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PHYCOREMEDIATION OF GREYWATER USING *SPIRULINA PLATENSIS* AND THE POTENTIAL APPLICATIONS OF THE PRODUCED BIOMASS

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ABSTRACT

Microalgae-based wastewater treatment represents an economical and sustainable solution to the global challenges of water and energy resource scarcity. This approach not only offers an eco-friendly method for biofuel production but also effectively removes nutrients and contaminants from wastewater. In this study, *Spirulina platensis* was cultivated in greywater (GW). Water quality was assessed by analyzing physicochemical parameters before and after the growth of *Spirulina*. The results showed substantial reductions in sodium (89%), chloride (72%), sulfate (76%), nitrogen (34%), and phosphate (45%). The study also showed decreases in Chemical Oxygen Demand (COD) and Biological Oxygen Demand (BOD) by 55%. The pH became more alkaline, while electrical conductivity (EC) decreased notably. Although GW proved to be a suitable medium for *Spirulina* growth, it was not as optimal as the control medium, as evidenced by marked reductions in optical density, dry weight, chlorophyll and carotenoid in Spirulina. However, the metabolic profile showed an increase significantly (p-value<0.0001)in protein, lipid, and carbohydrate levels. Based on these findings, it can be concluded that cultivating *Spirulina* in GW not only positively altered its physicochemical properties but also resulted in an enhanced yield of *Spirulina*.

Key Words: Greywater, microalgae, *Spirulina platensis*, water quality improvement, biomass production,

INTRODUCTION

The ongoing increase in the global population and economic growth has undeniably intensified the issues of environmental pollution and resource scarcity **(Tan *et al.*, 2021)**. According to the United Nations, by 2025, approximately 1.8 billion people worldwide will reside in regions suffering from water scarcity, with two-thirds of the global population living in water-stressed areas due to factors such as consumption, population growth, and climate change **(United Nations, 2016)**.

Greywater (GW) is wastewater generated from household activities excluding Blackwater (toilet wastewater), encompassing water from washing machines, dishwashers, showers, baths, and sinks. GW constitutes 30–50% of the organic load and 9–20% of the nutrient load of the total wastewater produced in a household. Interestingly, the availability of GW aligns with human activity, enabling a balance between water supply and demand when a modest buffering capacity is established **(Van de Walle *et al.*, 2023)**. In Egypt, GW treatment has the potential to provide approximately 4.15–8.30 billion cubic meters of water annually, which could significantly support the country's water resources **(Batisha, 2020)**.

Treating GW facilitates its potential reuse in domestic applications, thereby reducing stress on existing ecosystems. Three primary technologies are used in GW treatment: physical, chemical, and biological methods **(Awasthi *et al.*, 2023)**. Biological wastewater treatment is a promising and environmentally friendly approach that employs living microorganisms to degrade organic matter in the effluent through metabolic processes such as oxidation and cell synthesis **(Nagda *et al.*, 2022; Sravan *et al.*, 2024)**.

Microalgae-based wastewater treatment technology emerged in the 1950s as a synergistic approach to reducing environmental pollution while generating renewable fuels **(Paddock, 2019)**. Microalgae possess various attributes that make them valuable across multiple industries, including food, pharmaceuticals, cosmetics, health supplements, wastewater treatment, and biofuel production **(de Oliveira and Bragotto, 2022)**. A significant body of research has focused on using microalgae to treat GW because of their exceptional ability to recover nutrients and assimilate organic matter and nutrients from wastewater. They were also effective in removing pollutants such as dyes and toxic metals **(Manhaeghe *et al.*, 2020; Bhandari *et al.*, 2023)**. Phycoremediation, the use of microalgae for biological wastewater treatment, is among the most effective processes in this domain. Microalgae are cultivated in effluents with high inorganic and organic loads from various industries, including poultry, slaughterhouses, paper, textile, distilleries, supercritical water gasification, produced water from nonrenewable oil and gas extraction, and landfill leachate.

Biomass production for bioenergy, integrated with phycoremediation, offers a techno-economically viable pathway for commercialization and sustainable energy production, contributing to climate change mitigation, reducing competition with crop irrigation, and minimizing carbon and water footprints **(Borah *et al.*, 2023)**.

Microalgae are photosynthetic microorganisms classified into two categories: prokaryotes and eukaryotes. Prokaryotic microalgae, such as cyanobacteria, are also known as blue-green algae, while eukaryotic microalgae include diatoms and green algae **(Khavari *et al.*, 2021)**. These fast-growing organisms can adapt to a wide range of aquatic environments with varying temperatures, pH levels, and light intensities, thriving in freshwater, saltwater, marine water, and wastewater **(Gauthier *et al.*, 2020)**. Unlike conventional crops, microalgae do not require herbicides, pesticides, land, or specific seasons for growth **(Almomani *et al.*, 2023)**.

As photosynthetic autotrophs, microalgae efficiently harness solar energy to convert atmospheric CO_2 and available nutrients into oxygen and carbon-rich biomass, such as carbohydrates, lipids, proteins, pigments, vitamins, bioactive compounds, and antioxidants **(Khan *et al.*, 2018)**. After harvesting, this biomass can be used safely in producing value-added products, including animal feed, bio-fertilizers, and various biofuels such as bioethanol, biodiesel, bio-jet fuel, biomethane, and biohydrogen **(Aljabri *et al.*, 2022)**. Furthermore, microalgae have potential applications as antioxidants, anti-inflammatory agents, antitumor agents, anticancer agents, antimicrobials, antivirals, and anti-allergy agents, making them crucial in biomedical and pharmaceutical industries **(Bouyahya *et al.*, 2024)**.

Numerous microalgae species have demonstrated significant phycoremediation capabilities for removing heavy metals, emerging contaminants, and pathogens from wastewater. Examples include *Scenedesmus, Chlorella, Botryococcus, Phormidium, Limnospira* (formerly known as *Arthrospira* or *Spirulina*), and *Chlamydomonas* **(Ahmad *et al.*, 2021; López-Sánchez *et al.*, 2022)**.

Spirulina platensis (*S. platensis*) is a filamentous cyanobacterium commercially cultivated in both saline and freshwater, with semi- and mass-cultivation occurring in several countries. Dried *Spirulina* is a rich nutritional source, containing a high protein content (260-770 g/kg), representing 70% of its dry weight, along with a substantial fat content (10-140 g/kg) **(El-Shall *et al.*, 2023)**. *S. platensis* has been previously utilized for nutrient removal from piggery wastewater **(Liang *et al.*, 2023)**, the removal of nitrogen and phosphorus compounds from marine shrimp culture effluent **(Holanda *et al.*, 2022)**, black water treatment, and biodiesel production **(Salman *et al.*, 2023)**.

The objectives of this study focus on using *Spirulina platensis* in the treatment of GW and highly valuable products can be made from the algal biomass. These products such as pigments, protein, carbohydrates, and polyunsaturated fatty acids.

MATERIALS AND METHODS

1.1. Chemicals and reagents

Pure chloroform, ether, acetone, and methanol were obtained from E. Merck Co. (Germany) and distilled prior to use.

1.2. Greywater sampling and characterization

Over the course of a week, four in-house generated greywater (GW) samples (excluding toilet wastewater) were collected daily between 6:00 a.m. and 9:00 p.m. from a single location in Giza Governorate, Egypt. A total of 6 litres of the collected GW was used in this study: five litres were stored in a sterile glass bottle for microbiological analysis, while one litre was kept in a plastic bottle for physicochemical analyses. Immediately after collection, the samples were transported to the laboratory for analysis. The biological and physicochemical analyses of the water samples were conducted following the methods outlined by **APHA (2005).**

1.3. Preparation of algal inoculums

The cyanobacteria strain *Spirulina platensis* was acquired from the Microbiology Department of the Soils, Water, and Environment Research Institute (SWERI), Agricultural Research Centre (ARC), Giza, Egypt. *S. platensis* was cultivated in Zarrouk medium ***(Zarrouk, 1966)*** and incubated in a growth chamber with continuous shaking at 150 rpm, under 2000 Lux illumination, and maintained at a temperature of 25±1°C for 21 days.

1.4. Experimental design

Algae were inoculated at 20% (V_inoculation/V_media) in 500 ml Erlenmeyer flasks containing 200 ml of liquid media. The algal strain was then cultivated in 500 ml Erlenmeyer flasks, each containing a 200 ml greywater sample. The cultures were incubated at 25±1°C with continuous shaking at 150 rpm and under 2000 Lux illumination for 21 days. The experiment was conducted in triplicate, and average values were recorded.

1.5. Algal growth analyses

The analyses of algal growth parameters, including pH, electrical conductivity (EC), optical density (OD), dry weight (DW), and chlorophyll-a (Ch-a), were performed. The culture concentration was determined using optical density (OD) measured by a spectrophotometer at 560 nm for *Spirulina platensis* **(Leduy and Therien, 1977).**

Chlorophyll-a was quantified spectrophotometrically following extraction with absolute methanol, as described by Vonshak and Richmond (1988). pH values and algal dry weight were assessed according to **Vonshak (1986)**. Electrical conductivity (EC) was measured using a conductivity meter **(Systronics 304) (Kar *et al.*, 2007).**

1.6. Algal metabolic profile

1.6.1. Determination of crude lipid (Conventional method): The amount of crude lipid content was determined according to **Khilari and Sharma. (2016).**

1.6.2. Total Protein: It was measured by the Bradford assay according to **Bradford (1976).**

1.6.3. Total Carbohydrate: It was detected according to the method described by **Roberts and Elias (2011).**

1.7. Statistical analysis

Statistical analysis was performed by the SPSS 21.0 computer program (IBM Inc, Armonk, NY) **(IBM Corp, 2021).** The results were expressed as mean ± standard deviation (SD).

The obtained data was first tested for normality and homogeneity of variance and was found to be normal and homogenous. The independent-sample t-test for comparison between the GW and GW + *S. platensis*, *S. platensis*+ GW and, *S. platensis*+ control whereas analysis of variance (ANOVA) was used for comparison among control, GW and GW + *S. platensis*, followed by the Least Significant Difference (LSD) test at 0.05 levels, as recommended by **Snedecor and Cochran (1982).**

RESULTS

1. The pH and EC in raw GW, control and *S. platensis* cultured in GW during treatment:

The pH value of the microalgae culture was significant (p-value<0.0001). The pH shifted from neutral (7.16±0.03) to alkaline (9.30±0.01**)** before and after cultivation in GW and a significant decrease in electrical conductivity (EC), from 4.13±0.07 mS/m to 3.91±0.01 mS/m after *Spirulina* cultivation in GW, with a (p-value<0.0001), as in **Fig. (1).**

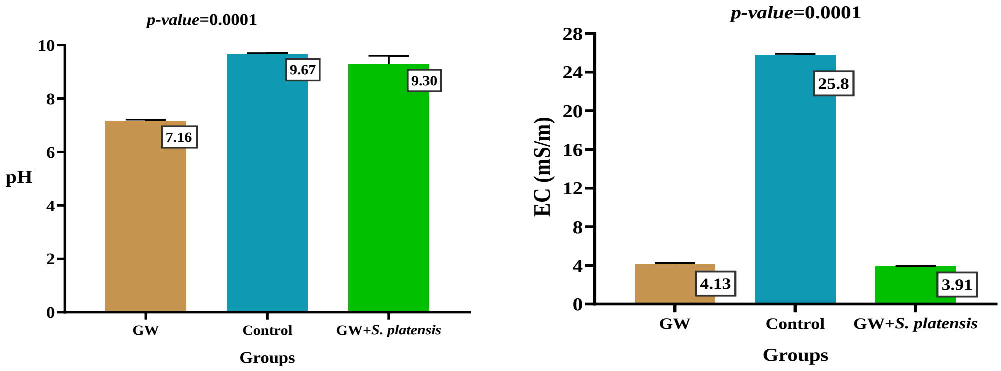
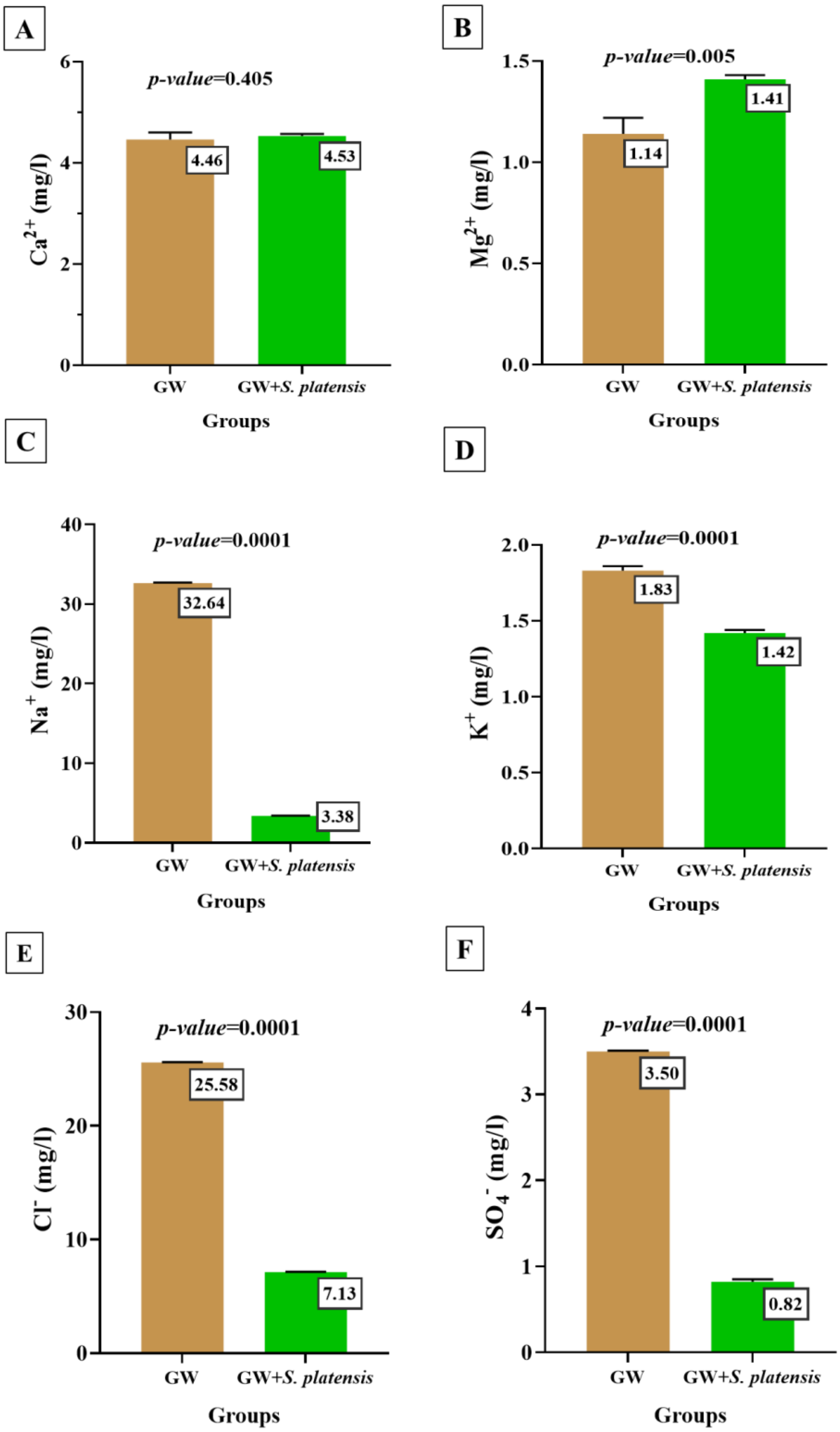


Fig. (1). The mean values of **(A)** pH and **(B)** EC in raw GW, control and *S. platensis* cultured in GW during treatment. The error bar represents the SD.

2. The Chemical parameters of GW before and after *S. platensis* treatment:

Calcium level was measured before and after *Spirulina* treatment and showed a non-significant increase from 4.46±0.08 to 4.53±0.02 $(t(4) = [0.930], p = .405)$. While magnesium level increased significantly from 1.14±0.05 to 1.41±0.01 $(t(4) = [5.629], p = 0.005)$. Sodium decreased significantly from 32.64±0.03 to 3.38±0.01, with $(t(4) = 815.203, p = 0.0001)$. Similarly, potassium was reduced significantly from 1.83±0.02 to 1.42±0.01 $(t(4) = [20.791], p = 0.0001)$. Chloride decreased significantly from 25.58±0.01 to 7.13±0.02, $(t(4) = [807.363], p = 0.0001)$. Further, sulphate decreased significantly from 3.50±0.01 to 0.82±0.01 $(t(4) = [171.200], p = 0.0001)$. Amniotic nitrogen (N-$NH_4^+$) decreased significantly from 22.50±0.06 to 14.85±0.01($t(4) = [131.040], p = 0.0001)$ after *Spirulina* cultivation, whereas nitrate nitrogen (N-NO_3^-) decreased significantly from 10.56±0.04 to 6.92±0.02 $(t(4) = [80.430], p = 0.0001)$. Phosphate decreased significantly from 8.05±0.02 to 4.70±0.35 $(t(4) = [9638], p = 0.001)$ after *Spirulina* cultivation after *Spirulina* cultivation**.**

The TDS value significantly decreased from 2678.97±4.61 to 2521.10±3.80 PPM $(t(4) = [26.437], p = 0.0001)$. COD declined significantly by more than 50% after *Spirulina* treatment from 246.33±2.33 to 110.00±0.58 mg/L $t(4) = [56.718], p = 0.0001)$. Similarly, BOD decreased significantly by more than 50% after *Spirulina* treatment from 171.97±0.98 to 76.00±0.58 mg l^{-1} $t(4) = [84.132], p = 0.0001)$ **(Fig. 2).**


A
p-value=0.405
Ca2+ (mg/l)
4.46
4.53
GW
GW+S. platensis
Groups
B
p-value=0.005
Mg2+ (mg/l)
1.14
1.41
GW
GW+S. platensis
Groups
C
p-value=0.0001
Na+ (mg/l)
32.64
3.38
GW
GW+S. platensis
Groups
D
p-value=0.0001
K+ (mg/l)
1.83
1.42
GW
GW+S. platensis
Groups
E
p-value=0.0001
Cl- (mg/l)
25.58
7.13
GW
GW+S. platensis
Groups
F
p-value=0.0001
SO4- (mg/l)
3.50
0.82
GW
GW+S. platensis
Groups

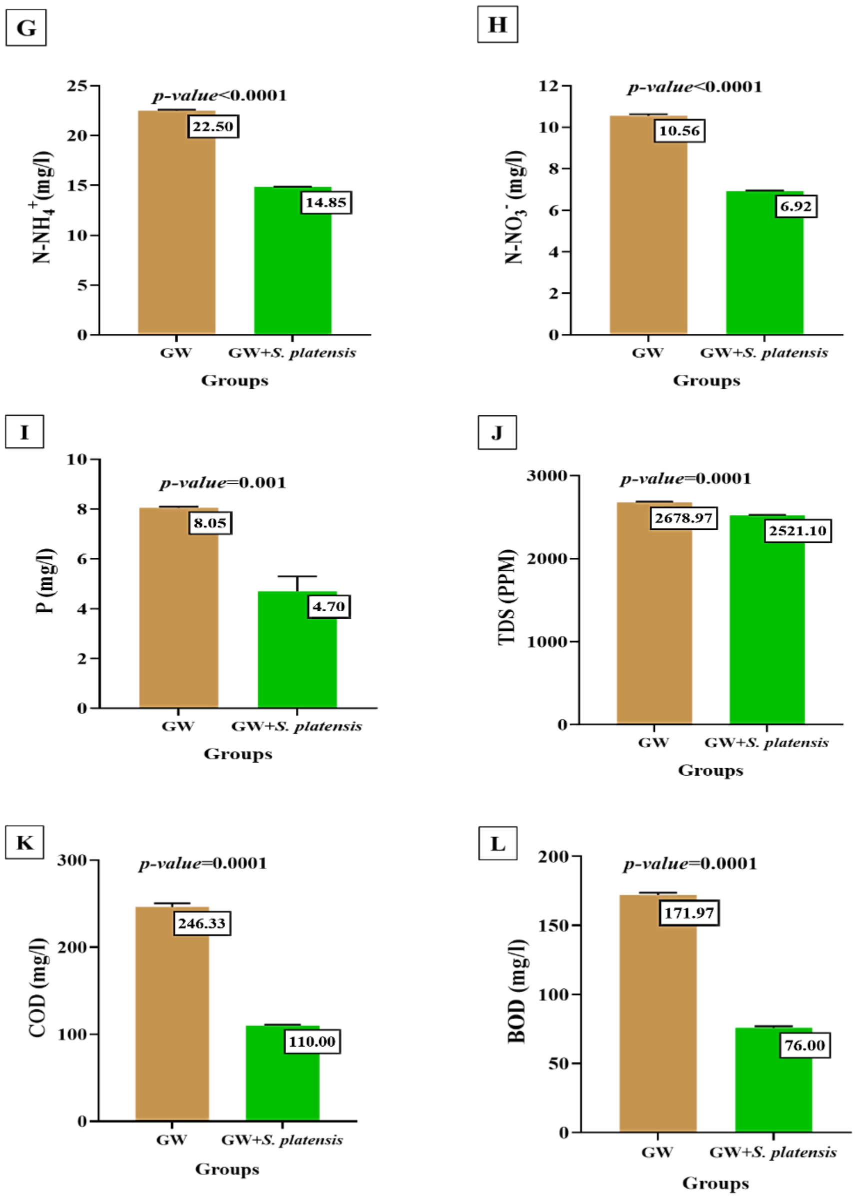


Fig. (2). The mean values of Ca^{+2}, Mg^{+2}, Na^{+}, K^{+}, Cl^{-}, SO_4^{+2}, N-NH_4, N-NO_3^{-}, P, TDS, COD, BOD in raw GW, and *S. platensis* cultured in GW**.** The error bar represents the SD

3. The growth parameters of *Spirulina platensis* cultivated in the synthetic control and GW

As in **Figure (3)**, *Spirulina* was cultivated in the GW and its control Zarrouk's medium under the same cultivation condition of temperature and lighting. *Spirulina* after cultivation in GW exhibited a significant decrease in the OD, DW, and chlorophyll content, compared to the control medium. Both chlorophyll A contents of *S. platensis* showed significant reductions from 0.69±0.02 to 0.25±0.03 ($t(4) = [12.127], p = 0.0001$). Chlorophyll B contents of *S. platensis* showed significant reductions from 0.71±0.02 to 0.42±0.10 ($t(4) = [2.843], p = 0.047$). However, the carotenoid content of *S. platensis* decreased significantly, ($t(4) = [1.080], p = 0.392$). The OD significantly decreased (from 0.23±0.03 to 0.14±0.01, ($t(4) = [2.900], p = 0.044$). the DW showed a significant decline from 3.63±0.21 to 1.26±0.07, ($t(4) = [10.913], p \leq 0.0001$).

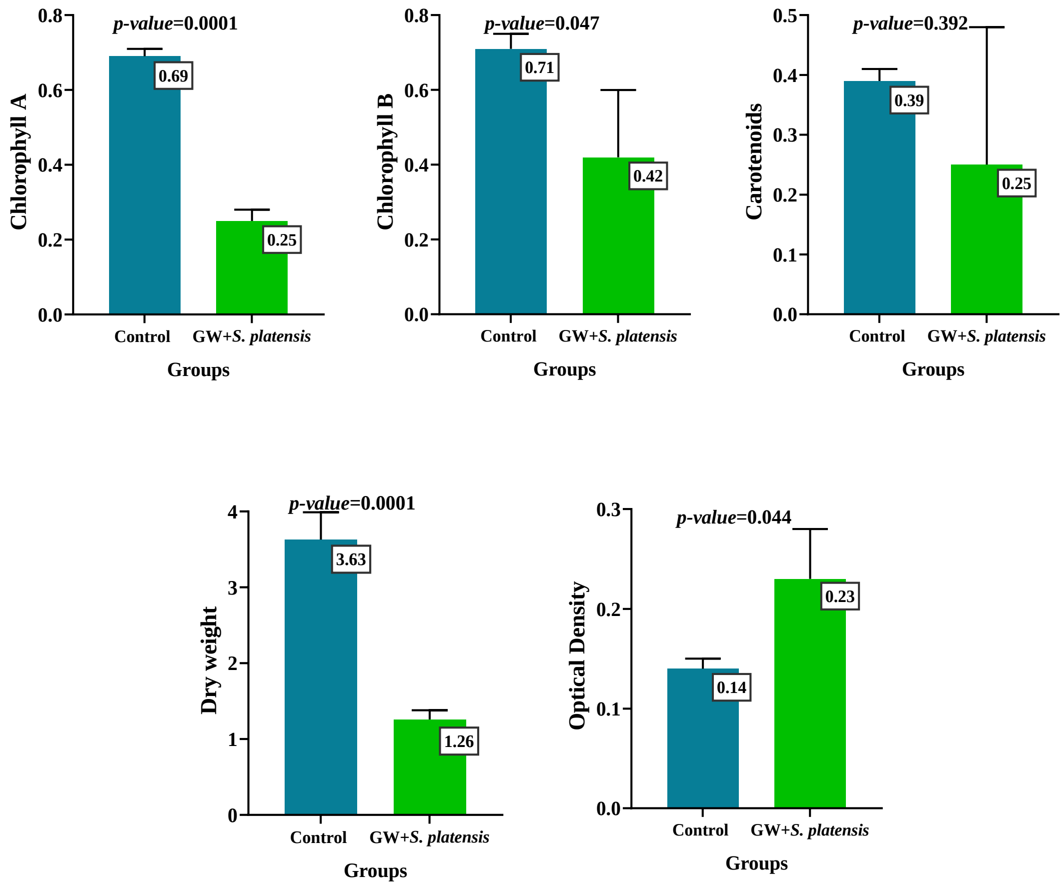


Fig.(3). The mean values of optical density (OD), dry weight (DW), chlorophyll A, chlorophyll B, and carotenoids in S. platensis which is cultured in control. and GW. The error bar represents the SD.

4 -The Metabolite profile

Data presented in **Fig. (4)** illustrated that *Spirulina platensis* showed a significant increase in protein, lipid, and carbohydrate contents after cultivation in GW, with values reaching 89.63±0.16, 56.10±0.23, and 25.07±0.11, respectively (p-value<0.0001).

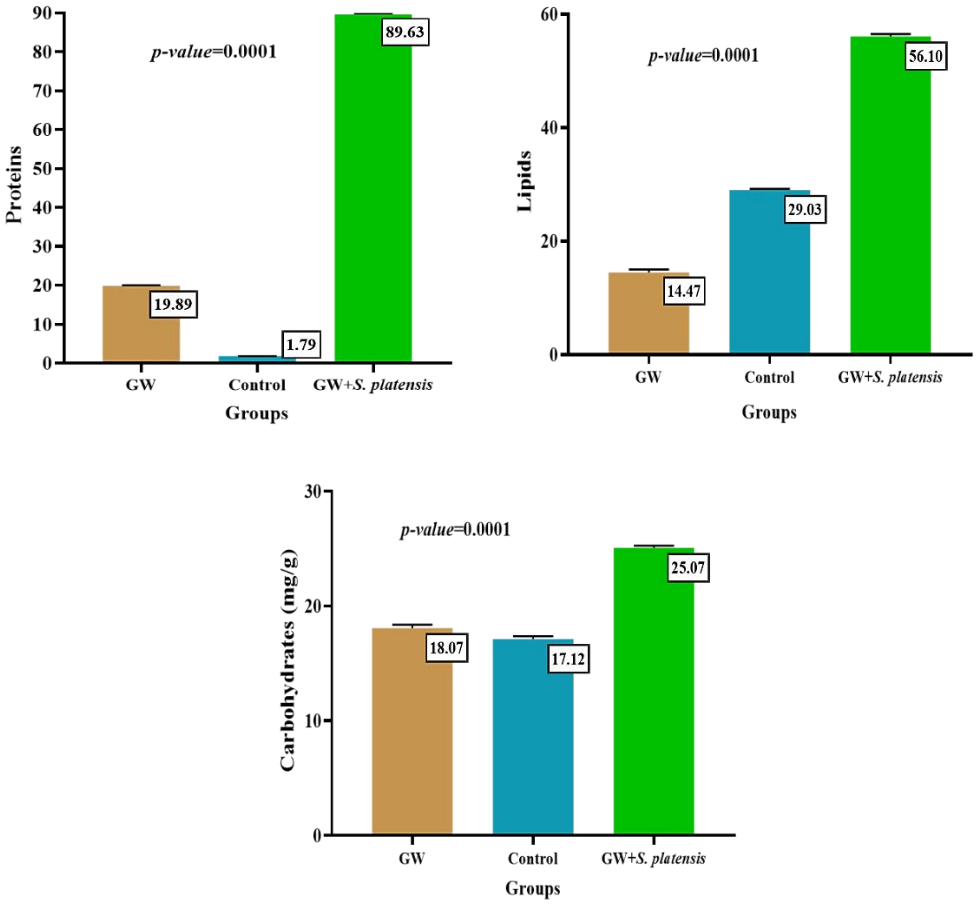


Fig. (4). The mean values of total protein, lipids, and carbohydrates of raw GW, control and *S. platensis* cultured in GW. The error bar represents the SD

DISCUSSION

1. pH

In the current study, the pH shifted from neutral to alkaline, this result was consistent with the findings of **El-Sheekh *et al.* (2021)**, who noted a slightly alkaline pH in wastewater following *Spirulina* cultivation. *S. platensis* thrives in a relatively high pH, which helps prevent contamination by other microalgae and enhances chlorophyll production. The optimal pH for its growth is between 8 and 11, and it also prefers temperatures ranging from 30°C to 35°C **(Kumar & Kumar, 2021)**. In contrast, *Spirulina* cultivation in household wastewater, which typically has a pH range of 4.5 to 6.0, leads to an increase in pH to a more neutral range **(Premalatha *et al.*, 2024)**.

In microalgae cultivation, the pH of the cultivation medium is a crucial factor that influences nutrient uptake, photosynthetic activity, and carbon sequestration efficiency **(Prasad *et al.*, 2020)**. Temperature can impact pH levels, which, in turn, affects enzymatic activity, biosynthetic potential, metabolism, and growth rates of microalgae in water samples **(Corredor *et al.*, 2021)**.

In algal cultures, an initially low pH value typically increases due to photosynthetic assimilation of CO_2 **(Alkhamis *et al.*, 2022)**. The exchange of inorganic and organic species between algal cells and their surrounding medium is essential for metabolic interactions and nutrient availability **(Tronci and Schaum, 2022)**. In a closed system, nutrient depletion can lead to changes in pH and alkalinity, influenced by factors such as the net flux of H+ across the plasma membrane and the dynamics of weak electrolytes like CO_2/HCO_3- and NH_4/NH_3 **(Omarjee *et al.*, 2020)**. Therefore, the chemical equilibrium between inorganic carbon and protons is critical for maintaining the natural pH buffering system in water **(Kochergina and Onina, 2020)**.

2. Electrical Conductivity (EC)

The results indicated a significant decrease in electrical conductivity This finding is consistent with **Kulkarni *et al.* (2016)**, who observed a reduction in conductivity when *Spirulina platensis* was cultivated in various concentrations of dairy wastewater. They reported a maximum decrease in EC of 55.71% at a 7.5% concentration of dairy effluent and a minimum reduction of 54.59% in the control set.

Electrical conductivity (EC) measures the ability of an aqueous solution to conduct an electric current, which depends on the presence and concentration of ions, their mobility and valence, and the solution's temperature **(Mizuhata, 2022)**. *Spirulina* cultivation in wastewater has been reported to lower concentrations of pollutants such as ammonia and nitrate **(Kumari *et al.*, 2023)**. Moreover, heavy metals like iron, copper, zinc, nickel, and chromium can significantly affect conductivity levels in wastewater **(Hailu and Nibret, 2023)**.

3. The chemical characteristics of raw and treated GW

Water quality parameters were assessed by analysing physicochemical attributes both before and after the growth of *Spirulina* **(Premalatha *et al.*, 2024)**. In this study, except for calcium and magnesium, significant reductions were observed in sodium, potassium, chloride, sulphate, nitrogen, phosphate, total dissolved solids (TDS), chemical oxygen demand (COD), and biological oxygen demand (BOD) in the greywater (GW) following *Spirulina* cultivation. Overall, cultivating *Spirulina* in GW led to significant changes in its physicochemical characteristics. The calcium level was measured before and after *Spirulina* treatment and showed a non-significant increase. Magnesium level increased significantly after *Spirulina* cultivation as

The observed increases in Ca^{2+} and Mg^{2+} levels suggest a rise in total water hardness. Total water hardness refers to the combined concentration of calcium and magnesium ions **(Aliberti *et al.*, 2023)**. Domestic greywater (GW) can contain various ions, including sodium, calcium, magnesium, chlorine, and boron, with detergents being a primary source of these ions. These ions contribute to water hardness and salinity **(Ghaly *et al.*, 2021)**. According to EPA (2024) classifications, water hardness can be categorized as follows: soft (0–60 mg/L), moderately hard (60–120 mg/L), hard (120–180 mg/L), or very hard (greater than 180 mg/L).

Sodium levels decreased significantly following *Spirulina* cultivation, Potassium levels decreased significantly after *Spirulina* cultivation; However, excessive potassium ions are generally not considered hazardous and can even benefit crop needs. Only potassium concentrations exceeding 50 mol/L are regarded as posing a serious risk in irrigation water **(Yaakob *et al.*, 2021)**.

Chloride levels decreased significantly after *Spirulina* cultivation, Chloride is a common issue in irrigation water, and excessive chloride concentrations can be toxic to crops. When chloride levels in the leaves exceed the crop's tolerance, observable signs such as leaf burn or drying of leaf tissue can occur. These indications are typically observed when chloride concentrations in the water reach between 0.3% and 1.0% **(Palanivel *et al.*, 2022)**.

Sulphate levels decreased significantly following *Spirulina* cultivation, Sulphate ions are a major contributor to salinity in irrigation waters. While Sulphate can be beneficial for fertility, as irrigation water often contains sufficient sulphates to support optimal crop production, excessive Sulphate concentrations can still impact water quality and crop health **(Mukhopadhyay *et al.*, 2022)**.

Ammoniacal nitrogen (N-NH_4^{+}) decreased significantly following *Spirulina* Cultivation Similarly, nitrate nitrogen (N-NO_3^{-}) also decreased significantly

Nitrate (NO_3^{-}) is the preferred nitrogen source for microalgae, and its utilization typically leads to an increase in pH values. In contrast, the use of ammonia is associated with the release of H^{+} ions, which can cause a decrease in pH **(Arumugam *et al.*, 2013).**

Phosphate levels decreased significantly after *Spirulina* cultivation. Elevated phosphorus levels can disrupt ecological balance and are a major factor contributing to the unwanted growth of aquatic weeds and algae **(Omarjee *et al.*, 2020)**.

The high nutrient levels in the greywater (GW) used in this study likely reflect the chemical characteristics of the wastewater source. Nutrients such as nitrogen, phosphorus, and other trace elements in GW are crucial for microalga growth and biomass production. Nitrogen is essential for cell formation, nucleic acids, and protein synthesis, and its deficiency can impact lipid synthesis in microalgae. Phosphorus plays a key role in various cellular processes, including the synthesis of phosphate compounds, sugars, nucleic acids, ATP (adenosine triphosphate), and phosphorylated enzymes **(Dammak *et al.*, 2023)**. *Spirulina* platensis is particularly effective at removing both organic and inorganic compounds from aqueous solutions due to its rapid biosorption rate and high biosorption capacity. Therefore, *Spirulina* is a promising candidate for water purification **(Dolatabadi and Hosseini, 2016)**. Additionally, (**Safrilia *et al.*, 2021)** found that *S. platensis* outperformed *Chlorella vulgaris* in treating organic wastewater, highlighting its effectiveness as a fast-growing microalga.

The total dissolved solids (TDS) value significantly decreased after *Spirulina* treatment. According to **(Palanivel *et al.*, 2022)**, irrigation water with TDS levels below 450 mg/L is considered good, whereas water with TDS levels above 2000 mg/L is deemed unsuitable for irrigation purposes. COD and BOD declined significantly by more than 50% after *Spirulina* treatment. These findings were consistent with **Premalatha *et al.* (2024)**, who observed decreases in TDS, chlorides, Sulphate, phosphate, and total alkalinity in household wastewater after *Spirulina* cultivation, leading to improvements in various chemical parameters. However, contrary to our findings, they reported an increase in sodium levels and a decrease in total hardness. Our results also align with **Dolatabadi and Hosseini (2016)**, who noted significant reductions in TDS and BOD after *Spirulina* treatment, though COD increased. Additionally, (**Mukhopadhyay *et al.* 2022)** reported that treatment with *Arthrospira* sp. achieved reductions of 96.82% in BOD and 87.5% in COD.

(Bhatt *et al.*, 2021) highlighted that kitchen waste typically contains high levels of proteins, lipids, phosphorus (50–70 mg/L), and nitrogen (20–40 mg/L). The blood from meats, washed in kitchen sinks, is a major source of nitrogen, while phosphorus is introduced through soaps and detergents. Grease and oil from cooking contribute to obnoxious odours and turbidity, resulting in higher COD and BOD levels. High BOD concentrations deplete dissolved oxygen in receiving waters, reducing pH values and inhibiting microbial growth, which can lead to the death of aquatic animals. Additionally, suspended solids from activities like floor cleaning and clothes washing contribute to the inorganic content of greywater, which includes calcium, sodium, magnesium, sulphur, potassium, phosphate, chlorine, ammonium salts, and various heavy metals. Detergents are primarily responsible for introducing these heavy metals into greywater.

4. Growth parameters

The growth of the microalgae was monitored using optical density (OD) at 560nm, biomass estimation and chlorophyll contents **(El-Sheekh *et al.*, 2021).** In this study, *Spirulina* was cultured in the GW and its control Zarrouk's medium under the same cultivation condition of temperature, and lighting. *Spirulina* after cultivation in GW exhibited significant decreases in the OD, DW, and chlorophyll content, compared to the control medium. The OD significantly decreased, and the DW showed a significant decline, Also, both the chlorophyll A and B contents of *S. platensis* showed significant reductions. However, the carotenoid content of *S. platensis* decreased non-significantly

The control medium used in this study was Zarrouk's medium, which is widely recognized as the optimal medium for cultivating *Spirulina platensis* **(Abd El-Monem *et al.,* 2019)**. It is expected that *Spirulina* exhibited superior growth in Zarrouk's medium compared to greywater (GW), as Zarrouk's medium provides more favourable conditions for growth.

Optical density (OD) is a crucial metric for evaluating the growth performance of *S. platensis* **(Zuki *et al.,* 2022)**. Photosynthetic pigments, including chlorophyll-a, chlorophyll-b, and carotenoids, are essential for efficient light absorption in cyanobacteria, facilitating photosynthesis **(Simkin *et al.,* 2022).** **(Jiang *et al.,* 2023)** noted that higher levels of these pigments correlate with improved photosynthetic performance, which enhances growth, biomass production, and overall yield. In contrast, a reduction in these pigments impairs light absorption and electron transfer, leading to decreased photosynthetic CO_2 fixation, slower growth, and reduced biomass accumulation **(Dong *et al.,* 2022)**.

In summary, laundry detergents, prevalent in domestic wastewater, can significantly impact microalgae growth by contributing to phytotoxicity. They cause substantial reductions in light-harvesting pigments such as chlorophyll-a, chlorophyll-b, and carotenoids, especially at higher concentrations. This impact results from the disruption of pigment synthesis and the accelerated degradation of pigments due to detergent-induced damage to pigment-protein complexes and increased photo-degradation (**Uzma** *et al*., 2018).

The optical density (OD) is a critical parameter for assessing the growth performance of *Spirulina platensis* **(Zuki *et al.,* 2022)**. Photosynthetic pigments, including chlorophyll-a, chlorophyll-b, and carotenoids, are vital for efficient light absorption and photosynthesis in cyanobacteria **(Simkin *et al.,* 2022)**. Higher levels of these pigments correlate with improved photosynthetic activity, leading to increased growth and biomass production **(Sherin *et al.,* 2022)**. Conversely, a decrease in these pigments impairs light absorption and electron transfer, slowing growth and reducing biomass accumulation **(Dong *et al.,* 2022)**.

Laundry detergents, common in domestic wastewater, can contribute to phytotoxicity. Light-harvesting pigments, including chlorophylls a and b and total carotenoids, are particularly sensitive to detergent stress, showing significant reductions in a dose-dependent manner with increasing concentrations of detergents, especially above 1 or 10 mg L−1 **(Uzma *et al.*, 2018)**. Detergents negatively impact photosynthetic pigments by inhibiting pigment biosynthesis and disturbing protein synthesis due to the surfactants present in the detergents. Additionally, surfactants accelerate the degradation of pigments by breaking pigment-protein complexes and increasing pigment photo-degradation rates.

At low concentrations, detergents alter membrane permeability by denaturing proteins, leading to cell permeabilization and lysis. At higher concentrations, they cause a progressive loss of cell bioactivity by removing phospholipids from the cell membrane, further disrupting cellular function **(Feijão *et al.*, 2022)**.

5. Metabolite profile

In this study, *Spirulina platensis* showed a significant increase in protein, lipid, and carbohydrate contents after cultivation in greywater (GW), These results are in line with **Cardoso *et al***. (2020), who reported higher biomass production and increased carbohydrate (69.77%) and lipid (12.77%) contents in *Spirulina* sp. cultivated in aquaculture wastewater compared to control conditions.

Nutrients in GW, such as phosphorus and nitrogen from various sources including food residues, oils, fats, and detergents, play a crucial role in microalgae growth and biochemical composition **(Yaakob *et al.*, 2021)**. High phosphorus levels can promote algal growth, while nitrogen from ammonia-containing products and proteins also supports this growth **(Ghaly *et al.*, 2021)**. Microalgae can utilize both inorganic and organic carbon sources in wastewater for synthesizing biomolecules like carbohydrates and lipids, either in heterotrophic or mixotrophic modes **(Goswami *et al.*, 2021)**.

Microalgae can uptake and store essential nutrients such as carbon (C), nitrogen (N), and phosphorus (P) from wastewater. They utilize both CO_2 and bicarbonate (HCO_3^-) for photosynthesis and can use nitrate ($NO3^-$), ammonium (NH_4^+), and nitrite (NO_2^-) as inorganic nitrogen sources. Phosphorus is vital for synthesizing nucleic acids, ATP, and phospholipids, which are crucial for energy and cellular membrane functions **(Su, 2021)**.

CONCLUSION

Microalgae-based greywater (GW) treatment offers an effective approach for wastewater management. In this process, GW serves as a suitable medium for cultivating *Spirulina*. In turn, *Spirulina* contributes to the improvement of GW's physicochemical characteristics by absorbing nutrients and pollutants and utilizing them for biomass formation. This not only enhances the quality of the GW but also reduces its pollutant load through nutrient uptake and algal growth. High valuable product can be made from the algal biomass. These products such as pigments, poly unsaturated fatty acids, biofuels (biodiesel, biogas bio hydrogen and bioethanol).

RECOMMENDATIONS

Phycoremediation using different types of microalgae is a suitable solution for treatment of the wastewater. Accordingly, phycoremediation of GW by algae was recommended and the clean water may be used for irrigation and watering plants and algae biomass can be used as a food additive for animals or as an energy source. Also, it has antioxidant, anti-inflammatory, antitumor properties, it can be used in pharmaceutical and cosmetics industry. Another benefit is using expanding microalgae mass as an energy source.

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معالجة المياه الرمادية باستخدام طحلب *سبيرولينا* بلاتنيسيس والتطبيقات المحتملة للكتلة الحيوية المنتجة

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المستخلص

تعد معالجة مياه الصرف الصحي بالطحالب الدقيقة حلاً فعالاً من حيث التكلفة ومستدامًا؛ نظرا للتحديات العالمية المتمثلة في ندرة موارد المياه والطاقة. تناولت هذه الدراسة زراعة طحلب *سبيرولينا بلاتنسيس في* المياه الرمادية. تم تقييم معايير جودة المياه من خلال تحليل المعايير الفيزيائية والكيميائية قبل وبعد نمو *سبيرولينا* . أظهرت الدراسة انخفاضا كبيرا في مستويات الصوديوم بنسبة 89٪، والكلوريد 72٪، والكبريتات 76 ٪، والنيتروجين 34 ٪، والفوسفات 45 ٪. كما انخفضت مستويات الأكسجين الكيميائي والأكسجين البيولوجي المطلوب بنسبة 55%. أصبح الرقم الهيدروجيني أكثر

قلوية، في حين انخفضت التوصيلية الكهربية بشكل ملحوظ. أوضحت النتائج أن المياه الرمادية وسطا مناسبا لنمو *سبيرولينا* ولكن ليست مثالية كما هو الحال في اوساط التحكم، حيث لوحظ انخفاض في الكثافة البصرية والوزن الجاف ومحتويات الكلورفيل والكاروتينويد في *سبيرولينا* . وفقا لنتائج الدراسة يمكن استنتاج أن زراعة *سبيرولينا* في المياه الرمادية قد حسنت بشكل إيجابي الخصائص الفيزيائية والكيميائية لهذه المياه، مما أدي إلى زيادة إنتاج *سبيرولينا* . كما يمكن الاستنتاج أن معالجة المياه الرمادية باستخدام طحلب *سبيرولينا* يحقق فوائد متعددة، بما في ذلك انخفاض تكلفة المعالجة وصلاحية المياه المعالجة للاستخدامات الزراعية، بالإضافة إلى احتوائها على البروتينات والكربوهيدرات والدهون ما يؤهلها لاستخدامها في تطبيقات متعددة منها إنتاج الديزل الحيوي والإيثانول الحيوي وكذلك كعلف.

الكلمات المفتاحية: المياه الرمادية، *سبيرولينا بلاتنيسيس*، تحسين جودة المياه، إنتاج الكتلة الحيوية