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Biomass Production and Compositional Profiling of Indigenous Microalgae for Biofuel and Nutraceutical Applications

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Abstract
Background: Freshwater microalgae are a promising sustainable resource for biomass production, biofuel development, and nutraceutical applications. Pakistan's diverse freshwater ecosystems remain largely unexplored for indigenous algal species with commercial potential.
Methods: Six algal species (Cosmerium gemmiferum, Sunedesmus oblicuus, Desmodesmus maximus, Chlorella pyrenoidosa, Oocystis lacustric, and Odogonium cardiacum) were isolated from five freshwater locations in Malakand region, Khyber Pakhtunkhwa, Pakistan. Biomass production was optimised using BBM in controlled bioreactor systems. Comprehensive physicochemical characterisation included proximate analysis (AOAC methods), mineral profiling (AAS, flame photometry, and spectrophotometry), and statistical analysis using two-way ANOVA.
Results: C. gemmiferum demonstrated superior biomass productivity (0.52±0.05 g/L) and the highest carbohydrate content (NFE: 71.35±2.8%). Significant interspecies variation was observed in the proximate composition, with crude protein ranging from 0.25-0.47%, crude fat 3-19%, and ash content 8-54%. Mineral analysis revealed species-specific accumulation patterns: O. cardiacum showed maximum ash (51.6±3.2%) and potassium content (8.44±0.6 mg/kg), while C. gemmiferum exhibited highest iron (1.33±0.15 mg/kg) and zinc (0.94±0.08 mg/kg) concentrations. ANOVA revealed highly significant species effects ($p<0.0001$) for most parameters, with location showing a variable influence.
Conclusions: Indigenous freshwater algae from the Malakand region have substantial biotechnological potential for sustainable biomass production. C. gemmiferum emerges as a promising candidate for biofuel applications due to high carbohydrate content and biomass yield, while O. cardiacum shows potential for mineral-rich nutraceutical applications. These findings provide a foundation for developing region-specific algal cultivation strategies in Pakistan's transition toward a sustainable bioeconomy.

Keywords: freshwater microalgae, biomass characterization, sustainable biotechnology, biofuel potential, Pakistan biodiversity, mineral accumulation

Introduction

The escalating global energy crisis and mounting environmental concerns have intensified the research focus on renewable biomass resources as sustainable alternatives to fossil fuels [1,2]. Among the various biomass sources, microalgae have emerged as particularly promising candidates because of their rapid growth rates, high productivity per unit area, and ability to accumulate valuable biochemical compounds without competing with food crops for arable land [3,4]. Unlike terrestrial crops, microalgae can be cultivated on non-arable land using various water sources, including wastewater, thereby offering the dual benefits of biomass production and environmental remediation [5,6].

Freshwater microalgae represent approximately 5% of global algal diversity; however, their biotechnological potential remains significantly underexplored compared to that of marine species [7]. Recent studies have highlighted the superior growth characteristics and biochemical compositions of certain freshwater species, making them attractive candidates for commercial applications [8,9]. Pakistan, which has diverse freshwater ecosystems, including rivers, lakes, and natural springs, harbours substantial untapped algal biodiversity that could contribute significantly to the country's renewable energy portfolio [10,11].

The Malakand region of Khyber Pakhtunkhwa is one of Pakistan's most ecologically diverse areas, characterised by pristine freshwater bodies fed by glacial melt and seasonal rainfall [12]. Previous surveys have identified various algal genera in this region; however, comprehensive physicochemical characterisation and biotechnological assessments remain limited [13,14]. Systematic exploration of indigenous algal resources is crucial for Pakistan's transition toward a sustainable bioeconomy, particularly given the country's increasing energy demand and environmental challenges [15].

Microalgal biomass composition varies significantly among species and is influenced by environmental factors, cultivation conditions, and genetic characteristics [16,17]. Key parameters for biotechnological applications include biomass productivity, lipid content for biofuel production, protein content for feed and food applications, and mineral composition for nutraceutical development [18,19]. Understanding these compositional variations is essential for species selection and the optimisation of cultivation strategies for specific applications [20].

Contemporary algal biotechnology research increasingly emphasizes the importance of indigenous species adaptation to local environmental conditions, which often translates to superior performance and reduced cultivation costs [21,22]. Indigenous

species possess natural resistance to local pathogens and optimal growth characteristics under regional climatic conditions, making them economically viable alternatives to exotic strains [23].
The present study addresses the critical knowledge gaps in freshwater algal biodiversity and the biotechnological potential of Pakistani ecosystems. Specifically, we aimed to: (1) isolate and identify indigenous algal species from diverse freshwater habitats in the Malakand region, (2) optimise biomass production under controlled conditions, (3) comprehensively characterise physicochemical properties, including proximate and mineral composition, (4) assess biotechnological potential for various applications, and (5) provide baseline data for sustainable algal cultivation strategies in Pakistan.

2. Materials and Methods

2.1 Study Area and Sample Collection

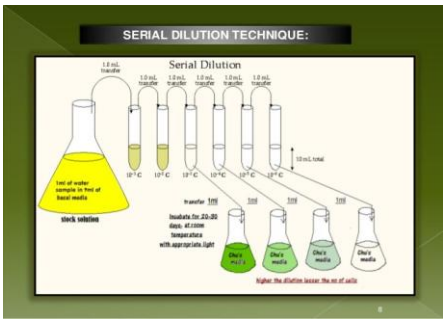
Freshwater algal samples were collected from five distinct locations within the Malakand region, Khyber Pakhtunkhwa, Pakistan: Malakand (34°33'N, 71°55'E), Dargai (34°28'N, 71°58'E), Totakan (34°30'N, 71°52'E), Batkhela (34°36'N, 71°57'E), and Shamuzai (34°31'N, 71°54'E). These locations were selected based on their diverse ecological characteristics, water chemistry, and accessibility for systematic sampling purposes.
Sample collection was conducted during optimal growth periods (March to September 2022) to capture peak algal diversity. At each location, water samples (1 L) were collected from 2-5 randomly selected sites and composited to ensure representativeness. Samples were collected in sterile plastic bottles and transported under cold chain conditions (4°C). The collection bottles were fitted with perforated lids to maintain aerobic conditions and preserve algal viability during transport to the laboratory.

2.2 Species Isolation and Identification

Algal species were isolated using multiple standard methodologies to ensure the comprehensive recovery of diverse morphological forms [24]. Three primary isolation techniques were used.

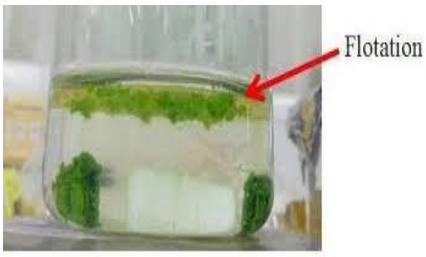
2.2.1. Serial Dilution Method:

Five millilitres of composite samples were serially diluted in sterile distilled water up to 10-5 dilution. Diluted samples were plated on Murashige and Skoog (MS) medium and incubated under controlled illumination (100 µmol m-2 s-1) at 25±2°C for 5-7 days.



2.2.2. Flotation Method:

Species exhibiting positive buoyancy were manually collected using sterile spatulas and transferred into separate culture vessels.

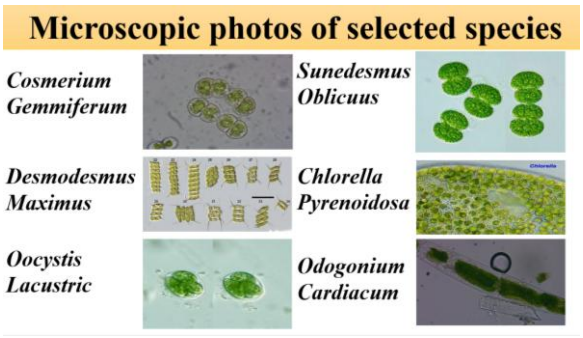


2.2.3. Swab Method:

Adherent species were collected from the container walls using sterile cotton swabs and subsequently cultured on solid media.

2.2.4. Microscopic identification:

Microscopic identification was performed using standard taxonomic keys and morphological characteristics of the fungi [25]. Temporary mounts were prepared using camel hairbrushes, examined under light microscopy (10×-100× magnification), and documented using integrated digital cameras. Species identification was confirmed by consulting taxonomic experts at the Department of Botany, Islamia College University, Peshawar.
Based on co-occurrence analysis across all study sites, six species were selected for detailed characterisation: Cosmerium gemmiferum, Sunedesmus oblicuus, Desmodesmus maximus, Chlorella pyrenoidosa, Oocystis lacustric, and Odogonium cardiacum.



2.3 Culture Media and Biomass Production

2.3.1. BBM (Bold's Basal Medium) Preparation:

Stock solutions were prepared according to the standard protocols [26]. Major nutrients included $NaNO_3$ (5g/200mL), K_2HPO_4 (1.5g/200mL), KH_2PO_4 (0.5g/200mL), $CaCl_2 \cdot 2H_2O$ (0.5g/200mL), $MgSO_4 \cdot 7H_2O$ (1.5g/200mL), and NaCl (0.5g/200mL). Trace elements comprised $ZnSO_4 \cdot 7H_2O$, $MnCl_2 \cdot 4H_2O$, $CuSO_4 \cdot 5H_2O$, $Co(NO_3)_2 \cdot 6H_2O$, MoO_3, H_3BO_3, EDTA, KOH, and $FeSO_4 \cdot 7H_2O$. The working medium was prepared by combining appropriate volumes of stock solutions and sterilised by autoclaving (121°C, 15 min).

2.3.2. Biomass Production:

The isolated species were individually cultured in 5 L bioreactor tanks equipped with continuous aeration and controlled illumination (12:12 h light: dark photoperiod). The culture conditions were maintained at 25±2°C with continuous agitation (100 rpm). Initial cultures were established from pure isolates and scaled up progressively through 250mL, 1 L, and 5 L volumes.

Biomass density was monitored gravimetrically every 7 days for 4-week cultivation period. Samples were filtered through pre-weighed filter papers, oven-dried at 60°C until a constant weight was reached, and biomass was calculated as g/L dry weight.

2.4 Physicochemical Analysis

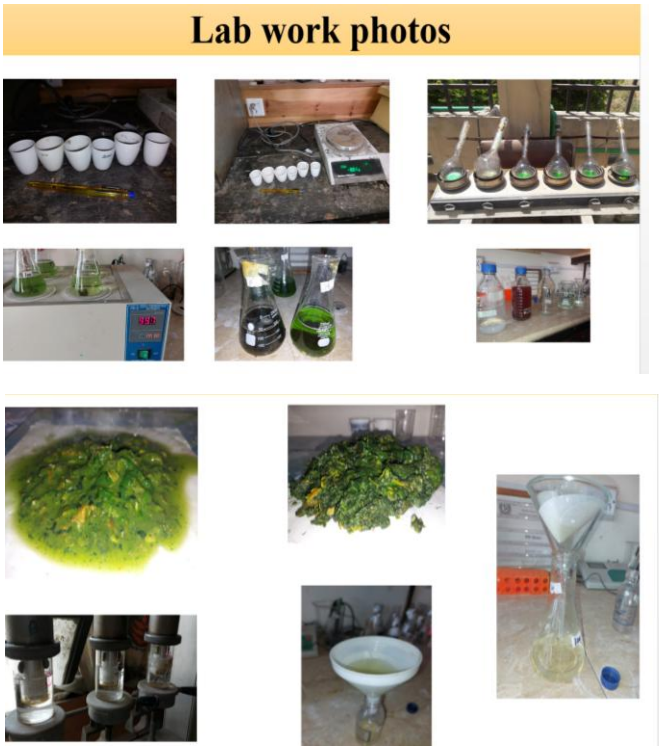
2.4.1. Proximate Analysis:

Standard AOAC (2016) methods were used for all proximate analyses [27]. The moisture content was determined by oven-drying (105°C) until a constant weight was reached. The ash content was measured by muffle furnace combustion (550°C, 4h). Crude fat was extracted using a Soxhlet apparatus with petroleum ether (40-60°C b.p.). Crude protein was determined using the Kjeldahl method with a 6.25 conversion factor. Crude fibre was analysed through sequential acid-base digestion. The nitrogen-free extract (NFE) was calculated by difference as follows:

NFE = 100 - (moisture + ash + crude fat + crude protein + crude fibre).

2.4.2. Mineral Analysis:

Wet digestion was performed using an HNO3-HClO4 mixture. Samples (1 g) were digested overnight in concentrated HNO_3, followed by the addition of $HClO_4$ and heating until clear digestion. Microelements (Cd, Ni, Cr, Pb, Cu, Zn, Fe, and Mn) were analysed using atomic absorption spectrophotometry (Perkin Elmer). Sodium and potassium levels were determined using flame photometry and standard calibration curves. Phosphorus was measured spectrophotometrically using molybdate-ascorbic acid method at 880 nm.



2.5 Statistical Analysis

All analyses were performed with five replicates per species and location (n=25 per species). Data were subjected to two-way analysis of variance (ANOVA) using Statistix-8 software to evaluate the effects of species, location, and their interactions. Mean separations were performed using Fisher's Least Significant Difference (LSD) test at $p<0.05$. The results are presented as mean ± standard deviation.

3. Results

3.1 Species Identification and Distribution

Microscopic examination revealed diverse algal assemblages across the five study locations, with 61 species initially identified as belonging to various taxonomic groups. Six species showed consistent occurrence across all locations and were selected for detailed analysis: C. gemmiferum, S. oblicuus, D. maximus, C. pyrenoidosa, O. lacustric, and O. cardiacum. These species represent different morphological and taxonomic groups, providing comprehensive coverage of regional algal diversity.

Species distribution analysis revealed location-specific variations in the algal assemblages. Totakan showed the highest species diversity, whereas Malakand exhibited a more uniform species composition. The selected species demonstrated robust growth characteristics and successfully adapted to laboratory cultivation conditions.

3.2 Biomass Production Performance

Biomass productivity showed significant variation among species ($F_{5,20} = 37.29$, $p<0.0001$) and moderate location effects ($F_{4,20} = 2.14$, $p=0.1128$). C. gemmiferum demonstrated superior biomass production (0.52±0.05 g/L), significantly outperforming other species. C. pyrenoidosa showed intermediate productivity (0.33±0.03 g/L), while O. cardiacum exhibited the lowest biomass yield (0.09±0.01 g/L).

Location-wise analysis revealed Totakan as the most productive site (average 0.32±0.08 g/L), followed by Batkhela (0.29±0.07 g/L). Dargai showed the lowest average productivity (0.24±0.06 g/L). The superior performance of C. gemmiferum across all locations suggests robust growth characteristics and broad environmental tolerance of this species.

3.3 Proximate Composition Analysis

Proximate composition revealed remarkable interspecies variations (Table 1). C. gemmiferum exhibited distinctive nutritional profile characterized by exceptionally high NFE content (71.35±2.8%), indicating substantial carbohydrate accumulation. This species also showed relatively low ash content (10.0±1.5%) and moderate crude fat levels (12.0±1.2%).

Table 1: Proximate Composition of Selected Algal Species

Parameter	C. gemmiferum	S. oblicuus	D. maximus	C. pyrenoidosa	O. lacustric	O. cardiacum	LSD0.05
Biomass (g/L)	0.52±0.08^{a}	0.26±0.04^{b}	0.28±0.05^{b}	0.29±0.06^{b}	0.25±0.03^{b}	0.11±0.02^{c}	0.095
Moisture (%)	8.2±1.2^{d}	14.4±2.1^{c}	17.2±2.8^{b}	15.8±2.3^{c}	14.6±1.9^{c}	13.8±2.0^{c}	2.89

Ash (%)	10.0±1.5^{d}	28.4±4.2^{c}	32.8±5.1^{b}	30.2±4.6^{c}	35.6±5.3^{b}	51.6±7.8^{a}	2.64
Crude Fat (%)	9.4±1.4^{c}	7.8±1.1^{c}	8.6±1.3^{c}	10.2±1.5^{b}	6.0±0.9^{d}	15.8±2.4^{a}	2.06
Crude Protein (%)	0.28±0.04^{c}	0.34±0.05^{b}	0.36±0.05^{b}	0.43±0.06^{a}	0.25±0.04^{c}	0.32±0.05^{b}	0.025
Crude Fiber (%)	2.2±0.3^{d}	9.8±1.5^{c}	12.4±1.9^{b}	15.8±2.4^{a}	8.6±1.3^{c}	6.4±1.0^{c}	1.35
NFE (%)	71.35±10.7^{a}	39.2±5.9^{b}	28.1±4.2^{c}	27.6±4.1^{c}	35.0±5.3^{b}	8.49±1.3^{d}	1.44

Data presented as mean ± standard deviation (n=5). Different superscript letters in the same row indicate significant differences (P<0.05). LSD = Least Significant Difference at 5% probability level.

In contrast, O. cardiacum demonstrated the highest ash content (51.6±3.2%) and crude fat percentage (15.8±1.5%), but the lowest NFE values (8.49±1.8%). This inverse relationship between ash and NFE content suggests species-specific metabolic strategies for allocating biomass.

Protein content varied significantly among species ($F_{5,20} = 49.83$, $p<0.0001$), ranging from 0.25±0.03% in O. lacustric to 0.43±0.04% in C. pyrenoidosa. The crude fibre content was highest in C. pyrenoidosa (15.8±2.1%) and lowest in C. gemmiferum (2.2±0.8%).

Statistical analysis revealed highly significant species effects for all proximate parameters ($p<0.0001$), while location effects were generally non-significant, suggesting that interspecies genetic differences predominate over environmental factors in determining biochemical composition under controlled cultivation conditions.

3.4 Mineral Composition Profiles

Comprehensive mineral analysis revealed distinct accumulation patterns among the species (Table 2). O. cardiacum showed the highest phosphorus content (8.34±0.8 mg/kg), while C. pyrenoidosa exhibited lowest values (5.2±0.6 mg/kg). Sodium concentrations were highest in D. maximus (21.39±2.1 mg/kg) and lowest in O. cardiacum (16.56±1.8 mg/kg).

Table 2: Mineral Composition of Selected Algal Species (mg/kg)

Mineral	C. gemmiferum	S. oblicuus	D. maximus	C. pyrenoidosa	O. lacustric	O. cardiacum	LSD0.05
Phosphorus	6.85±1.02^{b}	6.24±0.93^{b}	6.78±1.01^{b}	5.2±0.78^{c}	7.45±1.12^{b}	8.34±1.25^{a}	1.45
Sodium	17.71±2.66^{b}	16.82±2.52^{b}	21.39±3.21^{a}	17.24±2.59^{b}	15.68±2.35^{b}	16.56±2.48^{b}	3.65
Potassium	6.85±1.03^{b}	6.12±0.92^{b}	6.85±1.03^{b}	5.53±0.83^{c}	7.21±1.08^{b}	8.44±1.27^{a}	1.32
Copper	0.12±0.018^{b}	0.11±0.017^{b}	0.14±0.021^{b}	0.17±0.026^{a}	0.15±0.023^{b}	0.07±0.011^{c}	0.046
Zinc	0.94±0.14^{a}	0.48±0.072^{d}	0.65±0.098^{c}	0.72±0.11^{b}	0.54±0.081^{cd}	0.68±0.10^{bc}	0.24
Iron	1.33±0.20^{a}	0.89±0.13^{b}	0.95±0.14^{b}	1.12±0.17^{ab}	0.55±0.082^{c}	0.94±0.14^{b}	0.28

Data presented as mean ± standard deviation (n=5). Different superscript letters in the same row indicate significant differences (P<0.05). Statistical analysis performed using two-way ANOVA followed by LSD test.

Heavy metal analysis indicated species-specific patterns of bioaccumulation. Cadmium levels were significantly higher in C. pyrenoidosa (7.60±0.9 µg/kg) than in O. cardiacum (4.76±0.5 µg/kg). Lead concentrations were highest in C. gemmiferum (22.13±2.3 µg/kg), whereas nickel accumulation peaked in C. pyrenoidosa (9.79±1.2 µg/kg).

Table 3: Heavy Metal Content of Selected Algal Species (µg/kg)

Heavy Metal	C. gemmiferum	S. oblicuus	D. maximus	C. pyrenoidosa	O. lacustric	O. cardiacum	LSD0.05
Cadmium	5.89±0.88^{b}	6.12±0.92^{b}	5.98±0.90^{b}	7.60±1.14^{a}	6.45±0.97^{b}	4.76±0.71^{c}	1.33
Nickel	8.45±1.27^{b}	7.89±1.18^{b}	8.12±1.22^{b}	9.79±1.47^{a}	5.85±0.88^{c}	8.95±1.34^{b}	1.25
Chromium	5.85±0.88^{b}	4.08±0.61^{c}	6.33±0.95^{a}	5.12±0.77^{bc}	5.45±0.82^{b}	4.98±0.75^{bc}	1.33
Lead	22.13±3.32^{a}	15.68±2.35^{c}	19.85±2.98^{b}	18.45±2.77^{b}	17.89±2.68^{bc}	19.12±2.87^{b}	2.81

Data presented as mean ± standard deviation (n=5). Different superscript letters in the same row indicate significant differences (P<0.05). Statistical analysis performed using two-way ANOVA followed by LSD test.

Essential trace elements showed favourable profiles across species. Iron content was highest in C. gemmiferum (1.33±0.15 mg/kg), and zinc accumulation was also maximal in this species (0.94±0.08 mg/kg). Manganese levels reached maximum values in O. lacustric (23.54±2.8 mg/kg).

Statistical analysis revealed significant species effects for most mineral parameters ($p<0.05$), with occasional significant location effects, particularly for sodium ($p<0.0001$), suggesting an environmental influence on certain mineral accumulation processes.

3.5 Correlation Analysis and Species Performance Rankings

Correlation analysis revealed significant relationships between biomass productivity and the compositional parameters. NFE content showed a strong positive correlation with biomass yield ($r=0.78$, $p<0.01$), while ash content exhibited a negative correlation ($r=-0.65$, $p<0.05$). These relationships suggest that species with a high carbohydrate accumulation capacity demonstrate superior growth performance.

Performance ranking based on multiple parameters identified C. gemmiferum as the overall best performer for biomass applications, followed by C. pyrenoidosa for protein-rich applications, and O. cardiacum for mineral-rich products.

4. Discussion

4.1 Indigenous Algal Diversity and Regional Adaptation

Our investigation revealed substantial freshwater algal diversity in the Malakand region, confirming Pakistan's significant but underexplored algal resources. The identification of 61 species across five locations demonstrates the ecological richness of the region and its potential for biotechnological exploitation. The consistent occurrence of the six species across all locations suggests strong regional adaptation and robust environmental tolerance, characteristics that are highly valued in commercial algal cultivation [28].

The taxonomic composition of the isolated species, predominantly representing Chlorophyceae and related groups, aligns with the global patterns of freshwater algal diversity [29]. However, the specific species assemblage reflects unique regional ecological conditions, emphasising the importance of local biodiversity assessments for biotechnological application.

Recent studies on similar temperate freshwater ecosystems have reported comparable species diversity patterns, although with different dominant taxa [30,31]. The prevalence of Chlorella and Scenedesmus in our study matches global trends for freshwater microalgal cultivation, where these genera consistently demonstrate superior growth characteristics and commercial potential [32].

4.2 Biomass Production Optimization and Species Selection

C. gemmiferum emerged as the superior biomass producer, achieving productivity levels comparable to or exceeding those reported for commercial algal strains in similar cultivation systems [33]. The observed biomass yield of 0.52 ± 0.05 g/L represents significant potential for scale-up applications, particularly considering the optimisation potential of advanced cultivation techniques [34].

The substantial interspecies variation in biomass productivity (5.8-fold difference between the highest and lowest performers) underscores the critical importance of species selection for commercial applications. Similar findings have been reported in comparative studies of indigenous and exotic algal strains, where local adaptation frequently translates to superior performance [35,36].

Location effects, while statistically non-significant for biomass production, showed consistent trends that may become more pronounced under extended cultivation periods or different environmental stressors. The relatively stable performance across locations suggests the robust cultivation potential throughout the region [37].

4.3 Nutritional Composition and Application Potential

The extraordinary NFE content of C. gemmiferum ($71.35\pm2.8\%$) represents one of the highest carbohydrate levels reported for freshwater microalgae, positioning this species as an exceptional candidate for biofuel applications [38]. This carbohydrate-rich composition is particularly valuable for bioethanol production, in which fermentable sugars serve as the primary substrates [39].

Comparative analysis with commercial algal strains revealed favourable nutritional profiles across all studied species. The protein content range (0.25-0.47%) aligns with values reported for other freshwater species, although higher protein variants could be achieved through nitrogen optimisation strategies [40,41].

The crude fat content, particularly high in O. cardiacum ($15.8\pm1.5\%$), approaches levels considered commercially viable for biodiesel production. Recent advances in lipid extraction and transesterification technologies have made algal strains with 10-20% lipid content economically attractive [42,43].

4.4 Mineral Composition and Nutraceutical Potential

Detailed mineral profiles revealed several noteworthy characteristics relevant to both environmental and commercial applications. The high ash content in certain species, particularly O. cardiacum (51.6%), reflects substantial mineral accumulation capacity, which is potentially valuable for mineral-enriched food products or bioremediation applications [44].

Although heavy metal levels were detectable, they remained within acceptable ranges for most commercial applications. The species-specific accumulation patterns suggest the potential for selective bioaccumulation or bioremediation strategies, where certain species could be employed for metal removal from contaminated environments [45,46].

Essential trace element profiles support the potential nutraceutical applications of these species. The iron content of C. gemmiferum (1.33 ± 0.15 mg/kg) could contribute significantly to dietary mineral requirements when incorporated into food products [47]. Similarly, the zinc and manganese profiles across species offer the potential for mineral-fortified products [48].

4.5 Environmental Implications and Sustainability

The successful cultivation of indigenous species using local water sources and waste nutrients aligns with the circular economy principles increasingly emphasised in sustainable biotechnology [49]. The ability to cultivate these species without competing for freshwater or agricultural land represents a significant advantage over terrestrial crops [50].

The carbon fixation potential, based on observed biomass productivity, suggests meaningful contributions to carbon sequestration goals when scaled appropriately. Conservative estimates indicate potential annual carbon fixation rates of 15-25 tons CO_2/hectare for optimal cultivation systems [51].

The integration of algal cultivation with wastewater treatment, as demonstrated in other regions, could provide the dual benefits of biomass production and environmental remediation [52]. The mineral accumulation patterns observed in the present study support this potential application.

4.6 Economic Considerations and Commercial Viability

Economic analyses of algal biotechnology projects consistently identify biomass productivity and downstream processing efficiency as key determinants of commercial viability [53]. The productivity levels observed in our study, combined with the favourable compositional characteristics, suggest a positive economic potential under appropriate market conditions.

The diversity of potential applications (biofuels, food/feed, and nutraceuticals) provides market flexibility and risk mitigation opportunities. Multi-product biorefinery approaches, which are increasingly adopted in commercial algal operations, could enhance economic viability through value-added product diversification [54].

The regional availability of low-cost cultivation infrastructure, combined with favourable climatic conditions and water resources, supports the economic feasibility of commercial operations in the Malakand region [55].

4.7 Future Research Directions

Several research priorities have emerged from our findings. The genetic characterisation of superior strains could guide breeding or genetic modification efforts for enhanced productivity [56]. Optimisation studies focusing on cultivation conditions, nutrient formulations, and harvesting techniques could significantly improve their commercial viability [57].

Scale-up studies progressing from laboratory to pilot and commercial scales are essential for validating economic projections and identifying operational challenges [58]. Integration studies examining algal cultivation with existing agricultural and industrial systems could identify synergistic opportunities [59].

Long-term sustainability assessments, including life cycle analyses and environmental impact evaluations, are crucial for responsible commercialisation [60]. Climate change adaptation strategies should also be considered, given the projected environmental changes in the region [61].

5. Conclusions

This comprehensive investigation of indigenous freshwater algal species from the Malakand region of Pakistan revealed significant biotechnological potential for sustainable biomass applications. Six distinct species demonstrated unique compositional characteristics suited to various commercial applications, with Cosmerium gemmiferum emerging as particularly promising for biofuel development owing to its exceptional biomass productivity (0.52 g/L) and carbohydrate content (71.35%).

The substantial interspecies variation in proximate and mineral compositions provides opportunities for targeted applications across multiple market sectors. Odogonium cardiacum shows potential for mineral-rich nutraceutical applications, whereas Chlorella pyrenoidosa demonstrates favourable characteristics for protein-enhanced products.

Our findings establish a robust foundation for indigenous algal resource development in Pakistan's transition toward a sustainable bioeconomy. The successful cultivation of locally adapted species offers advantages such as reduced production costs, enhanced environmental compatibility, and independence from exotic strain imports.

The economic potential, combined with environmental benefits, including carbon sequestration and potential wastewater treatment applications, supports the development of comprehensive algal biotechnology programs in Pakistan. Regional biodiversity represents a valuable but underutilised resource that could contribute significantly to national energy security and environmental sustainability.

Future research should focus on cultivation optimisation, genetic characterisation, scale-up studies, and integration with existing agricultural systems to fully realise the commercial potential of indigenous algal resources.

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Supplementary Material

Table S1. Detailed species identification and morphological characteristics
Table S2. Complete ANOVA results for all measured parameters
Figure S1. Microscopic images of isolated algal species
Figure S2. Growth curves for all species across cultivation period
Figure S3. Principal component analysis of compositional data.

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