Natural Resources for Human Health
eISSN: 2583-1194

Original Research

Received 15 July 2026
Revised 27 Aug 2026
Accepted 11 Sep 2026

Natural Resources for Human Health 2026, Vol. 6 (21s): 666–686

KEYWORDS:
Spirulina platensis, Biochemical Composition, Nutritional Quality, Zarrouk's Medium, Animal Waste-Based Medium, Protein Content, Phycobilin and Carotenoid Content, Mineral Enrichment

Comparative Biochemical Analysis of Spirulina Platensis Grown in Zarrouk's Medium and Cow Dung-Based Medium

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ABSTRACT: Spirulina platensis is a nutritionally important cyanobacterium widely recognized for its high-value bioactive compounds, including proteins, amino acids, pigments, carbohydrates, essential minerals, and antioxidant molecules. The present study aimed to evaluate the biochemical composition and nutritional quality of S. platensis cultivated under different culture conditions using Zarrouk's medium and an animal waste-based medium. The study also focused on maintaining culture purity, controlling contamination, and analyzing major biochemical constituents. The results demonstrated significant variation in biochemical parameters between the two media. Spirulina grown in Zarrouk's medium showed higher protein content (54.13 ± 0.31%), amino acid content (1.72 ± 0.063 mg/g), carotenoid content (5.51 ± 0.11 µg/ml), carbohydrate content (11.3 ± 0.17%), and phycobilin concentration (0.12 ± 0.002 mg/g), indicating enhanced metabolic activity under optimized nutrient conditions. In contrast, animal waste medium supported higher accumulation of certain minerals, including iron, zinc, and copper, suggesting its potential for mineral enrichment of Spirulina biomass. Chlorophyll content was comparatively higher in animal waste medium, reflecting variation in photosynthetic pigment production under different nutrient environments. The findings highlight that culture conditions strongly influence the biochemical profile of S. platensis. Optimized cultivation strategies can improve the production of nutritionally enriched Spirulina biomass for applications in food, health supplements, pharmaceuticals, and biotechnology.

2. INTRODUCTION:

Spirulina platensis (syn. *Arthrospira platensis*) is a filamentous cyanobacterium valued for its high nutritional content and diverse bioactive compounds. It is widely used in food, pharmaceutical, nutraceutical, and biotechnology industries due to its rich composition of proteins, carbohydrates, lipids, pigments, vitamins, minerals, and antioxidants (Musa *et al.*, 2025; Spínola *et al.*, 2024). Proteins constitute approximately 55–70% of the dry biomass and contain all essential amino acids, making *Spirulina* an excellent nutritional supplement. Carbohydrates account for 15–25% of the biomass and mainly occur as polysaccharides with reported antioxidant, antiviral, and immunostimulatory properties. Lipids comprise about 5–14% of dry weight and are

rich in polyunsaturated fatty acids, particularly γ-linolenic acid (GLA), which contributes to various health benefits (Musa *et al*., 2025).

The characteristic blue-green colour of *Spirulina* is due to the presence of chlorophyll, carotenoids, and phycobiliproteins, especially C-phycocyanin, a potent antioxidant with significant therapeutic potential. In addition, *Spirulina* contains several vitamins, including B-complex vitamins, vitamin E, and provitamin A, along with essential minerals such as iron, calcium, magnesium, zinc, and selenium (Salih *et al*., 2025; Marjanović *et al*., 2024).

The biochemical composition of *S. platensis* is influenced by environmental and cultivation conditions. Zhou *et al.* (2018) reported that zinc exposure enhanced pigment and fatty acid production despite reducing growth, while Shabana *et al.* (2017) observed that moderate gamma irradiation increased protein, phenolic content, vitamins, and minerals. Therefore, the evaluation of biochemical constituents under different cultivation conditions is important for optimizing biomass quality and enhancing the commercial value of *Spirulina platensis*.

3. MATERIAL AND METHODS

3.1 Preparation of Zarrouk's Medium

Zarrouk's medium was prepared according to the method described by A. Sharma, Kaur, Sibbal Chatli *et al.* (2019). The medium was prepared by dissolving sodium bicarbonate (16.0 g), dipotassium hydrogen phosphate (0.5 g), sodium nitrate (2.5 g), potassium sulfate (1.0 g), sodium chloride (1.0 g), magnesium sulfate (0.2 g), calcium chloride (0.04 g), and ferrous sulfate (0.01 g) in 1000 mL of distilled water with continuous stirring to ensure complete dissolution of all components. The pH of the medium was adjusted to 9.0–11.0 using sodium carbonate or dilute hydrochloric acid, as required. The prepared medium was sterilized by autoclaving at 121°C for 15 min and allowed to cool to room temperature. Actively growing *Spirulina platensis* culture was then inoculated aseptically into the prepared medium. and incubated under controlled conditions of light and temperature. The optimal temperature for Spirulina platensis culture is in the range of 30- 35°C.

3.2 Preparation of Animal Waste-Based Medium

Fresh cow dung was collected, air-dried, and mixed with distilled water in a ratio of 1:10 (w/v) to prepare a slurry. The slurry was allowed to settle, after which the supernatant was separated and filtered through muslin cloth to remove coarse particles. The filtrate was further diluted with distilled water to obtain the required concentration of the animal waste-based medium. The prepared medium provided nutrients such as nitrogen, phosphorus, carbon, and trace elements required for the growth of *Spirulina platensis*. The pH of the medium was adjusted to the alkaline range of 9.0–9.5. The prepared medium was sterilized or subjected to the required treatment before inoculation with actively growing *S. platensis* culture. (Sofi Mohammad Yasin *et al*., 2012)

3.3 Inoculation and Cultivation of *Spirulina platensis*

An actively growing laboratory culture of *Spirulina platensis* was used as the inoculum for cultivation in Zarrouk's medium and animal waste-based medium. For each culture medium, three separate flasks were maintained as independent experimental replicates. Each flask contained 150 mL of the respective culture medium supplemented with 100 mL of actively growing *S. platensis* inoculum, giving a total culture volume of 250 mL per flask.

The inoculated cultures were maintained under the respective cultivation conditions and were manually shaken periodically to ensure uniform mixing of the culture and adequate exposure to light. The cultures were allowed to grow until sufficient biomass development was achieved. Growth was monitored at regular intervals by recording the optical density of the cultures, and the observations were used to evaluate the growth pattern of *S. platensis* in the two culture media.

3.4 Growth Monitoring

The growth of *Spirulina platensis* was monitored throughout the cultivation period in both Zarrouk's medium and animal waste-based medium. Culture growth was recorded at regular intervals by measuring the optical density of the cultures at the selected wavelength. The recorded values were used to construct growth curves by plotting optical density against cultivation time. The growth patterns obtained in the two culture media were compared to evaluate differences in growth and biomass development during cultivation.

3.5 Harvesting of *Spirulina platensis* from Zarrouk's Medium

Fresh Spirulina grows on the surface of the culture media. To harvest *Spirulina platensis*, muslin cloth was used within 10–15 days. Above layer of *Spirulina platensis* was removed from the medium and allowed to dry completely at room temperature. *Spirulina platensis* sample was scraped out from the foil or muslin cloth after drying completely at room temperature. Then, the sample was grinded by using pestle mortar to make it a fine powdered. The dried form of *Spirulina platensis* was stored in Eppendorf tubes or vials. (A. Sharma, Kaur, Sibbal Chatli, *et al*., 2019)

3.6 Harvesting of *Spirulina platensis* from Animal Waste-Based Medium

Cultures were harvested after sufficient biomass development, usually within 10–15 days, depending on environmental conditions. Harvesting was carried out by filtration through nylon cloth or sieve, followed by repeated washing with clean water to remove excess salts and impurities. The harvested biomass was dried either by solar drying or in a hot air oven at 45–50°C and stored for further biochemical analysis. The use of cow dung-based medium significantly reduced cultivation cost while supporting effective biomass production of *Spirulina platensis*. (Sofi Mohammad Yasin *et al*., 2012)

3.7 Determination of Total Biomass and Biomass Productivity

The final dry biomass produced in each culture medium was determined gravimetrically after complete drying of the harvested biomass. The biomass obtained from each culture was weighed and expressed as grams (g) per culture.

Biomass productivity was calculated using the following equation:

Biomass productivity (g day^{-1}) = Final dry biomass (g) / Cultivation period (days)

The final biomass production and biomass productivity of *S. platensis* cultivated in Zarrouk's medium and animal waste-based medium were compared.

3.8 Preparation of Biomass for Biochemical Analysis

The dried *S. platensis* biomass was finely powdered using a pestle and mortar and stored in clean, dry containers until biochemical and mineral analyses. The powdered biomass was used for estimation of protein, total amino acids, carbohydrates, carotenoids, chlorophyll, phycobilin pigments, and mineral elements.

3.9 Biochemical Test

(a) Protein estimation (Lowery *et al*., 1951)

(i) Alkaline Copper Reagent

- Solution A: 2% Na_2CO_3 in 0.1 N NaOH
- Solution B: 1% $CuSO_4 \cdot 5H_2O$
- Solution C: 2% sodium potassium tartrate
- Mix A: B:C in the ratio 100:1:1 just before use

(ii) Procedure

Protein content of *Spirulina platensis* biomass was estimated using the Lowry method. Dried algal biomass (50–100 mg) was homogenized in distilled water and centrifuged to obtain a clear supernatant, which was used for analysis. An aliquot of the extract (1 mL) was mixed with freshly prepared alkaline copper reagent and incubated at room temperature for 10 minutes to allow the Biuret reaction to occur. Subsequently, diluted Folin–Ciocalteu reagent was added, mixed immediately, and the reaction mixture was incubated in the dark for 30 minutes for color development. The intensity of the blue color formed was measured spectrophotometrically at 660 nm. Protein concentration was determined using a standard curve prepared with bovine serum albumin (BSA), and results were expressed as milligrams of protein per gram of dry biomass.

(b) Amino Acid estimation

Total amino acid content of *Spirulina platensis* biomass was estimated using the ninhydrin method (Moore & Stein, 1948). Dried algal biomass (50–100 mg) was homogenized in distilled water and hydrolyzed with 6 N hydrochloric acid at 100–110 °C for 18–24 hours to release free amino acids. After hydrolysis, the sample was neutralized and filtered to obtain a clear extract. An aliquot of the extract was reacted with ninhydrin reagent and heated in a boiling water bath for 15 minutes to develop a purple-colored complex. After cooling, the absorbance was measured spectrophotometrically at 570 nm. Total amino acid concentration was calculated using a standard curve prepared with a known concentration of glycine, and the results were expressed as milligrams of amino acids per gram of dry biomass.

(c) Total Carotene content (Lichtenthaler, 1987)

Total carotene content of *Spirulina platensis* biomass was estimated using the solvent extraction and spectrophotometric method. Dried algal biomass (50–100 mg) was homogenized with chilled 80% acetone until complete pigment extraction was achieved. The homogenate was centrifuged, and the supernatant was collected. Extraction was repeated until the residue became colourless, and all supernatants were pooled. The absorbance of the extract was measured at appropriate wavelengths (470, 663 and 645 nm) using a UV–Visible spectrophotometer, and total carotene content was calculated using standard equations. The results were expressed as milligrams of carotene per gram of dry biomass.

First, chlorophyll a and b are calculated:

$$\text{Chlorophyll a (µg/mL)} = 12.7(A_{663}) - 2.69(A_{645})$$
$$\text{Chlorophyll b (µg/mL)} = 22.9(A_{645}) - 4.68(A_{663})$$

Then, total carotenoids (carotenes) are calculated as:

$$\text{Total carotenoids (µg/mL)} = \frac{1000(A_{470}) - 1.82(\text{Chl a}) - 85.02(\text{Chl b})}{198}$$

Expression of Results

$$\text{Total carotene (mg/g dry weight)} = \frac{\text{Carotenoids (µg/mL)} \times V}{1000 \times W}$$ Type equation here.

Where:

- $A_{470}, A_{663}, A_{645}$= absorbance at respective wavelengths
- V= final volume of extract (mL)
- W= weight of dry sample (g)

(d) Carbohydrate estimation (Total Soluble Sugar)

Total soluble sugars in *Spirulina platensis* were estimated following ethanolic extraction. Dried algal biomass (50 g) was homogenized with 20 mL of 80% ethanol using a pestle and mortar to ensure complete extraction of soluble sugars. The homogenized sample was kept overnight at room temperature to enhance extraction efficiency. The mixture was then centrifuged at 1200 rpm for 15 minutes, and the supernatant was collected carefully. The ethanolic extract was concentrated on a water bath following the method of (Loomis & Shull, 1973). The concentrated extract was finally diluted with distilled water to make a known volume of 50 mL and used for quantitative estimation of total soluble sugars using standard colorimetric procedures.

(e) Total Chlorophyll Content (Arnon I. Daniel, 1948)

Total chlorophyll content of *Spirulina platensis* biomass was estimated using the acetone extraction method. Fresh or dried algal biomass (50–100 mg) was homogenized thoroughly with 80% acetone under low-light conditions to prevent pigment degradation. The homogenate was centrifuged to obtain a clear supernatant, and extraction was repeated until the residue became colorless. The pooled extract was made up to a known volume, and absorbance was measured spectrophotometrically

at 663 nm and 645 nm. Total chlorophyll content was calculated using standard equations, and the results were expressed as milligrams of chlorophyll per gram of dry biomass.

Chlorophyll content was calculated using the formula as described below:

$$\text{Total chlorophyll (mg/ g F.wt.)} = \frac{20.2 \times A663 + 8.02\ A645 \times V}{1000 \times W}$$

Where,

A = absorbance at specific wave length

V = final volume (ml) of chlorophyll extract with 80% acetone = 10

W = weight (g) of fresh leaf tissue. = 200mg = 0.2g

(f) Phycobilin content estimation (Bennett & Bogorad, 1973; Silveira *et al.*, 2007, **Moraes *et al.*, 2011**)

Phycobilins content of *Spirulina platensis* was estimated using aqueous extraction followed by spectrophotometric analysis. Dried algal biomass (50–100 mg) was suspended in phosphate buffer (0.05 M, pH 6.8) and subjected to repeated freezing and thawing to facilitate complete cell disruption and pigment release. The extract was centrifuged to obtain a clear supernatant containing phycobiliproteins. Absorbance of the extract was measured at 562 nm, 615 nm, and 652 nm using a UV–Visible spectrophotometer. Concentrations of phycocyanin, allophycocyanin, and phycoerythrin were calculated using standard equations, and total phycobilins content was expressed as milligrams per gram of dry biomass.

Phycocyanin (PC)

$$\text{PC (mg/mL)} = \frac{A_{615} - 0.474(A_{652})}{5.34}$$

Allophycocyanin (APC)

$$\text{APC (mg/mL)} = \frac{A_{652} - 0.208(A_{615})}{5.09}$$

Phycoerythrin (PE)

$$\text{PE (mg/mL)} = \frac{A_{562} - 2.41(\text{PC}) - 0.849(\text{APC})}{9.62}$$

Total Phycobilins

$$\text{Total phycobilins (mg/mL)} = \text{PC} + \text{APC} + \text{PE}$$

Expression on Dry Weight Basis

$$\text{Phycobilins (mg/g dry weight)} = \frac{\text{Total phycobilins (mg/mL)} \times V}{W}$$

Where:

- $A_{562}, A_{615}, A_{652}$= absorbance at respective wavelengths
- V= final volume of extract (mL)

- W= dry weight of biomass (g)

(g) Mineral Analysis (Kambou Sansan Philippe *et al.*, 2018)

Mineral content of *Spirulina platensis* biomass was determined using acid digestion followed by instrumental analysis. Dried algal biomass was finely powdered, and a known quantity (0.5–1.0 g) was digested with a mixture of concentrated nitric acid and perchloric acid until a clear solution was obtained. After cooling, the digest was filtered and diluted to a known volume with distilled water. The concentrations of major and trace minerals such as calcium, magnesium, iron, sodium, potassium, zinc, and copper were estimated using atomic absorption spectrophotometry (AAS) or flame photometry, as appropriate. Analysis proceeds using a flame atomic absorption spectrophotometer, where specific wavelengths are selected for each mineral—such as 766.5 nm for potassium, 422.7 nm for calcium, 285.2 nm for magnesium, 248.3 nm for iron, 279.5 nm for manganese, 589 nm for sodium, and others—to measure optical densities and determine concentrations via comparison to standards. Phosphorus is often quantified separately by colorimetry, while quality checks include recovery tests and replicates for accuracy. Mineral content was calculated using standard calibration curves prepared from known standard solutions, and the results were expressed as milligrams per gram of dry biomass.

3.10. Statistical Analysis

All experiments were performed in triplicate (n = 3), and the results are presented as mean ± standard deviation (SD). Statistical analysis was carried out using GraphPad Prism version 8 (GraphPad Software Inc., San Diego, CA, USA). Differences between *Spirulina platensis* cultivated in Zarrouk's medium and Animal waste medium were analyzed using an independent two-tailed Student's *t*-test. One-way analysis of variance (ANOVA) was also performed to compare the two culture media. Since only two experimental groups were compared, both statistical tests yielded equivalent conclusions. A probability value of $p < 0.05$ was considered statistically significant

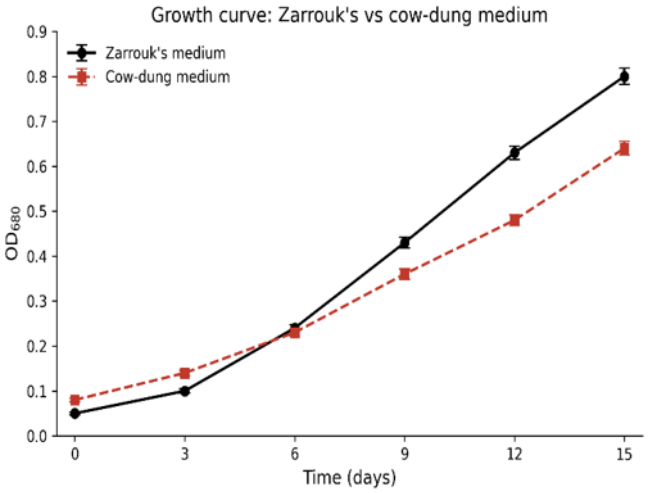
4. RESULT:

4.1. Growth Curve of *Spirulina platensis*

The growth pattern of *Spirulina platensis* was monitored periodically during cultivation in Zarrouk's medium and animal waste-based medium by measuring the optical density of the cultures. The changes in optical density over the cultivation period were used to construct the growth curves.The growth curves showed the variation in *S. platensis* growth with cultivation time in the two culture media. The growth of *Spirulina platensis* was monitored by measuring OD_{680} at regular intervals during the 15-day cultivation period. In Zarrouk's medium, the OD_{680} increased gradually from 0.050 ± 0.003 on day 0 to 0.800 ± 0.018 on day 15**.** Similarly, in cow-dung medium, the OD_{680} increased from 0.080 ± 0.004 to 0.640 ± 0.015 during the same period. The progressive increase in OD_{680} indicated continuous growth of *S. platensis* in both culture media. **(Table 1)(Graph-1)**

Table 1. Growth of *Spirulina platensis* in Zarrouk's and cow-dung media over 15 days.

DAYS	Zarrouk's medium (OD_{680})	cow-dung medium (OD_{680})
0	0.050 ± 0.003	0.080 ± 0.004
3	0.100 ± 0.005	0.140 ± 0.006
6	0.240 ± 0.008	0.230 ± 0.008
9	0.430 ± 0.012	0.360 ± 0.012
12	0.630 ± 0.015	0.480 ± 0.012
15	0.800 ± 0.018	0.640 ± 0.015



Graph-1: Growth of *Spirulina platensis* in Zarrouk's and cow-dung media, measured as OD_{680} (mean ± SD, n = [3]).

4.2. Biomass Production of *Spirulina platensis*

The highest dry biomass was obtained in Zarrouk's medium (2.69 g), while the animal waste-based medium produced 1.45 g. Thus, Zarrouk's medium resulted in approximately 1.85-fold higher biomass production. The higher biomass may be associated with the balanced nutrient availability of Zarrouk's medium (Gami *et al.*, 2011; Sharma *et al.*, 2014). Although animal waste can provide nutrients for algal growth, variations in nutrient composition and availability may explain the lower biomass observed in the present study (Markou & Georgakakis, 2011; Madkour *et al.*, 2012).

4.3. Biochemical Test of *Spirulina platensis*

4.3.1 Protein estimation

The protein content of *Spirulina platensis* cultivated in different media was estimated using the Lowry method. The results revealed that protein concentration varied significantly depending on the nutrient composition of the culture medium. The protein estimation assay is shown in **Figure 1**, while the comparative effect of culture media on protein content is presented in **Graph 2** and **Table 2**. The maximum protein content was observed in Zarrouk's medium (54.13 ± 0.31%), whereas the minimum value was recorded in Animal waste medium (37.96 ± 0.23%).

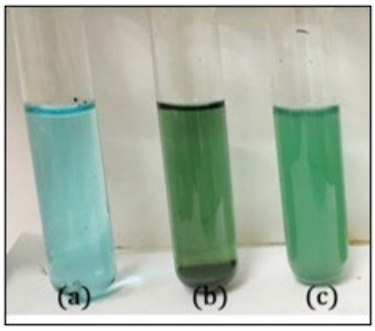
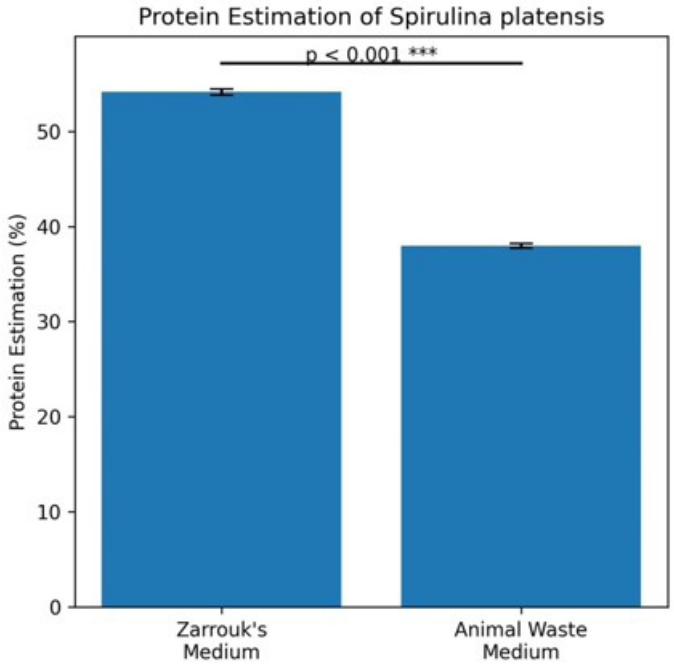


Figure 1: Protein estimation of *Spirulina platensis* (a) Control (b) Zarrouk's Media (c) Animal Media



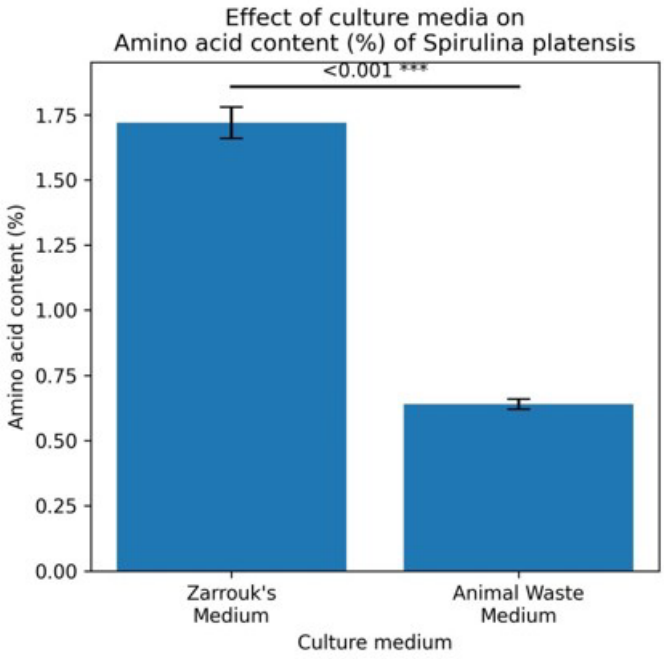
Graph-2: Effect of culture media on the protein content of *Spirulina platensis*. Values are expressed as mean ± SD (n = 3). Statistical significance between Zarrouk's medium and Animal waste medium was determined using an independent two-tailed Student's *t*-test. $P < 0.001$.

The comparatively higher protein content in Zarrouk's medium indicates better nitrogen availability and favourable growth conditions. These findings suggest that nutrient-rich media enhance protein biosynthesis in *Spirulina platensis*, which is consistent with its known high protein nature.

4.3.2 Amino Acid Content

The amino acid composition of *Spirulina platensis* cultivated under different media conditions was analyzed to evaluate its nutritional quality. The results revealed significant variation in amino acid content among the different treatments. The total amino acid content was found to be highest in Zarrouk's medium (1.72 ± 0.063 mg/g), whereas the lowest value was recorded in Animal waste medium (0.643 ± 0.016 mg/g) **(**Graph-3**).**

The concentration of amino acids was comparatively higher in Zarrouk's medium, suggesting that nutrient-rich conditions favour enhanced protein synthesis and amino acid accumulation **Graph-3.**



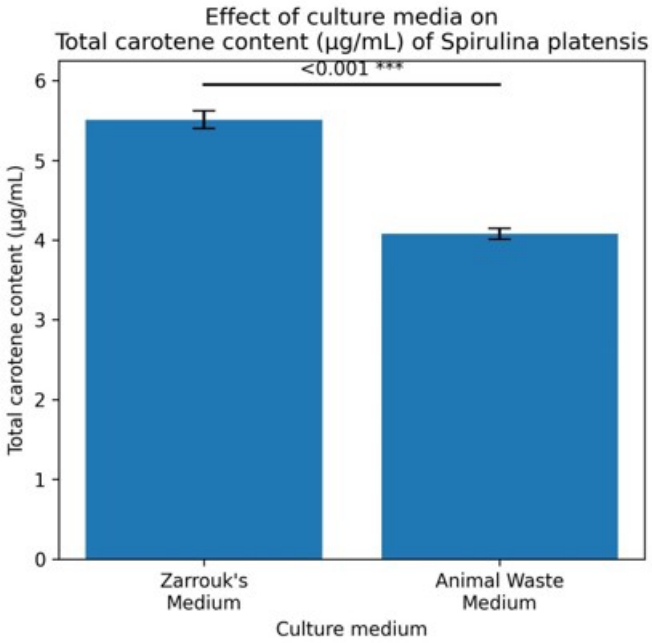
Graph-3: Effect of culture media on the amino acid content of *Spirulina platensis*. Values are expressed as mean ± SD (n = 3). Error bars represent standard deviation. Statistical significance was determined using an independent two-tailed Student's t-test. $P < 0.001$.

4.3.3 Total Carotene content

Carotenoid content showed significant variation among the experimental samples (Figure-2). The maximum carotenoid concentration was observed in Zarrouk's medium (5.51 ± 0.11 µg/ml), while the minimum was recorded in Animal waste medium (4.15 ± 0.07 µg/ml) (Graph-4).Higher carotenoid levels suggest enhanced protective mechanisms against oxidative stress and improved light-harvesting efficiency.



Figure 2: Total Carotenoid Content of *Spirulina platensis* (a) Control (b) Zarrouk's Media (c) Animal Media



Graph-4: Effect of culture media on the total carotene content of *Spirulina platensis*. Values are expressed as mean ± SD (n = 3). Error bars represent standard deviation. Statistical significance was determined using an independent two-tailed Student's t-test. $P < 0.001$.

4.3.4 Carbohydrate estimation

(i) Total Soluble Sugar

Total soluble sugar was estimated using the phenol–sulphuric acid method(Figure 3). The results indicated a noticeable variation among different culture conditions. The highest carbohydrate content was recorded in Zarrouk's medium (11.3 ± 0.17 %), while the lowest was observed in Animal waste medium (8.97 ± 0.11 %) (Graph-5). The increased carbohydrate accumulation in Zarrouk's medium may be attributed to optimal carbon availability and metabolic activity. These observations indicate that carbohydrate synthesis in *Spirulina* is highly influenced by nutrient composition and environmental conditions.

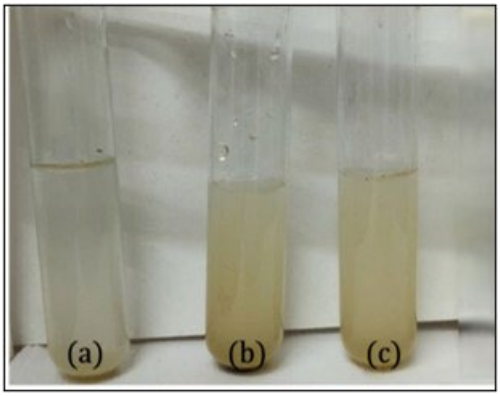
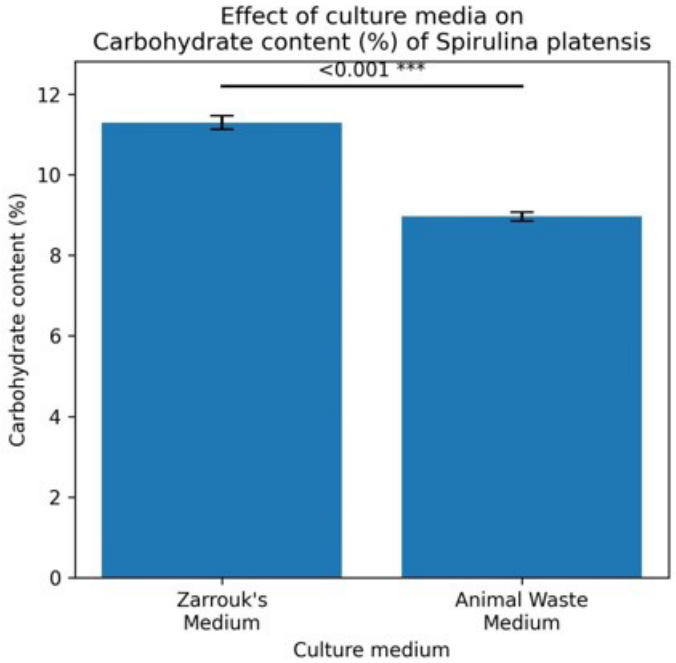


Figure 3: Total Soluble sugar of *Spirulina platensis* (a) Control (b) Zarrouk's Media

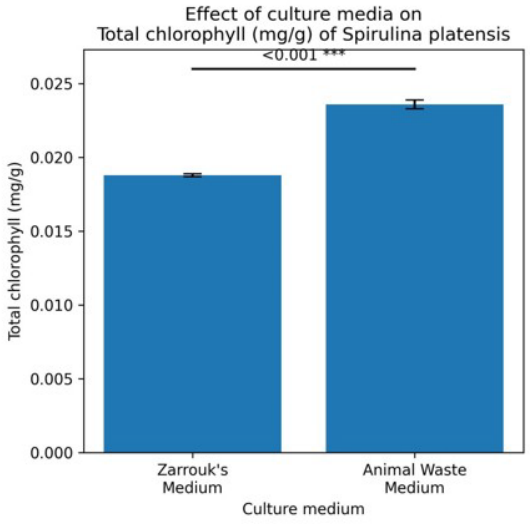
(c) Animal Media



Graph-5: Effect of culture media on the carbohydrate content of *Spirulina platensis*. Values are expressed as mean ± SD (n = 3). Error bars represent standard deviation. Statistical significance was determined using an independent two-tailed Student's t-test. $P < 0.001$.

4.3.5 Total Chlorophyll Content

The total chlorophyll content of *Spirulina platensis* was estimated spectrophotometrically. Among the different treatments, the highest chlorophyll content was observed in Animal waste medium (0.0236 ± 0.0003 mg/g), whereas the lowest was recorded in Zarrouk's medium (0.0189 ± 0.0003 mg/g) (Graph-6). The variation in chlorophyll content indicates differences in photosynthetic efficiency under varying nutrient conditions.



Graph-6: Effect of culture media on the total chlorophyll content of *Spirulina platensis*. Values are expressed as mean ± SD (n = 3). Error bars represent standard deviation. Statistical significance was determined using an independent two-tailed Student's t-test. $P < 0.001$.

4.3.6 Phycobilin content estimation

Phycobilin pigments were estimated to evaluate the accessory photosynthetic pigments in *Spirulina* (Figure 4). The results indicated that phycocyanin content was highest in Zarrouk's medium (0.12 ± 0.002 mg/g or mg/ml), while the lowest was observed in Animal waste medium (0.095 ± 0.001 mg/g or mg/ml) as shown in Graph 7. Similar trends were observed for allophycocyanin and phycoerythrin. The higher phycobilin content reflects better physiological and metabolic activity of the algal cells under optimized conditions.

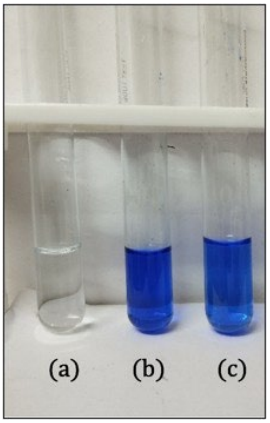
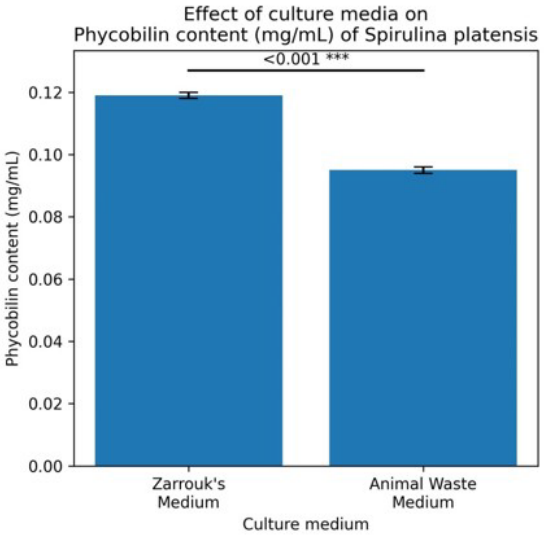


Figure 4: Total Phycobilin Content of *Spirulina platensis* (a) Control (b) Zarrouk's Media (c) Animal Media



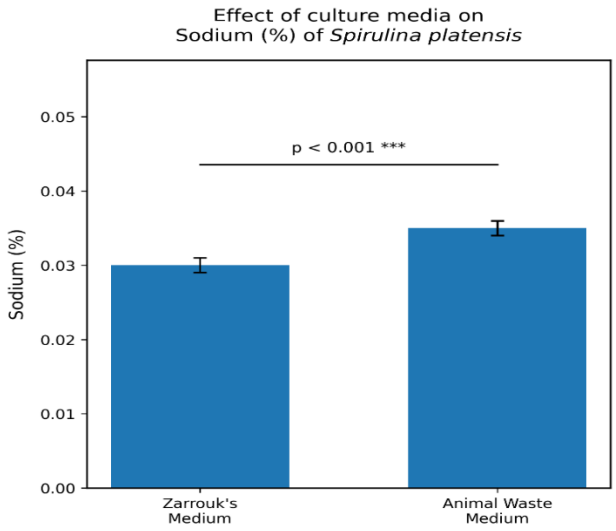
Graph-7: Effect of culture media on the phycobilin content of *Spirulina platensis*. Values are expressed as mean ± SD (n = 3). Error bars represent standard deviation. Statistical significance was determined using an independent two-tailed Student's t-test. $P < 0.001$.

4.3.7 Mineral Analysis

The mineral composition of *Spirulina platensis* cultivated in different media was analyzed to evaluate its nutritional and elemental profile. The results indicated significant variation in macro- and micro-element content depending on the culture medium.

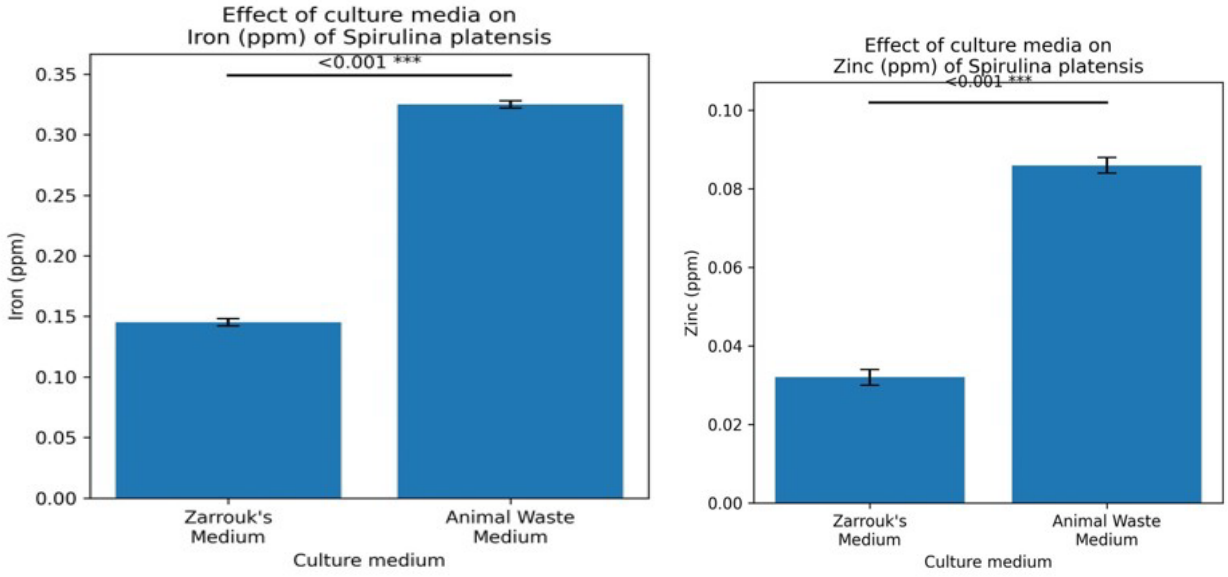
Natural Resources for Human Health
eISSN: 2583-1194

Among the macronutrients, sodium (Na) content was found to be moderate in Zarrouk's medium (0.03 ± 0.001 %), while a comparatively higher sodium content of 0.035 ± 0.001% was observed in animal waste medium**.** (Graph-8). The variation in sodium content may be attributed to differences in salinity and nutrient composition of the respective media.



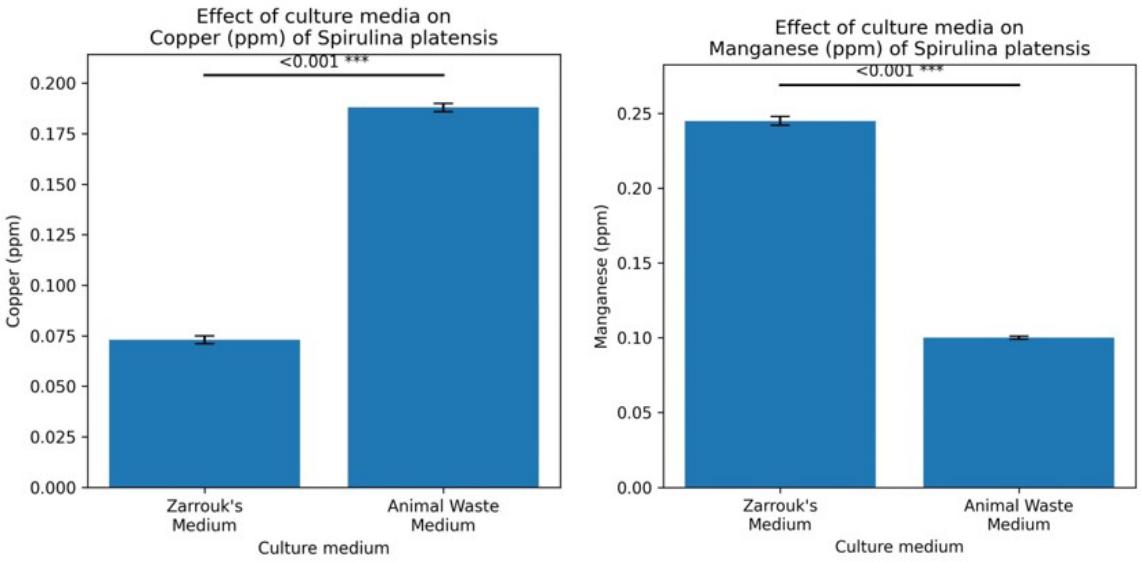
Graph-8: Effect of culture media on the sodium content of *Spirulina platensis*. Values are expressed as mean ± SD (n = 3). Error bars represent standard deviation. Statistical significance was determined using an independent two-tailed Student's t-test. *P* **< 0.001**

The micronutrient analysis revealed that iron (Fe) content was highest in Animal waste medium (0.325 ± 0.003 ppm), whereas the lowest value was recorded in Zarrouk's medium (0.145 ± 0.002 ppm) (Graph-9) . Similarly, zinc (Zn) content was observed to be lowest in Zarrouk's medium (0.034 ± 0.002 ppm) and highest in Animal waste medium (0.087 ± 0.002 ppm) as shown in Graph 10. Copper (Cu) concentration also showed variation, with the maximum value recorded in Animal waste medium (0.188 ± 0.002 ppm) and the minimum in Zarrouk's medium (0.073 ± 0.002 ppm)(Graph -11).



Graph-9 & Graph-10: Effect of culture media on iron accumulation (Graph-9) & zinc accumulation (Graph-10) in *Spirulina platensis*. Values are expressed as mean ± SD (n = 3). Error bars represent standard deviation. Statistical significance was determined using an independent two-tailed Student's *t*-test. *P* **< 0.001**

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eISSN: 2583-1194



Graph-11 & Graph-12: Effect of culture media on Copper accumulation (Graph-11) & Manganese accumulation (Graph-12) in *Spirulina platensis*. Values are expressed as mean ± SD (n = 3). Error bars represent standard deviation. Statistical significance was determined using an independent two-tailed Student's *t*-test. $P < 0.001$

Manganese (Mn) content followed a similar trend, where Zarrouk's medium exhibited the highest concentration (0.248 ± 0.003 ppm), while Animal waste medium showed the lowest value (0.101 ± 0.002 ppm) (Graph -12). The presence of these essential trace elements highlights the nutritional importance of *Spirulina*, as they play a crucial role in enzymatic activities and metabolic processes.

Overall, the results clearly demonstrate that *Spirulina platensis* grown in Animal waste medium exhibited a comparatively higher accumulation of essential minerals, whereas Zarrouk's medium showed relatively lower mineral content. This variation emphasizes the significant influence of culture conditions on mineral uptake and accumulation in microalgal biomass.

Table 2: Biochemical and mineral composition of *Spirulina platensis* cultivated in different culture media

Parameter		Zarrouk's Medium (Mean ± SD)	Animal Waste Medium (Mean ± SD)	*p*-value
Protein Estimation (%)		54.13 ± 0.31	37.96 ± 0.23	<0.001***
Amino acid Estimation (%)		1.72 ± 0.06	0.64 ± 0.02	<0.001***
Total Carotene content (µg/ml)		5.51 ± 0.11	4.08 ± 0.07	<0.001***
Carbohydrate Estimation (TSS) (%)		11.30 ± 0.17	8.97 ± 0.11	<0.001***
Total Chlorophyll content (mg/g)		0.0188 ± 0.0001	0.0236 ± 0.0003	<0.001***
Phycobilin content (mg/ml)		0.119 ± 0.001	0.095 ± 0.001	<0.001***
Mineral Analysis	**Zinc (ppm)**	0.032 ± 0.002	0.086 ± 0.002	<0.001***
	Copper (ppm)	0.073 ± 0.002	0.188 ± 0.002	<0.001***

	Iron (ppm)	0.145 ± 0.003	0.325 ± 0.003	<0.001***
	Manganese (ppm)	0.245 ± 0.003	0.100 ± 0.001	<0.001***
	Sodium (%)	0.030 ± 0.001	0.035 ± 0.001	<0.001***

Table 2 Footnote: Values are expressed as mean ± standard deviation (SD) of three independent experiments (n = 3). Statistical significance between Zarrouk's medium and Animal waste medium was determined using an independent two-tailed Student's t-test. One-way ANOVA was also performed to confirm the statistical differences. Significance was considered at $p < 0.05$. NS = not significant; $p < 0.05$*, $p < 0.01$**, and $p < 0.001$***

4.4 Statistical Analysis

Statistical analysis revealed that the biochemical composition of *Spirulina platensis* was significantly influenced by the culture medium (**Table 2) .** Protein, amino acid, total carotene, carbohydrate, phycobilin, zinc, copper, iron, sodium and manganese contents differed significantly between Zarrouk's medium and Animal waste medium ($p < 0.001$). Total chlorophyll content was also significantly different between the two media ($p < 0.001$), with higher chlorophyll content observed in the Animal waste medium.

5. Discussion

The present investigation provides comprehensive insights into the biochemical composition and nutritional quality of *Spirulina platensis* cultivated under different culture conditions. The findings demonstrate that culture medium composition significantly influences the accumulation of proteins, amino acids, pigments, carbohydrates, and minerals, highlighting the importance of optimized cultivation strategies for producing nutritionally enriched biomass. The comparative analysis between Zarrouk's medium and animal waste-based medium revealed distinct biochemical profiles, each offering specific advantages for different applications.

5.1. Protein and Amino Acid Composition

The protein content of *Spirulina platensis* varied considerably between the two media, with Zarrouk's medium supporting significantly higher protein accumulation (54.13 ± 0.31%) compared to the animal waste-based medium (37.96 ± 0.23%). This substantial difference can be attributed to the optimized nutrient composition of Zarrouk's medium, which provides readily available nitrogen sources (sodium nitrate), carbon sources (sodium bicarbonate), and essential minerals required for protein biosynthesis (Dineshkumar *et al.*, 2016; Soni *et al.*, 2019). The protein content observed in Zarrouk's medium falls within the range reported in previous studies (55-70% dry weight), confirming the high nutritional potential of *S. platensis* as a protein-rich microbial food source (Becker, 2013; Chang & Liu, 2024; Dolganyuk *et al.*, 2020).

The reduced protein content in the animal waste-based medium (37.96%) suggests nitrogen limitation or suboptimal nutrient availability in the cow dung extract. Although cow dung contains organic nitrogen and other nutrients that can support algal growth (Markou & Georgakakis, 2011; Madkour *et al.*, 2012), the nitrogen release kinetics and stoichiometric ratios may not align optimally with *Spirulina* requirements for maximum protein synthesis. Previous studies by Sofi Mohammad Yasin *et al.* (2012) and Michael *et al.* (2019) reported successful cultivation of *Spirulina* using animal waste-based media, but the present findings indicate that while such media support growth, they may not achieve the protein levels possible with synthetic media. Ragaza *et al.* (2020) similarly noted that alternative media often result in reduced protein content compared to standard media, though they offer significant cost advantages for large-scale production.

The amino acid analysis revealed that Zarrouk's medium supported higher total amino acid content (1.72 ± 0.063 mg/g) compared to the animal waste medium (0.643 ± 0.016 mg/g). The presence of essential amino acids including lysine, leucine, isoleucine, and valine in appreciable amounts confirms the superior nutritional value of *Spirulina* biomass, consistent with the findings of Aouir *et al.* (2017) and Chang & Liu (2024). The higher amino acid accumulation in Zarrouk's medium reflects enhanced nitrogen assimilation and protein synthesis under optimized nutrient conditions. Shanthi *et al.* (2018) reported that amino acid supplementation can further enhance growth and biochemical composition of *Spirulina*, suggesting that nitrogen management is critical for optimizing protein quality.

5.2. Pigment Composition

The pigment analysis revealed interesting patterns of variation between the two media. Carotenoid content was significantly higher in Zarrouk's medium (5.51 ± 0.11 µg/ml) compared to the animal waste medium (4.15 ± 0.07 µg/ml), indicating enhanced production of these important antioxidant pigments under optimized conditions. Carotenoids serve multiple functions in *Spirulina*, including photoprotection, light-harvesting, and antioxidant defense (Kumar *et al.*, 2022; Mary Leema *et al.*, 2010). The higher carotenoid accumulation in Zarrouk's medium suggests improved photosynthetic efficiency and enhanced protective mechanisms against oxidative stress. This observation aligns with Shabana *et al.* (2017), who reported that moderate stress conditions could enhance pigment production, though the present results indicate that nutrient optimization is equally important for carotenoid biosynthesis.

Phycobilin pigments, particularly phycocyanin, showed higher concentrations in Zarrouk's medium (0.12 ± 0.002 mg/ml) compared to the animal waste medium (0.095 ± 0.001 mg/ml). Phycocyanin is a valuable blue pigment with significant therapeutic potential, including antioxidant, anti-inflammatory, and hepatoprotective properties (Kumar *et al.*, 2022; Marjanović *et al.*, 2024). The higher phycocyanin content in Zarrouk's medium reflects better physiological and metabolic activity under optimized conditions, consistent with the findings of Manirafasha *et al.* (2018) and Bachchhav *et al.* (2016), who reported enhanced phycocyanin production through light and nutrient optimization.

Interestingly, chlorophyll content was higher in the animal waste medium (0.0236 ± 0.0003 mg/g) compared to Zarrouk's medium (0.0189 ± 0.0003 mg/g). This observation suggests that the altered nutrient composition or potential mild stress conditions in the animal waste medium may stimulate chlorophyll synthesis as an adaptive response. This finding aligns with Çelekli *et al.* (2016) and Nyabuto *et al.* (2015), who reported that nutrient stress can influence pigment synthesis pathways in microalgae. The variation in chlorophyll content indicates differences in photosynthetic efficiency under varying nutrient conditions and highlights the complex regulatory mechanisms governing pigment biosynthesis.

5.3. Carbohydrate Accumulation

Carbohydrate content was significantly higher in Zarrouk's medium (11.3 ± 0.17%) compared to the animal waste medium (8.97 ± 0.11%), reflecting better carbon utilization and metabolic activity under optimized conditions. Carbohydrates serve as energy reserves and contribute to stress adaptation in microalgae (Markou & Georgakakis, 2011; Spínola *et al.*, 2024). The higher carbohydrate accumulation in Zarrouk's medium can be attributed to the adequate supply of inorganic carbon (bicarbonate) and favorable environmental conditions that promote photosynthetic carbon fixation and carbohydrate biosynthesis.

The observed carbohydrate levels in Zarrouk's medium (11.3%) fall within the range reported for *Spirulina* (15-25% dry weight) in previous studies (Chang & Liu, 2024). The reduced carbohydrate content in the animal waste medium suggests limitations in carbon availability or metabolic efficiency, possibly due to the complex nature of organic matter in cow dung extract. However, the ability of *S. platensis* to accumulate carbohydrates under different nutrient conditions demonstrates its metabolic flexibility and potential for producing value-added polysaccharides with reported antioxidant, antiviral, and immunostimulatory properties (Marjanović *et al.*, 2024).

5.4. Mineral Composition

The mineral analysis revealed distinct patterns of element accumulation between the two media. The animal waste medium supported significantly higher accumulation of iron (0.325 ± 0.003 ppm), zinc (0.087 ± 0.002 ppm), and copper (0.188 ± 0.002 ppm) compared to Zarrouk's medium. This enhanced mineral uptake and storage in the animal waste medium can be attributed to the mineral-rich nature of cow dung extract, which contains substantial quantities of these essential trace elements (Kambou Sansan Philippe *et al.*, 2018; Kumari *et al.*, 2015). The ability of *S. platensis* to accumulate essential minerals increases its potential as a functional food supplement, particularly for addressing micronutrient deficiencies in human and animal nutrition.

Conversely, Zarrouk's medium exhibited higher manganese content (0.248 ± 0.003 ppm) compared to the animal waste medium (0.101 ± 0.002 ppm), while sodium content remained relatively similar between the two media (approximately 0.03%). These variations indicate that culture media composition directly influences mineral uptake and storage within Spirulina cells, likely through mechanisms involving metal transporters and chelation (Zhou *et al.*, 2018; Cardoso *et al.*, 2022).

The enhanced iron accumulation in the animal waste medium is particularly noteworthy, as iron deficiency is a common nutritional problem worldwide, and *Spirulina* enriched with iron could serve as an effective dietary supplement (Ragaza *et al*., 2020; Becker, 2013).

The presence of zinc and copper in substantial amounts in the animal waste medium-grown biomass highlights the potential for producing mineral-enriched Spirulina through cost-effective cultivation strategies. Zinc plays crucial roles in enzymatic activities, immune function, and cellular metabolism, while copper is essential for iron metabolism and antioxidant defense (Fields *et al*., 2014; Marjanović *et al*., 2024). The accumulation of these minerals in *Spirulina* biomass suggests that cultivation in animal waste-based media could provide dual benefits: bioremediation of nutrient-rich waste and production of value-added biomass for nutritional supplementation.

5.5. Nutritional and Commercial Implications

The biochemical profile variations observed between the two media have important implications for the nutritional and commercial applications of *Spirulina platensis*. Zarrouk's medium appears more suitable for producing high-protein biomass with enhanced carotenoid, phycocyanin, and amino acid content, making it ideal for health supplements, nutraceuticals, and pharmaceutical applications where protein quality and bioactive pigment content are paramount (Chang & Liu, 2024; Kumar *et al*., 2022). The superior protein and pigment profile of Zarrouk's medium-grown Spirulina aligns with the findings of Soni *et al*. (2017) and Dolganyuk *et al*. (2020), who emphasized the importance of optimized cultivation for producing high-value bioactive compounds.

The animal waste-based medium, despite lower overall protein and pigment content, offers distinct advantages for producing mineral-enriched biomass, particularly for applications where micronutrient fortification is desired. The cost-effectiveness of using agricultural waste as a nutrient source also makes this approach attractive for small-scale producers and developing regions where synthetic media may be economically prohibitive (Michael *et al*., 2019; Madkour *et al*., 2012). The ability to combine waste treatment with biomass production aligns with circular economy principles and sustainable production practices (Markou & Georgakakis, 2011; Ragaza *et al*., 2020).

The higher chlorophyll content observed in the animal waste medium-grown biomass may also offer advantages for applications where green coloration is desired, though the lower phycocyanin content suggests reduced production of this valuable pigment. This trade-of between different biochemical components highlights the need for targeted cultivation strategies based on desired end-use applications. For example, producers focusing on phycocyanin extraction for natural food coloring or therapeutic applications would benefit from Zarrouk's medium optimization, while those targeting mineral supplementation or cost-effective biomass production might prefer the animal waste-based approach (Bachchhav *et al*., 2016; Manirafasha *et al*., 2018).

6. CONCLUSION

In conclusion, the present study demonstrates that cultivation conditions significantly influence the biochemical composition and nutritional quality of *Spirulina platensis*. Zarrouk's medium proved superior for producing high-protein biomass with enhanced carotenoids, phycobilins, amino acids, and carbohydrates, making it suitable for nutraceutical and pharmaceutical applications. In contrast, the animal waste-based medium, while yielding lower levels of major biochemical compounds, promoted enhanced accumulation of essential minerals such as iron, zinc, and copper, offering advantages for mineral supplementation and cost-effective production. The findings highlight the importance of selecting appropriate cultivation media based on desired nutritional and commercial properties of *Spirulina* biomass. Successful contamination control and maintenance of suitable culture conditions further supported reliable biomass production. These results contribute to the sustainable utilization of *S. platensis* as a functional food ingredient, nutritional supplement, and source of bioactive compounds, with implications for food security, human health, and biotechnological applications.

Declarations

Ethics Approval and Consent to Participate

This study did not involve human participants, human subjects, or live animals. Therefore, ethical approval and consent to participate were not required.

Consent for Publication

Not applicable. This manuscript does not contain any personal information or identifiable images of individuals requiring consent for publication.

Competing Interests

Rashmi Gupta, Harish Chandra Singh, and Sanjeev Kumar declare that they have no competing financial or non-financial interests that could have influenced the work reported in this manuscript.

Funding

Rashmi Gupta, Harish Chandra Singh, and Sanjeev Kumar received no specific financial support or funding from any government agency, commercial organisation, or other external funding body for this study.

Authors' Contributions

Rashmi Gupta carried out the experimental work, collected and analysed the data, performed the biochemical investigations, and prepared the initial manuscript. Harish Chandra Singh provided scientific supervision and guidance throughout the study and contributed to the interpretation of the results and revision of the manuscript. Sanjeev Kumar contributed to the chemical and mineral analyses, interpretation of the analytical results, and critical revision of the manuscript. All three authors, Rashmi Gupta, Harish Chandra Singh, and Sanjeev Kumar, reviewed and approved the final manuscript.

Data Availability

The data generated and analysed during this study are included in the manuscript. Additional information or supporting data may be obtained from the corresponding author, Rashmi Gupta, upon reasonable request.

Declaration of Originality

Rashmi Gupta, Harish Chandra Singh, and Sanjeev Kumar confirm that the manuscript represents original work carried out by the authors. The manuscript has not been published previously and is not currently under consideration for publication in another journal. All relevant sources and previous studies used in the preparation of this manuscript have been appropriately acknowledged and cited.

Acknowledgements

The authors would like to thank the Department of Botany and the Department of Chemistry, J.S. University, Shikohabad, Uttar Pradesh, India, for providing the academic and laboratory support required to carry out this study.

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