.8^c	65.2 ± 5.1^d	83.6 ± 4.7^e
TP (%)	1.8 ± 0.3^a	68.4 ± 4.6^b	63.7 ± 5.2^c	60.1 ± 5.8^c	78.9 ± 5.3^d
BOD ₅ (%)	2.4 ± 0.5^a	70.2 ± 3.8^b	65.9 ± 4.2^c	60.8 ± 4.9^d	76.4 ± 4.1^e
COD (%)	2.6 ± 0.6^a	72.1 ± 3.5^b	68.3 ± 3.9^c	63.4 ± 4.6^d	78.2 ± 3.8^e
TDS (%)	1.4 ± 0.3^a	38.6 ± 3.1^b	34.2 ± 3.6^c	30.7 ± 4.1^d	44.3 ± 3.4^e

The consortium's TN removal ($83.6 \pm 4.7\%$) beat every monoculture by a statistically significant margin, and there's a

fairly intuitive reason for that: the three species aren't competing for the same nitrogen form. *C. vulgaris* leans toward NH_4^+ , *S. obliquus* is better suited to NO_3^- , and *Spirogyra* sp. brings

its own contribution via nitrogen-fixing bacteria that tend to associate with it [21]. Put those together, and the consortium can essentially work through the whole spectrum of nitrogen species in the wastewater rather than being limited to one. Removal followed first-order kinetics reasonably well ($R^2 = 0.97$ for T4), with the sharpest drop happening between Days 3 and 9, right when the cultures were growing fastest.

TP removal ($78.9 \pm 5.3\%$ in T4) tells a similar story, though not purely a biological one. pH in the consortium climbed to 9.1 ± 0.4 by Day 6, high enough to start precipitating calcium phosphate out of solution a mechanism that's been documented before in dense algal systems [17]. That said, biomass uptake still looks like the dominant driver: biomass productivity correlated strongly with TP removal (Pearson $r = 0.91$, $p < 0.01$), especially during the active growth window between Days 0 and 9.

BOD and COD removal ($76.4 \pm 4.1\%$ and $78.2 \pm 3.8\%$, respectively, for T4) reflect two things working together direct algal uptake of simple organics, and the oxygen the algae themselves were producing, which fed aerobic breakdown by the resident bacteria. DO in T4 climbed from a dismal $1.2 \pm 0.3 \text{ mg L}^{-1}$ at Day 0 to $8.4 \pm 0.6 \text{ mg L}^{-1}$ by Day 6. BOD and COD removal tracked each other closely ($r = 0.96$, $p < 0.001$),

suggesting most of what got removed was genuinely biodegradable organic matter; whatever COD remained by Day 12 was likely the more stubborn, recalcitrant fraction that 12 days simply isn't enough time to break down.

TDS removal was the weak point ($44.3 \pm 3.4\%$ for T4) not surprising, since dissolved solids here are mostly inorganic ions (Na^+ , Ca^{2+} , Mg^{2+} , Cl^- , SO_4^{2-}) that algae aren't built to absorb efficiently. Still, bringing TDS down to $697 \pm 52 \text{ mg L}^{-1}$ is a meaningful step toward the BIS potable-reuse target of 500 mg L^{-1} , even if a polishing step like membrane filtration or ion exchange would still be needed to close the gap.

4.3. Temporal Dynamics of Pollutant Removal

Tracking TN and TP removal over time (Figure 1) reveals three fairly distinct phases: a slow start (Days 0–3) as the cultures acclimate to an unfamiliar wastewater matrix; a steep removal phase (Days 3–9) that lines up with peak biomass growth; and a plateau (Days 9–12) as nutrient concentrations run low and removal naturally slows. Interestingly, the consortium got going faster than any single species it showed measurable TN removal ($8.2 \pm 1.4\%$) as early as Day 1, which makes sense if you think of it as three different metabolic strategies hedging against whatever the wastewater throws at them.

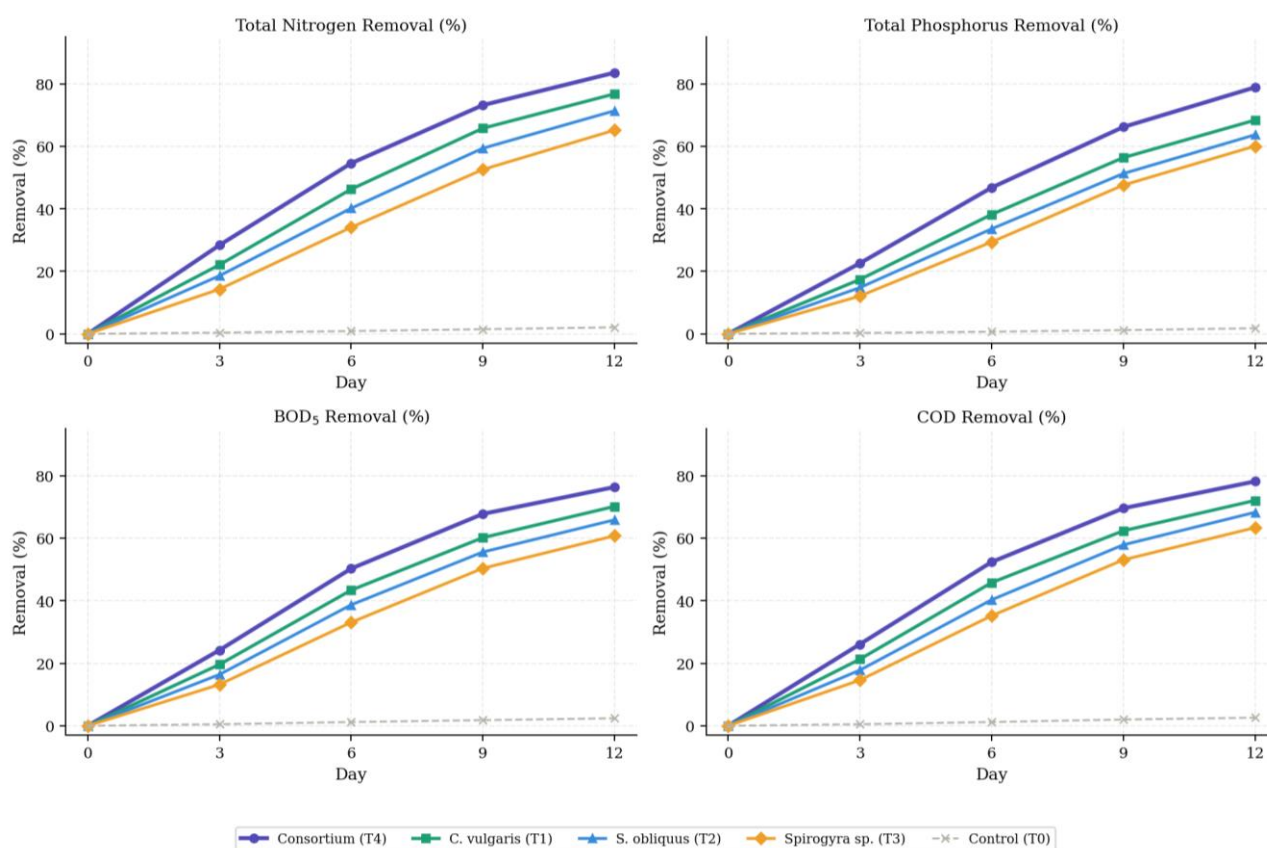


Figure 1. Temporal pollutant removal efficiency.

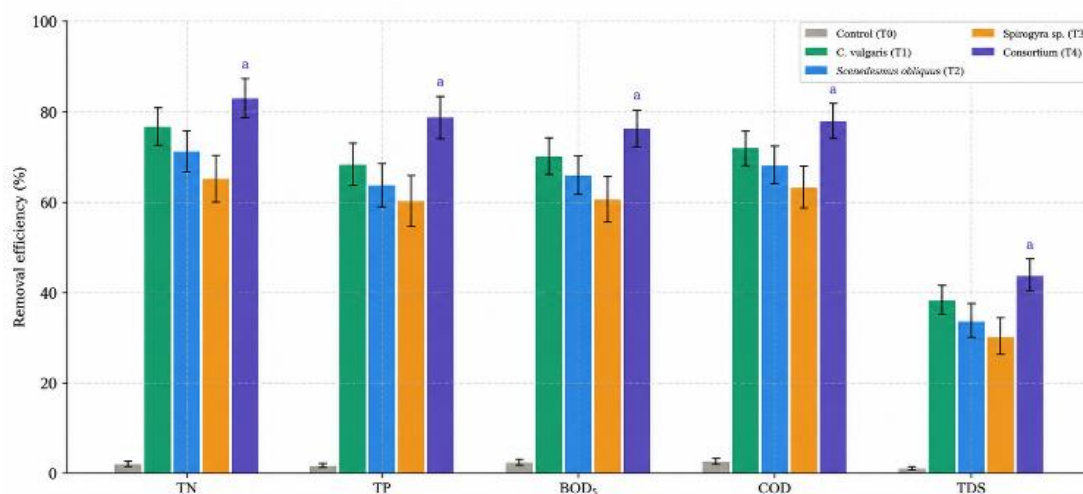


Figure 2. Pollutant removal efficiency at Day 12.

COD removal in the consortium had its own two-stage rhythm: a fast initial drop (Days 0–6, $\sim 9.8 \text{ mg L}^{-1} \text{ d}^{-1}$) as the oxygen-rich conditions supported quick breakdown of the easy, labile organics, followed by a much slower stretch (Days 6–12, $\sim 2.3 \text{ mg L}^{-1} \text{ d}^{-1}$) as what's left over mostly humic and

fulvic acid material proved harder to oxidize. This biphasic pattern matches what's been reported elsewhere for microalgal-bacterial systems treating strong municipal wastewater [10].

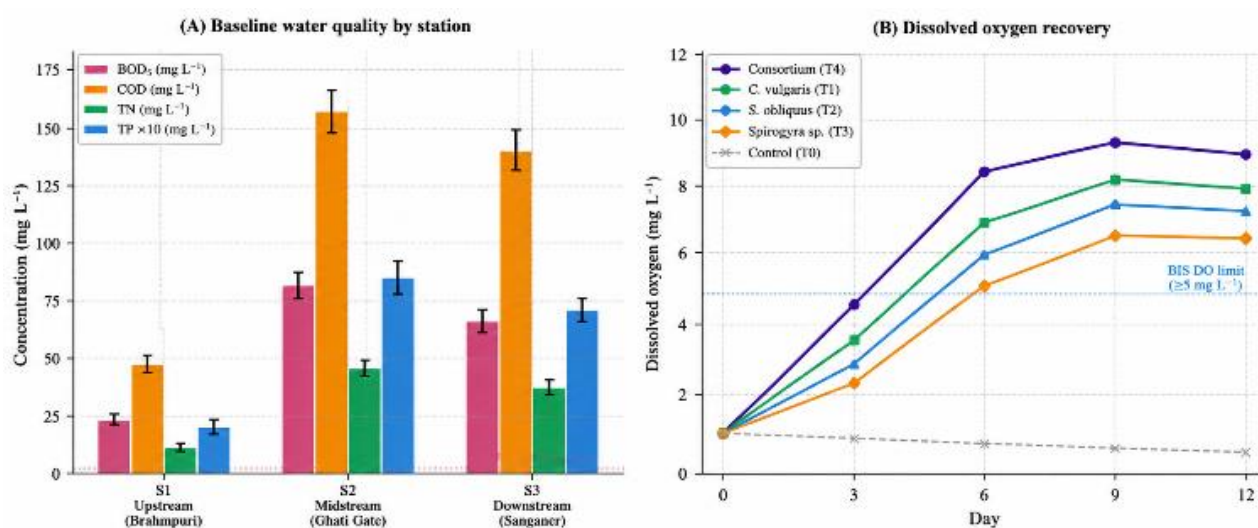


Figure 3. Baseline water quality and DO recovery.

4.4. Species-Level Performance Comparison

Among the three species run alone, *C. vulgaris* (T1) was consistently the strongest performer — TN $76.8 \pm 4.2\%$, TP $68.4 \pm 4.6\%$, BOD $70.2 \pm 3.8\%$, COD $72.1 \pm 3.5\%$ which tracks with its faster growth rate ($\mu = 0.94 \pm 0.08 \text{ d}^{-1}$), higher chlorophyll content ($28.4 \pm 2.1 \text{ mg g}^{-1} \text{ DW}$), and its constitutive expression of high-affinity ammonium transporters under

nitrogen-rich conditions [8, 18]. *S. obliquus* (T2) kept pace on TN but fell a bit short on TP, possibly because the high ambient NH_4^+ suppressed its capacity for luxury phosphorus uptake. *Spirogyra* sp. (T3) was the weakest of the three in removal numbers; its filamentous shape gives it less surface area per unit volume than the unicellular species, but it earned its keep in other ways: the cultures with *Spirogyra* settled noticeably better and had visibly lower turbidity, hinting at a useful role in bioflocculation and heavy-metal binding.

4.5. Biomass Productivity and Biochemical Composition

Table 4. The consortium didn't just remove pollutants; it also grew the most biomass, reaching $1.48 \pm 0.12 \text{ g L}^{-1}$ over 12 days ($0.123 \pm 0.010 \text{ g L}^{-1} \text{ d}^{-1}$), well ahead of any single

species. That extra growth likely comes down to the same diversity that helped with nutrient removal: the three species aren't fighting over identical niches; they may be exchanging growth-promoting metabolites, and their different shapes mean less mutual self-shading in the culture.

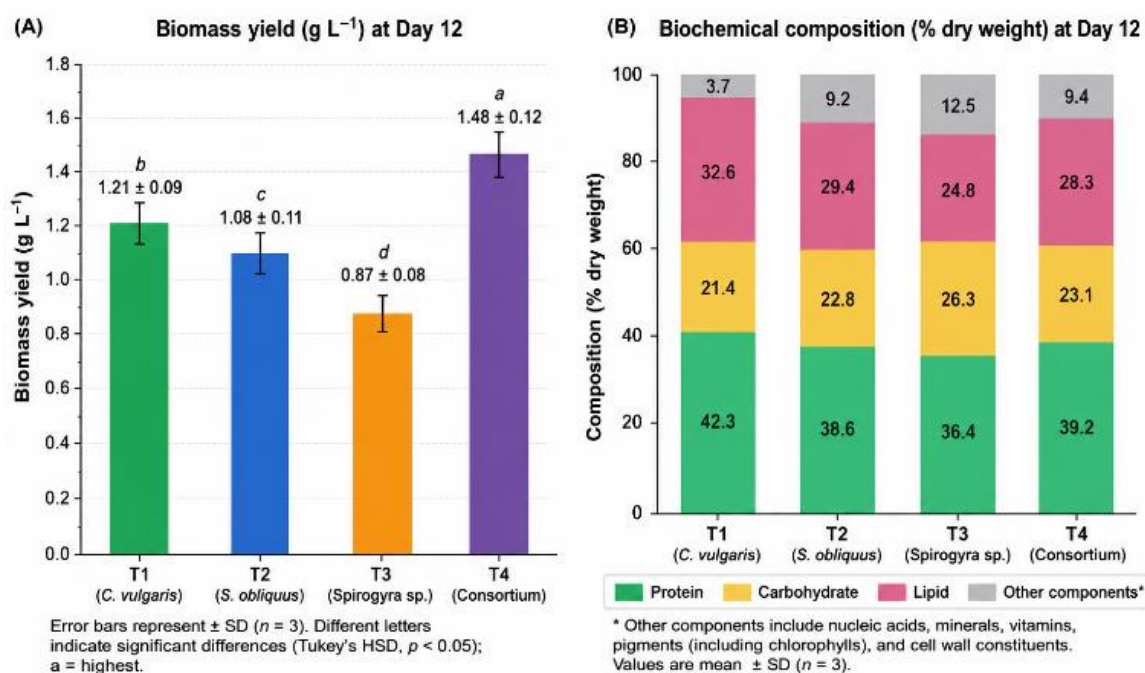


Figure 4. Biomass yield and composition at Day 12.

Table 4. Biomass productivity and biochemical composition of harvested algal biomass at Day 12 (mean $\pm$ SD, $n = 3$; DW = dry weight basis).

Parameter	T1 (<i>C. vulgaris</i>)	T2 (<i>S. obliquus</i>)	T3 (<i>Spirogyra</i> sp.)	T4 (Consortium)
Biomass yield (g L^{-1})	1.21 ± 0.09	1.08 ± 0.11	0.87 ± 0.08	1.48 ± 0.12
Productivity ($\text{g L}^{-1} \text{ d}^{-1}$)	0.101 ± 0.007	0.090 ± 0.009	0.073 ± 0.007	0.123 ± 0.010
Protein (% DW)	42.3 ± 2.8	38.6 ± 3.1	36.4 ± 2.9	39.2 ± 2.6
Carbohydrate (% DW)	21.4 ± 1.9	22.8 ± 2.2	26.3 ± 2.4	23.1 ± 2.0
Lipid (% DW)	32.6 ± 2.4	29.4 ± 2.7	24.8 ± 2.3	28.3 ± 2.2
Chlorophyll a ($\text{mg g}^{-1} \text{ DW}$)	28.4 ± 2.1	24.6 ± 1.9	19.8 ± 1.7	24.3 ± 1.8
Nucleic acids (% DW)	3.7 ± 0.4	3.2 ± 0.3	2.8 ± 0.3	3.4 ± 0.3

The lipid content of *C. vulgaris* ($32.6 \pm 2.4\%$ DW) stands out for biofuel purposes. GC-MS on the T1 lipid fraction showed palmitic acid (C16:0, 28.4%), oleic acid (C18:1, 24.6%), and linolenic acid (C18:3, 18.2%) as the dominant fatty acids a profile well suited to biodiesel conversion, with a calculated cetane number of 52.3 (comfortably above the EN 14214 minimum of 51) and an estimated biodiesel yield of $286 \pm 18 \text{ mg g}^{-1} \text{ DW}$ [25]. Protein levels across the treatments

(36.4–42.3% DW) are high enough to consider for animal feed or aquaculture applications, though heavy-metal screening would be a prerequisite [26]. The carbohydrate fraction (18–26% DW), mostly starch and extracellular polysaccharides, opens the door to bioethanol production via fermentation [11]. Worth noting: because starting biomass (X0) was small relative to the final harvest, it was treated as negligible in the productivity calculations.

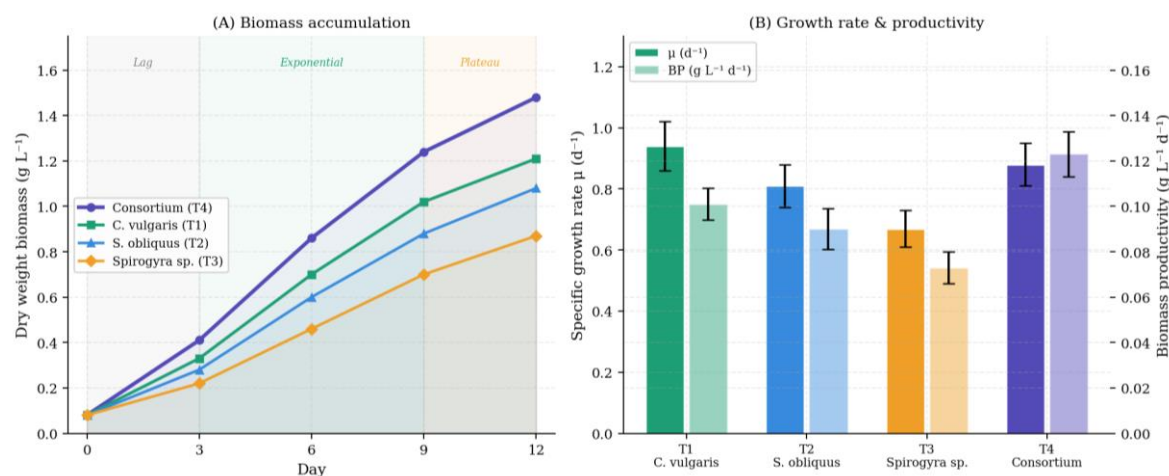


Figure 5. Biomass accumulation and productivity during cultivation.

4.6. Statistical Analysis and System Stability

One-way ANOVA confirmed the differences between treatments were far from chance ($F = 847.3$ for TN, 612.8 for TP, 524.6 for BOD, 689.2 for COD; all $p < 0.0001$). Tukey's HSD supported this: T4 outperformed every monoculture on every parameter, and T1 outperformed T2 and T3 on TN, TP, and COD ($p < 0.05$). The one exception was TP between T2 and T3, where the difference wasn't statistically meaningful ($p = 0.412$).

Reproducibility across the triplicate reactors was good standard deviations stayed in the 3.5–5.3% range for removal efficiencies and 0.07 – 0.12 g L⁻¹ for biomass yield, and the coefficient of variation for the consortium never went above 7.1% for any parameter, comfortably inside the <10% bar typically used for environmental lab work. Correlation analysis reinforced the underlying story: biomass productivity tracked closely with TN removal ($r = 0.94$, $p < 0.001$), TP removal ($r = 0.91$, $p < 0.001$), and COD removal ($r = 0.88$, $p < 0.001$) in

other words, however much the algae grew is a pretty good predictor of how much pollution they cleared.

4.7. Comparison With Literature Values

Set against published numbers, the consortium's TN removal ($83.6 \pm 4.7\%$) holds up well it's in the same range or better than what's reported for non-native *Chlorella* strains treating municipal wastewater under comparable retention times (68–82%), and it clearly outperforms conventional activated-sludge treatment at similar loading (45–65%) [7, 9, 27]. TP removal ($78.9 \pm 5.3\%$) likewise edges out standard biological nutrient removal systems that don't rely on chemical precipitation (typically 60–75%) [15]. COD removal ($78.2 \pm 3.8\%$) is roughly on par with aerobic membrane bioreactors (75–90%), but those systems burn through 0.8 – 1.5 kWh m⁻³ compared to under 0.1 kWh m⁻³ for aeration in the solar-driven algal system [6]. Taken together, this makes phycoremediation a reasonably strong, lower-energy option for a resource-constrained, semi-arid city like Jaipur.

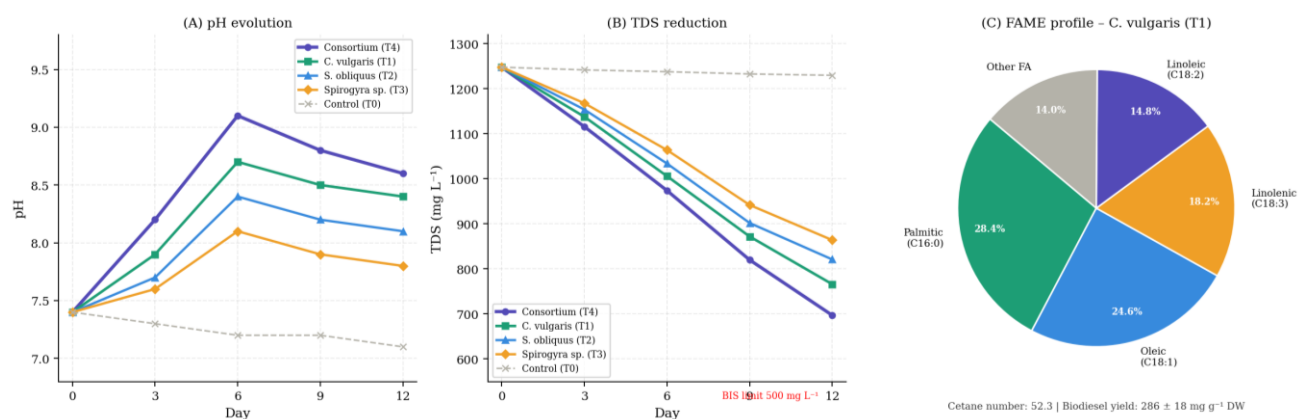


Figure 6. pH, TDS removal, and FAME composition.

4.8. Implications for Scale-Up and Circular Bioeconomy

Moving from lab flasks to field ponds isn't a simple matter of scaling up volume a few practical questions need answering first. The two obvious commercial options are open raceway ponds (0.2–0.3 m deep, paddle-wheel mixed) or closed tubular photobioreactors [28]. Raceways are far cheaper to build (roughly USD 200,000 per hectare versus USD 1–5 million for closed systems) but come with real downsides: contamination, temperature swings, and zooplankton grazing. On the harvesting side, the consortium may actually have an edge: the settling behaviour contributed by *S. obliquus* and *Spirogyra* sp. could cut harvesting costs, which normally eat up 20–30% of total production expenses in microalgal systems, by allowing gravity settling instead of energy-intensive centrifugation [19, 20].

A rough back-of-envelope economic picture also looks encouraging. At the observed productivity of $0.123 \text{ g L}^{-1} \text{ d}^{-1}$ and a modest treatment volume of 1 MLD, the system would produce around 123 kg of algal biomass per day. Given current prices for biodiesel feedstock (USD 0.6–0.8 kg^{-1}), biofertilizer (USD 0.4–0.6 kg^{-1}), and algal protein supplement (USD 2–5 kg^{-1}), that biomass stream could go a long way toward offsetting operating costs potentially even making the system close to cost-neutral at scale, which would matter a lot for cash-strapped municipal bodies [12, 29].

5. Future Directions and Recommendations

Getting this technology from where it stands now (roughly TRL 3, lab-scale) to something field-ready (TRL 5–6) will take work in a few specific directions.

The most immediate need is outdoor pilot testing 500-L raceway modules run across all four seasons, since Jaipur's climate is genuinely extreme: summer temperatures past 45 °C, monsoon dilution, and winter nights dropping close to 5 °C will all affect how the algae behave. Strategies like supplementary CO₂ dosing, timed harvesting to avoid culture crash, and possibly bio augmenting with cold-tolerant strains during winter deserve testing [28].

It's also worth exploring the consortium's capacity to remove heavy metals, given the documented chromium and lead contamination from the Sanganer textile tributaries. Understanding the biosorption isotherms and kinetics for Cr(VI), Pb(II), and Cd(II) would open the door to using this same system for multi-contaminant treatment [14].

A proper life-cycle assessment and techno-economic analysis covering energy use, what happens to the biomass afterward, land footprint, and carbon accounting would go a long way toward building the case needed for policy buy-in. Some early evidence from comparable Indian river projects suggests that pairing phycoremediation with anaerobic digestion of

harvested biomass for biogas could achieve a positive net energy balance once systems exceed 100 MLD [29].

There's also a practical near-term option worth exploring: using phycoremediation as a polishing step *after* conventional secondary treatment rather than a replacement for it. Adding algal high-rate ponds downstream of the existing oxidation ponds at Jaipur's Delawas plant could bring effluent quality into compliance while still generating saleable biomass without tearing out infrastructure that's already there [6, 28].

Longer term, genetic and metabolic engineering of the local strains aimed at boosting lipid accumulation or heavy-metal tolerance, along with synthetic-ecology approaches to designing more stable, higher-performing artificial consortia, could push performance well past what naturally occurring strains can offer [30].

6. Conclusion

This study is, as far as we can tell, the first systematic look at using indigenous microalgae to treat the badly polluted Dravyawati River in Jaipur. A few things stand out:

1. The three-species consortium *Chlorella vulgaris*, *Scenedesmus obliquus*, and *Spirogyra* sp. removed $83.6 \pm 4.7\%$ of TN, $78.9 \pm 5.3\%$ of TP, $76.4 \pm 4.1\%$ of BOD₅, and $78.2 \pm 3.8\%$ of COD over a 12-day retention time, clearly ahead of any single species and of conventional secondary treatment benchmarks.
2. *C. vulgaris* was the standout individual performer, thanks to its fast growth, efficient nitrogen uptake machinery, and mixotrophic flexibility; it's effectively the workhorse of the consortium.
3. With biomass productivity of $0.123 \text{ g L}^{-1} \text{ d}^{-1}$ and lipid content at $28.3 \pm 2.2\%$ DW, the consortium shows real promise for bioenergy, on top of possible uses in biofertilizer, animal feed, and bioethanol production all fitting neatly into a circular bioeconomy model.
4. Because these indigenous strains are already adapted to the Dravyawati's specific water chemistry, temperature swings, and light conditions, they outperformed what non-native strains typically achieve a strong argument for screening and using local algal communities as a starting point for any phycoremediation program, rather than importing standard lab strains.
5. Statistical testing (ANOVA and Tukey's HSD) confirmed these differences were real ($p < 0.0001$), and the low variability across replicates ($\text{CV} \leq 7.1\%$) suggests the system behaves consistently and reproducibly.

Taken together, this points to indigenous microalgal consortia as a genuinely promising route for restoring urban rivers in semi-arid regions like this one scientifically sound, economically reasonable, and environmentally aligned. With the pilot-scale work and LCA/TEA studies recommended above, phycoremediation could become a standard

part of urban water management in Rajasthan and similar river basins elsewhere, contributing directly to SDG 6 (clean water), SDG 11 (sustainable cities), and SDG 13 (climate action).

Abbreviations

BOD	Biochemical Oxygen Demand
COD	Chemical Oxygen Demand
TN	Total Nitrogen
TP	Total Phosphorus
TDS	Total Dissolved Solids
DO	Dissolved Oxygen
MLD	Million Litres per Day
BIS	Bureau of Indian Standards
NbS	Nature-based Solutions
CapEx	Capital Expenditure
APHA	American Public Health Association
HDPE	High-Density Polyethylene
CTAB	Cetyltrimethylammonium Bromide
PCR	Polymerase Chain Reaction
OD	Optical Density
PAR	Photosynthetically Active Radiation
VVM	Volume of Gas per Volume of Liquid per Minute
GC-MS	Gas Chromatography–Mass Spectrometry
FAME	Fatty Acid Methyl Ester
SD	Standard Deviation
ANOVA	Analysis of Variance
HSD	Honest Significant Difference
BP	Biomass Productivity
RE	Removal Efficiency
CV	Coefficient of Variation
BNR	Biological Nutrient Removal
LCA	Life Cycle Assessment
TEA	Techno-Economic Analysis
TRL	Technology Readiness Level
SDG	Sustainable Development Goal

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Author Contributions

Prity Sharma: Conceptualization, Project administration, Supervision, Writing – original draft

Chandan Singh: Formal Analysis, Investigation, Methodology

Arun Jain: Resources, Validation

Teena Pareek: Writing – review & editing

Preeti Sharma: Data curation, Methodology

Shalini Jauhari: Investigation, Writing – review & editing

Vinika Manglani: Software, Visualization

Pratima Vijavargiya: Investigation, Software

Kalpna Dhayal: Visualization, Writing – review & editing

Data Availability Statement

The datasets generated and analysed during the current study are available from the corresponding author upon reasonable request.

Conflicts of Interest

The authors declare no conflict of interest.

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