# EFFECTS OF RED, GREEN AND BLUE LED LIGHT ON GROWTH, ASTAXANTHIN ACCUMULATION AND PROTEIN CONTENT IN *HAEMATOCOCCUS PLUVIALIS*

**Dr. Gökçe KENDİRLİOĞLU ŞİMŞEK**

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## PREFACE

Today, microalgae-based biotechnological research continues to gain momentum with the support of multidisciplinary collaborations. These studies involve not only biologists and bioengineers, but also professionals from the fields of energy, agriculture, aquaculture, and pharmaceutical sciences. In recent years, due to their rapid growth, photosynthetic efficiency, and ability to produce high-value metabolites such as astaxanthin, microalgae have attracted increasing interest in both academic and industrial domains. In this context, understanding the biochemical responses of microalgae under various environmental conditions has become essential for the development of sustainable and efficient production models. In this study, the growth responses of *Haematococcus pluvialis* under red, blue and green LED lighting conditions were experimentally investigated. The findings revealed that red light promoted biomass accumulation most effectively, while blue light was associated with increased pigment synthesis. This work aims to contribute to the academic literature by addressing both the theoretical background and experimental data regarding the role of light spectrum in microalgal cultivation. Furthermore, it presents insights into how LED-based lighting systems can be used strategically to direct algal metabolism toward target compounds such as astaxanthin. It is hoped that this book will serve as a valuable resource for students, researchers and practitioners working in the fields of algal biotechnology and renewable bio-based production. In this regard, I believe that the content presented here in will support readers in understanding the dynamic relationship between light conditions and algal physiology and will contribute to the design of innovative strategies for optimizing metabolite yield in microalgal systems.

08.09.2025

**Dr. Gökçe KENDİRLİOĞLU ŞİMŞEK**

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# EFFECTS OF RED, GREEN AND BLUE LED LIGHT ON GROWTH, ASTAXANTHIN ACCUMULATION AND PROTEIN CONTENT IN *HAEMATOCOCCUS PLUVIALIS*

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## INTRODUCTION

The growing global population, climate change, uncontrolled land use and global health crises indicate that much higher demands will arise in the coming years in the food, energy, pharmaceutical, and healthcare sectors. Although current solution strategies focus on increasing agricultural productivity and the efficient use of fossil resources, the limited progress of technological advancements may prove insufficient to meet this increasing demand. Therefore, the development of new biological resources that are sustainable, economical, non-toxic, environmentally compatible and easy to produce has become a critical necessity for ensuring energy and food security. In this context, algae have emerged as promising alternative organisms due to their high photosynthetic capacity, rapid growth rates, and ability to produce valuable metabolites (Bhattacharya et al., 2020; Goswami et al., 2021; Ambati et al., 2014).

As key components of aquatic ecosystems, algae not only serve as a source of organic nutrients for heterotrophic organisms but also contribute significantly to oxygen production in their environment through photosynthesis (Barsanti & Gualtieri, 2021).

Lacking true root, stem and leaf structures, algae are classified into two main groups based on their size characteristics: microalgae and macroalgae (Cirik & Cirik, 2011). Today, both microalgae and macroalgae are attracting considerable attention in biotechnological applications due to their high biomass yields, rapid proliferation and rich biochemical compositions (Spolaore et al., 2006). Among these organisms, microalgae in particular are considered potential bioresources in many fields, including sustainable biofuel production, dietary supplements, animal feed, pharmaceuticals and cosmetics, owing to their unicellular structures, ease of cultivation and modifiable metabolic processes (Becker, 2007; Mata et al., 2010).

In the face of a growing population and the consequent rise in energy and food demand, efforts to increase the efficiency of current agricultural production systems and fossil fuel sources may prove inadequate in the long term. Therefore, the need for alternative biological resources that are environmentally sustainable, economical, non-toxic, and easy to produce is steadily increasing. In this context, microalgae have become the focus of numerous scientific studies due to their cellular contents of high biotechnological value, such as carbohydrates, proteins, lipids, and chlorophyll. These components make microalgae attractive raw materials to produce high-value commercial products such as biofuels, functional foods, nutraceuticals, and cosmetics. As photosynthetic organisms, microalgae not only possess the ability to convert inorganic substances into organic compounds but are also favored in various sectors due to advantages

such as rapid growth, high biomass production, low production costs, ease of accessibility, and safe use across wide application areas (Ho et al., 2013; Lim et al., 2022; Han et al., 2018). Moreover, they offer significant benefits in terms of environmental sustainability. Specifically, their effective role in the removal of pollutants such as nitrogen and phosphorus in wastewater treatment contributes to improved water quality (Pittman et al., 2011). In addition, algae-based production systems hold promise in areas such as greenhouse gas emission reduction and renewable energy generation. From the perspective of biodiesel production, algae stand out for their high energy efficiency. On an industrial scale, production processes are generally conducted through open pond systems established in temperate climate zones or photobioreactors situated under greenhouse conditions (Borowitzka, 1995; Chisti, 2007).

Metabolites synthesized by microalgae are classified into two main groups: primary metabolites, which are essential for the organism's fundamental life processes (proteins, carbohydrates, and lipids), and secondary metabolites, which possess biological activity related to physiological functions (carotenoids, astaxanthin and polyhydroxyalkanoates – PHA) (Japar et al., 2021; Liu et al., 2022). In terms of primary metabolites, microalgae hold significant potential due to their high content of functional proteins and their role as a natural source of protein (Piasecka & Baier, 2022).

Microalgae are microscopic, photosynthetic organisms involved in the synthesis of various biomolecules such as proteins, amino acids, lipids, polyunsaturated fatty acids, and carotenoids. The biologically active compounds produced by these organisms have found applications in numerous fields including biotechnology, aquaculture, biochemistry, the pharmaceutical industry, nutraceutical applications, and bioenergy production (Katiyar et al., 2017). Proteins obtained from microalgal species are known to possess low isoelectric points (approximately pI 4.0), low molecular weights (Al-Zuhair et al., 2017), and high levels of hydrophilic amino acids (Grossmann, Hinrichs & Weiss, 2019). These properties allow the proteins to dissolve easily in neutral pH solutions and be efficiently absorbed by the human body. Moreover, these proteins have been reported to support the immune system, exhibit protective effects against cancer, slow down the aging process, and reduce physical fatigue. Unlike commonly used animal-derived proteins such as collagen, microalgal proteins are also reported to possess antioxidant, anti-angiotensin converting enzymatic, anti-inflammatory, and antibacterial properties (Alzahrani et al., 2019; Zhou et al., 2023). It is also evident that microalgal proteins are being utilized in biomedical applications. These proteins are employed in the development of various hydrogel structures, in the investigation of antitumor effects in breast cancer models, and in evaluating their beneficial effects in hyperglycemia and hypolipidemia associated with obesity (de Melo et al., 2019). Accordingly, they are reported to hold significant potential in the fields of dietary supplementation and biomedicine (Hildebrand et al., 2020).

Efforts to develop bioplastics as environmentally friendly alternatives to petroleum-based synthetic polymers have increased interest in microalgae-based biopolymer production. Proteins synthesized from microalgal species have been used in bioplastic production, and these polymers stand out for their eco-friendly properties. Furthermore, these microalgae-derived bioplastics are not only biodegradable but also align with circular economy principles due to the potential for integrating their production processes with carbon capture and wastewater treatment (Karan et al., 2019).

Lipids derived from microalgae exhibit chemical structures similar to those of vegetable oils and are considered environmentally friendly and sustainable alternative sources to produce various biofuels, including biodiesel, bioethanol, biogas, and biohydrogen. One of the most important components of these lipids is triglycerides, which make microalgae suitable raw materials for biodiesel production. Owing to their chemical resemblance to vegetable oils, microalgal lipids are widely evaluated as promising feedstocks for the generation of different biofuel types, serving as sustainable alternatives to fossil fuels. Particularly, microalgae are regarded as natural lipid sources of strategic importance to the biofuel industry. The lipid content in microalgae can vary depending on the species' genetic characteristics and the cultivation conditions applied, reaching up to 60% of the dry biomass (Mata et al., 2010). Within algal cells, lipids are mainly stored in the form of triglycerides, phospholipids, and glycolipids.

The unsaturated fatty acids present in these lipid fractions possess chemical properties that are especially suitable for biodiesel production. Lipid synthesis and accumulation are significantly enhanced under environmental stress conditions such as nitrogen deficiency, high light intensity, and prolonged illumination (Hu et al., 2008). Thanks to these adaptive traits, microalgae are regarded as promising biological resources for sustainable energy production.

Algae are among the richest photosynthetic organisms in terms of pigment diversity, and these pigments are considered key elements in the taxonomic classification of algal species. In addition to chlorophyll-a, which is common to all photosynthetic organisms, different algal groups contain various pigments that differ in type and concentration. While all algal groups contain chlorophyll-a, members of Chlorophyta and Euglenophyta also contain chlorophyll-b; in contrast, the Bacillariophyta group may include additional pigments such as chlorophyll-c, chlorophyll-d, and chlorophyll-e (Tunail, 2009). According to the literature, chlorophyll typically constitutes approximately 0.5% to 1.5% of the cell's dry weight (Gouveria et al., 2008). Moreover, chlorophyll-a is known to be an important indicator of photosynthetic capacity and biomass productivity.

In addition to chlorophyll, carotenoids are also major pigments found in algae. Among them, astaxanthin is a promising carotenoid due to its potent antioxidant activity. As a secondary metabolite, astaxanthin is naturally synthesized by microalgae and possesses high commercial value.

Its production serves as a noteworthy example in terms of sustainability and the circular economy. Although there are more than 750 naturally occurring carotenoids in nature, astaxanthin stands out among compounds such as lutein, β-carotene, zeaxanthin, and canthaxanthin due to its lipophilic structure, strong antioxidant properties, and biological activities (Ulianova et al., 2020). Astaxanthin has been reported to exhibit antioxidant activity approximately 10 times stronger than other carotenoids such as β-carotene, zeaxanthin, canthaxanthin, and lutein, and up to 1000 times more effective than vitamin E (Patel et al., 2022). This carotenoid has the molecular structure 3,3′-dihydroxy-β,β′-carotene-4,4′-dione and is widely used in the food, feed, cosmetic, aquaculture, and pharmaceutical industries (Li et al., 2011). The antioxidant capacity of natural astaxanthin is not only superior to that of synthetic astaxanthin but also surpasses that of other biological compounds such as vitamin C, β-carotene, canthaxanthin, zeaxanthin, lutein, and α-tocopherol (Borowitzka, 2013; Koller et al., 2014; Miki, 1991; Pérez-López et al., 2014).

Astaxanthin ($C_{40}H_{52}O_4$) is a keto-carotenoid known as one of the most powerful natural antioxidants and plays a critical role in reducing cellular oxidative stress (Lim et al., 2018). Due to these properties, it has demonstrated protective effects against a variety of conditions including cardiovascular, gastrointestinal, and liver diseases, as well as cancer.

It has also been shown to be effective in therapeutic applications such as enhancing immune response, anti-inflammatory action, treating muscle pain, carpal tunnel syndrome, and regulating immune responses against tumor cells (Praveenkumar et al., 2015; Wayama et al., 2013). Today, astaxanthin is widely used not only in the health sector but also in the production of feed additives, pharmaceutical products, and functional foods.

The freshwater green microalga *Haematococcus pluvialis* is recognized as one of the richest natural sources of astaxanthin, a compound well known for its potent antioxidant properties. *H. pluvialis* is a unicellular green microalga belonging to the class Chlorophyceae and the order Volvocales, typically found in freshwater habitats (Kobayashi et al., 1997; Lorenz, 1999). This species can be observed in various freshwater environments such as puddles, rainwater collections, artificial ponds, and natural or artificial pools, and is distributed across regions of Europe, Africa, North and South America, Australia, and parts of India (Burchardt et al., 2006). The chloroplasts of *H. pluvialis* are cup-shaped and contain multiple pyrenoids. Its contractile vacuoles are irregularly and diffusely distributed near the surface of the protoplast. The organism has two equal-length flagella in the apical position, a nucleus, and is surrounded by a cellulose-based cell wall with large pores. While the cell wall has a thick, gelatinous structure, the protoplast displays a filamentous appearance (Boussiba, 2000).

Under favorable environmental conditions, cells in the green vegetative phase undergo morphological and physiological changes induced by stress factors such as temperature and salinity, transforming into the red cyst phase. This transformation is part of the remarkable life cycle of *H. pluvialis*, enabling adaptation to changing environmental conditions. Astaxanthin production in *H. pluvialis* cells is not only influenced by the nutrients and their concentrations in the culture medium but is also associated with the morphological, physiological and biochemical changes observed throughout the species' complex life cycle and environmental factors (Shah et al., 2016). The life cycle of *H. pluvialis* consists of two distinct stages: the green vegetative growth phase and the red cyst phase. Notably, the growth performance achieved during the green phase has a direct effect on the level of astaxanthin accumulation in the subsequent red cyst phase, indicating a positive correlation between the two stages (Acheampong et al., 2024). Under stress conditions, the planktonic cells of *H. pluvialis* gradually transform into immobile cysts, during which astaxanthin can accumulate up to 4% of the dry weight of the biomass. The production levels of bioactive compounds in microalgae vary depending on the culture conditions in which they are cultivated (Mustafa et al., 2013). In this regard, studies have shown that factors such as light type and intensity, photoperiod, salinity, temperature, pH level, and the composition, concentration, and quality of nutrients present in the medium significantly influence the synthesis of target compounds (Wahidin et al., 2013; Mandotra et al., 2016; Shen et al., 2016).

Light is a fundamental environmental factor for the survival of photosynthetic organisms. Since photosynthetic growth is directly associated with the light energy absorbed by cells, providing optimal light conditions in microalgae cultivation systems is of critical importance. Various studies have demonstrated that the light source directly affects the photosynthetic processes specific to microalgae. Accordingly, many researchers have examined the role of light intensity in microalgal development and have concluded that light intensity significantly impacts photosynthetic efficiency (Juneja et al., 2013). To achieve optimal growth rates in photosynthetic microorganisms, the light source used must possess certain essential characteristics. These include high energy efficiency, low heat emission, reliability, long lifespan, high stability, cost-effectiveness, and compatibility with the absorption spectrum of the target organism (Bertling et al., 2006). Moreover, it has been emphasized that the design and selection of effective white light depend on factors such as the type and intensity of the light source, spectral quality of the light, and the specific pigment composition of the microalgae. These parameters are reported to have a direct and determinative impact on the growth performance and metabolic activities of microalgal species (Bertling et al., 2006).

In general, microalgae utilize the light spectrum in the range of 400–700 nm during photosynthetic processes; however, the sensitivity of different species to specific wavelengths within this range may vary.

Some studies have also revealed that microalgae can exhibit developmental responses to specific spectral ranges outside of this interval. In particular, red light (600–700 nm) and blue light (400–500 nm) have been reported in numerous studies to support microalgal growth and significantly influence growth rates and lipid content (Chen et al., 2011; Wang & Lan, 2011; Das et al., 2011; Cheirsilp & Torpee, 2012).High light stress is a commonly applied condition for increasing astaxanthin accumulation in *Haematococcus pluvialis* (Lv et al., 2016; Su et al., 2016; Christian et al., 2018). For achieving rapid growth during the green motile cell phase of *H. pluvialis*, the optimal light intensity is below 80 μmol photons·$m^{-2}$·$s^{-1}$. In contrast, cells in the red spore stage are more tolerant to higher light intensities. Studies have shown that high light intensities stimulate astaxanthin biosynthesis, and the light intensity range that typically induces stress is between 300–500 μmol photons·$m^{-2}$·$s^{-1}$. Motile green cells of *H. pluvialis* primarily contain chlorophyll, but under stress conditions such as nutrient deficiency and high light intensity, they initiate astaxanthin biosynthesis.

Recently, light-emitting diodes (LEDs) have been used as light sources in algae cultivation due to their favorable advantages such as high flexibility in bioreactor design, excellent controllability for indoor cultivation, high photosynthetically active radiation (PAR) efficiency, and low heat emission.

It has been reported that blue LED light enhances astaxanthin accumulation, whereas red LED light is preferred for promoting microalgal growth (Katsuda et al., 2004; Lababpour et al., 2005; Xi et al., 2016). In recent years, the effects of different LED wavelengths on microalgae have been intensively investigated. Studies have shown that light quality plays a determining role in both growth and metabolite production. Karagülle and Telli (2024) reported that adding green light in specific proportions to red and blue light combinations significantly enhanced biomass production and chlorophyll content in *Haematococcus pluvialis*. Similarly, Ma et al. (2018) reported that medium-intensity red LED light was suitable for astaxanthin accumulation, while a red-blue LED combination yielded the best results in terms of both biomass and pigment production. Blue LED light significantly elevates reactive oxygen species (ROS) levels within *Haematococcus pluvialis* cells, leading to increased malondialdehyde (MDA) concentrations and catalase (CAT) enzyme activity—responses associated with astaxanthin biosynthesis as a photoprotective mechanism (Chen et al., 2023).Furthermore, research conducted on various microalgal species has revealed that red light provides the highest growth rate, white light enhances protein synthesis, and blue light promotes carbohydrate and secondary metabolite production (Kwan et al., 2021). In general, red light optimizes growth performance, while blue light enhances pigment production; a combination of red and blue light helps establish a balance between these two parameters. These findings suggest that LED lighting is a strategic tool for directing metabolite production in microalgal biotechnology.

The primary objective of this study is to evaluate the effects of LED lights with different wavelengths on the growth capacity and biochemical components of *Haematococcus pluvialis*. Natural astaxanthin derived from microalgae is a high-value carotenoid with broad applications in the pharmaceutical, food, cosmetic, and animal feed industries. Due to its strong antioxidant properties and beneficial health effects, the global demand for this compound continues to increase. Thus, its sustainable and cost-effective production is considered a priority research area in biotechnology. Since synthetic production methods present economic and environmental limitations, the development of renewable and ecologically safe alternatives is of critical importance.

In this context, considering the key role of the light spectrum in directing metabolic responses, LED-based lighting systems stand out due to their energy efficiency and spectral control advantages. The results of this study are expected to contribute to the optimization of pigment production processes based on microalgae and to provide a foundation for the development of applicable strategies for industrial-scale natural astaxanthin production.

## 2. Materials and Methods

### 2.1 Algal Species and Culture Conditions

The microalgae species *Haematococcus pluvialis* was obtained from the stock cultures of the Algal Biotechnology Laboratory at Fırat University.

The cultures were incubated in BG11 medium at 23 ± 1 °C under a 16:8 h light/dark photoperiod with an illumination intensity of 3000 lux in an incubator (Nüve ES 120/252). Experimental cultures were prepared in 250 mL volumes by inoculating the BG11 medium with stock cultures at a 1:1 (v/v) ratio. The BG11 medium composition per 1000 mL included: 4.0 g $K_2HPO_4$, 7.5 g $MgSO_4 \cdot 7H_2O$, 3.6 g $CaCl_2 \cdot 2H_2O$, 0.10 g $Na_2EDTA$, 2.00 g $Na_2CO_3$, 2.86 g $H_3BO_3$, 1.81 g $MnCl_2 \cdot 4H_2O$, 0.22 g $ZnSO_4 \cdot 7H_2O$, 0.39 g $Na_2MoO_4 \cdot 2H_2O$, 0.08 g $CuSO_4 \cdot 5H_2O$, 0.05 g $Co(NO_3)_2 \cdot 6H_2O$, and 75.0 g $NaNO_3$.

## 2.2 LED Light Applications and Experimental Setup

In the experimental studies, the cultures were exposed to three different LED light conditions: red (~660 nm), blue (~470 nm), and green (~520 nm). The light intensity was fixed at 3000 lux for all groups. The photoperiod was adjusted to 16 hours light and 8 hours dark (16:8) to match the stock culture conditions. Throughout the experiment, the ambient temperature was maintained at 23 ± 1 °C. Mixing was conducted using magnetic stirrers to ensure homogeneous light distribution and proper gas exchange. In the experimental setup, the LED lights were positioned to ensure equal light exposure across the cultures, and light intensity was measured at regular intervals to ensure stability. Separate groups were established for each LED color, and the experiments were conducted in triplicate. Cultures grown under white fluorescent light served as the control group.

## 2.3. Biochemical Analyses

For optical density measurements of *Haematococcus pluvialis*, cultures were homogenized and 2 mL samples were transferred into quartz cuvettes using a micropipette. Measurements were performed at 680 nm using a UV/VIS spectrophotometer (ISOLAB UV/VIS) for 10 days in triplicates.

In the protein analysis, 100 µL of Deoxycholate Solution (DOC, Sigma-Aldrich) was added to 1 mL of sample and mixed thoroughly, followed by the addition of 100 µL Trichloroacetic Acid (TCA, Sigma-Aldrich). The samples were then centrifuged at 2000 rpm for 10 minutes. After discarding the supernatant, 1 mL of Lowry reagent was added to the pellet and incubated for 20 minutes. Then, 1 mL of Folin & Phenol reagent (Sigma-Aldrich Total Protein Kit) was added, and the mixture was incubated for an additional 30 minutes. Absorbance was measured at 750 nm. Protein content was calculated using a protein standard curve based on absorbance values (Lowry et al.,1951)

For chlorophyll analysis, 2 mL of sample was centrifuged at 2000 rpm for 10 minutes, and the supernatant was discarded. Then, 2 mL of 99.9% methanol was added to the pellet, and the samples were incubated in an oven at 45 °C in the dark for 24 hours. After incubation, the samples were centrifuged again, and absorbance of the supernatant was measured at 665 nm and 652 nm (Lichtenthaler, 1987). Pigment contents were calculated using the following formulas:

**Chlorophyll a (mg $L^{-1}$)** = 16.72 × A665.2 − 9.16 × A652.4

**Chlorophyll b (mg $L^{-1}$)** = 34.09 × A652.4 − 15.28 × A665

In lipid analysis, 5 mL of sample was mixed with methanol–chloroform (1:2, v/v), followed by the addition of 2 mL of 0.4% $CaCl_2$ solution. The mixture was filtered through Schleicher & Schuell filter paper (125 mm), then incubated in an oven at 105 °C for 2 hours and left in the dark for 24 hours. The next day, the methanol-water upper phase was removed using a separating funnel. The remaining phase was incubated in a hot water bath at 80 °C, followed by heating at 90 °C in an oven for 1 hour to evaporate all chloroform (Bligh &Dyer,1959) After cooling, the tubes were weighed with a precision balance, and the lipid content was calculated using the formula below:

Lipid content (%) = (Weightofflask+ lipids)−Weightofflask(Weight of flask + lipids) − Weight of flask(Weightofflask+lipids)−Weightofflask × 100 / Sample weight (g)

For astaxanthin analysis, 5 mL of sample was centrifuged at 2000 rpm for 10 minutes. The pellet was mixed with 5 mL of 5% KOH in 30% methanol (v/v) and incubated at 65 °C for 15 minutes. After centrifugation, the supernatant was discarded, and 5 mL of DMSO was added to the pellet. Samples were then sonicated using a 3.0 kHz ultrasonic homogenizer (Bandelin HD2070) for 10 minutes. This process was repeated until visible coloration of the samples was achieved (Boussiba&Vonshak,1991). Astaxanthin concentration was then measured at 490 nm and calculated using the following formula; c (mg/L) = 4.5 × A490 × Va / Vb (Davies,1976).

## 2.4. Statistical analysis

All analyses were performed in triplicate. Data are presented as mean ± SD. The correlation heatmap shows the relationships between variables in the dataset. Each cell in this heatmap represents the correlation coefficient between two variables; the black and white colors represent the strength and direction of this correlation. In a correlation heatmap, the correlation coefficient ranges from -1 to 1.

A value of 1 indicates a perfect positive correlation, meaning that as one variable increases, the other variable also increases. A value of 0 indicates no relationship between the variables, meaning there is no correlation. A value of -1 indicates a perfect negative correlation, meaning that as one variable increases, the other decreases. All correlation heatmaps were created using Matplotlib (Version 7.4.1)

## 3. Results and Discussion

### 3.1 The Effect of Blue, Red and Green LED Light Wavelengths on the Growth Performance of *Haematococcus pluvialis*

In this study, the changes in the growth of the microalga *Haematococcus pluvialis* cultivated for ten days under different LED light wavelengths (blue, green, red – 3000 lux) were investigated. Optical density (OD) measurements at 680 nm were taken from the cultures using a spectrophotometer in triplicate. White, fluorescent light at 3000 lux was used as the control group.

On the day of inoculation, the OD values of all samples were similar, ranging from 0.255 to 0.262. By the second day, an increase in OD was observed in the cultures illuminated with red (660 nm) and blue (470 nm) LED light compared to the control group. On the fourth day, a notable increase was observed in the cultures illuminated with red LED light, followed by the cultures exposed to blue LED light. On the sixth day, maximum OD values were recorded in all groups, with the highest optical density reaching 0.859 in the red LED-illuminated culture. Growth under green LED light (520 nm) was more limited, and by day 10, the OD value reached 0.563. Based on these findings, it was concluded that red light wavelength promotes the growth of *Haematococcus pluvialis* (Fig.1).

These results support the idea that light spectrum plays a critical role in algal growth and that red light is the most favorable wavelength for stimulating the development of *H. pluvialis*.

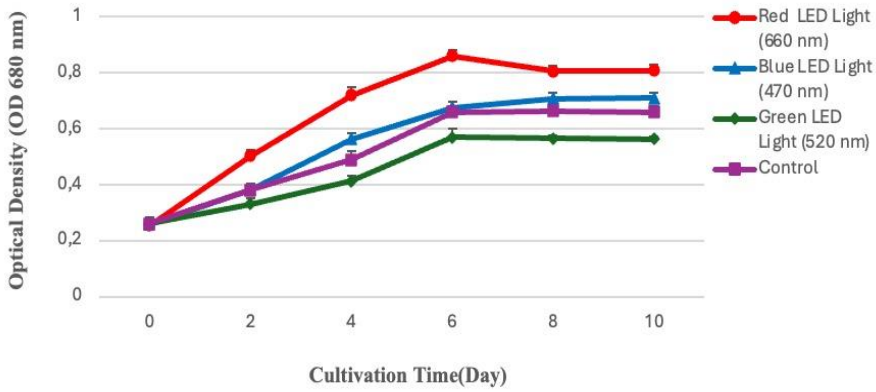


**Figure 1**. The Effect of Different Light Wavelengths on The Growth of *Haematococcus pluvialis* Over Ten-day Period

The findings obtained in this study demonstrate the growth-promoting effect of red LED light on *Haematococcus pluvialis* and are consistent with similar studies reported in the literature. Indeed, it has been reported that red light significantly enhances autotrophic growth and biomass production, and this effect is associated with increased carbonic anhydrase activity and $CO_2$ fixation efficiency (Li et al., 2023). Similarly, previous studies have indicated that red LED light results in higher photosynthetic oxygen production in *Chlorella kessleri* compared to blue light, which positively influences algal development (Wati, Rusva, & Umar, 2019). Moreover, it has been emphasized in a comprehensive review involving various microalgal species that red light stimulates cell division, thereby increasing photosynthetic efficiency and biomass production (Maltsev et al., 2021). In this context, the present findings support the enhancing effect of red LED light on the growth of *H. pluvialis*.

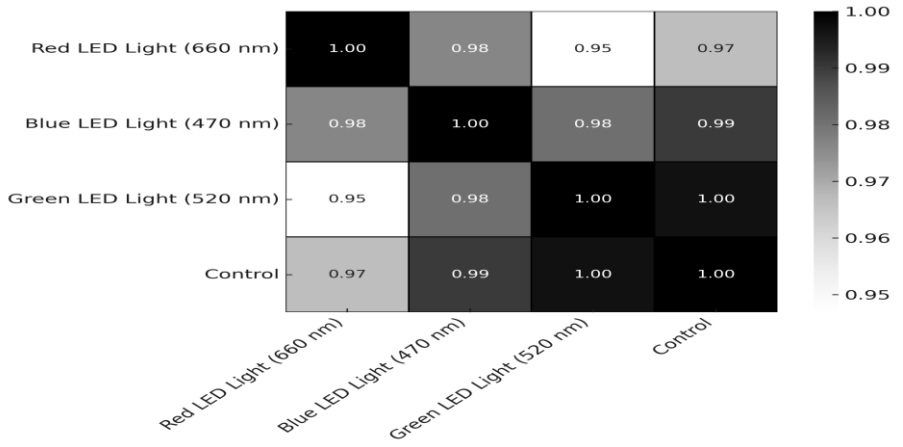


**Figure 2.** Heatmap Correlation Analysis of Time-Dependent Optical Density Values According to Different LED Light Sources

In this study, a correlation-based heatmap was employed to statistically analyze the effects of different LED light wavelengths on the growth of *Haematococcus pluvialis*. The heatmap graph visualizes the correlation between optical density (OD) values measured over time (days 0, 2, 4, 6, 8 and 10), allowing for a comparative assessment based on light types. The graph displays the strength of the linear relationship between variables using a Pearson correlation coefficient (r), which ranges from –1 to +1. This provides quantitative support for identifying the days and light conditions under which the highest growth rates occurred. Examination of the heatmap reveals that cultures exposed to red LED light (660 nm) exhibited progressively increasing OD values that were strongly correlated across days. This indicates that cell growth under red light was steady and continuous. Similarly, cultures illuminated with blue LED light (470 nm) also demonstrated high correlation values over time, reflecting a controlled and consistent growth process. In contrast, cultures exposed to green LED light (520 nm) displayed lower correlation values, suggesting that growth under this condition was irregular, with stagnation observed on certain days. In the control group, correlation coefficients were observed at more moderate levels, implying that cultures exposed to ambient white light exhibited more variable growth kinetics. This supports the notion that microalgal development is directly influenced by light spectrum.

Overall, the heatmap correlation analysis indicates that red and blue LED lights exert a more positive and consistent influence on the growth of *Haematococcus pluvialis*, whereas green LED light results in comparatively limited development (Fig 2.).

### 3.2 The Effect of Blue, Red, and Green LED Light Wavelengths on the Protein Content of *Haematococcus pluvialis*

In order to determine the effect of LED lights with different wavelengths on the protein content of *Haematococcus pluvialis*, analyses were conducted over a 10-day period and the variations in protein levels are presented in Figure 3. At the beginning of the experiment, all groups exhibited similar protein concentrations (ranging from 0.645 to 0.665 µg/mL). However, over time, different trends emerged depending on the light treatments applied. In the cultures illuminated with red LED light (660 nm), protein levels reached the highest values. In this group, protein concentration increased from 0.884 µg/mL on day 2 to 1.644 µg/mL on day 4 and peaked at 2.014 µg/mL on day 6. In the following days (days 8 and 10), a slight decline was observed (1.812 and 1.808 µg/mL, respectively), suggesting that protein synthesis may plateau or become limited after a certain point.In cultures exposed to blue LED light (470 nm), protein concentration increased from 0.662 µg/mL at the beginning to 1.412 µg/mL on day 4 and reached 1.802 µg/mL on day 6. These results indicate that blue light also supports protein synthesis, albeit to a lesser extent compared to red light. Overall, the findings demonstrate that red LED light promotes an increase in protein content and has a positive effect on protein biosynthesis in *Haematococcus pluvialis*.

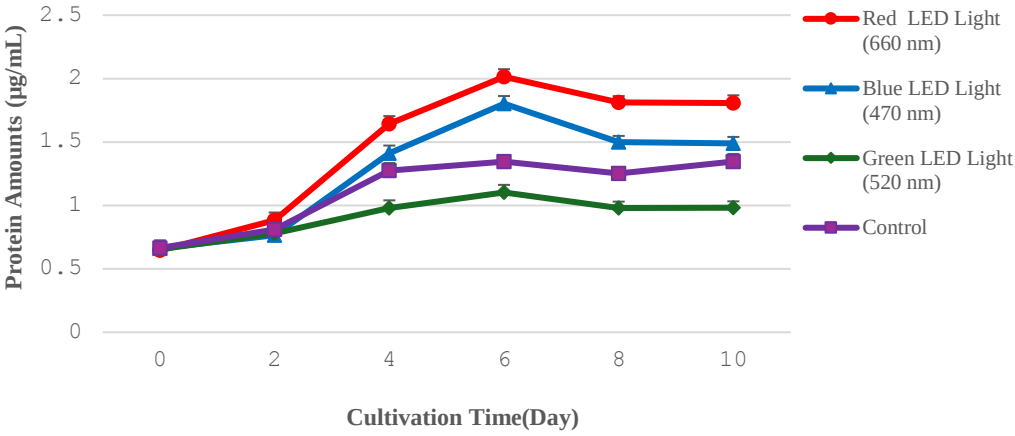


**Figure 3.** The Effect of Red, Blue and Green LED Light on the Protein Content of *Haematococcus pluvialis*

The effect of the red light spectrum on protein metabolism in microalgal cultures has been gaining increasing attention in the literature. In a study conducted on *Chlorella vulgaris*, the protein content of cultures incubated under red LED light was measured as 35.0% ± 1.0, which was found to be comparable to that under control conditions (white light). Furthermore, in terms of protein productivity, a high value of 200.7 mg $L^{-1}$ $day^{-1}$ was achieved in the red light group, surpassing the control group (Six et al., 2024). Similarly, in another study conducted on *Scenedesmus* sp., the application of a red LED-supported luminant solar concentrator (LSC) not only increased biomass but also enhanced protein content by approximately 15%. This increase was further supported by up to a 35% rise in protein productivity (Raeisossadati et al., 2020).

In line with these findings, the present study also observed that red light not only promoted cell growth but also supported protein synthesis, thereby enhancing the total protein content. These results suggest that red light is a favorable light source in microalgal biotechnology, offering advantages in terms of both carbon and nitrogen metabolism.

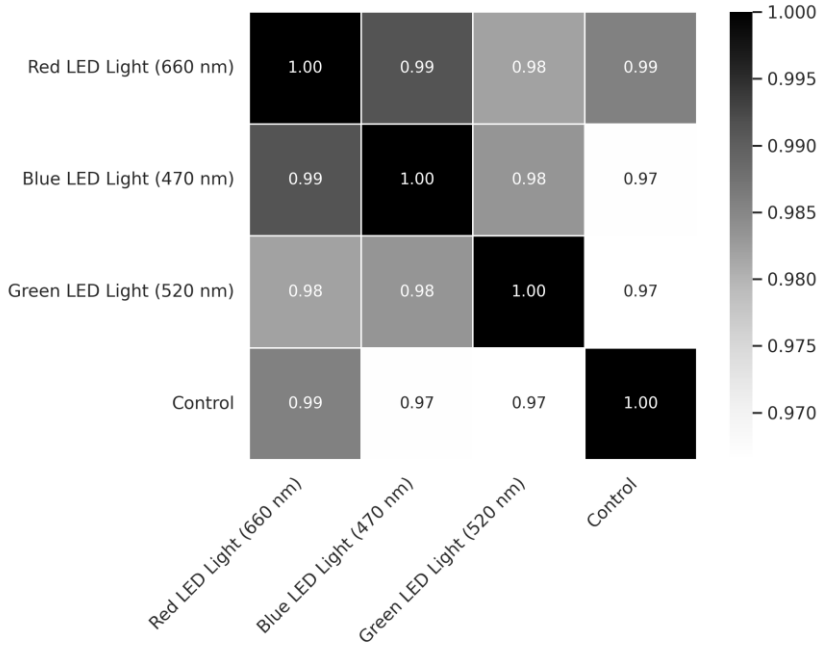


**Figure 4.** Correlation Heatmap of Protein Content under Different LED Light Wavelength Applications

The correlation heatmap illustrates the pairwise relationships among protein content measurements obtained under different LED light wavelengths—red (660 nm), blue (470 nm), green (520 nm)—and a control group (white fluorescent light).

Pearson correlation coefficients, ranging from 0.97 to 1.00, indicate very strong positive correlations across all experimental groups. These values suggest that protein accumulation trends were generally consistent over time, though subtle differences were observed depending on the light condition applied. The red LED light (660 nm) showed exceptionally strong correlations with all other treatments, particularly with the blue LED (r = 0.99) and control (r = 0.99) groups. This indicates a high degree of similarity in protein accumulation trends between these conditions. Notably, red LED light was the treatment under which the highest protein content was recorded, confirming its positive influence on protein biosynthesis. The blue LED light (470 nm) also displayed a perfect internal correlation (r = 1.00) and high correlations with red (r = 0.99) and green (r = 0.98) light treatments. However, its correlation with the control group was slightly lower (r = 0.97), suggesting some divergence in protein accumulation dynamics compared to white light. The green LED light (520 nm) exhibited slightly lower correlations with other treatments, including r = 0.98 with red and blue LEDs and r = 0.97 with the control. This reflects a relatively less consistent pattern in protein synthesis under green light, which aligns with earlier findings that green light is less effective at stimulating photosynthetic pigment and protein accumulation in microalgae. The control group, exposed to white fluorescent light, demonstrated high correlations with all LED treatments, especially red light (r = 0.99). This may be due to the composite spectral nature of white light, which includes red wavelengths, thus partially mimicking the effect of monochromatic red light (Fig.4).

### 3.3 Effects of Different LED Light Wavelengths on Astaxanthin Accumulation in *Haematococcus pluvialis*

In this study, the influence of LED light sources with different wavelengths namely red (660 nm), blue (470 nm), and green (520 nm) as well as a white light control group, on astaxanthin accumulation in *Haematococcus pluvialis* was investigated over a 10-day cultivation period. The experimental results demonstrated that the highest astaxanthin content was achieved under red LED light, with a maximum concentration of 0.984 µg/mL recorded on day 6. This value was substantially higher than that measured in the control group on the same day (0.555 µg/mL), indicating a significant stimulatory effect of red light on astaxanthin biosynthesis. A gradual increase in astaxanthin levels was observed throughout the red light treatment, starting from 0.554 µg/mL on day 2 and reaching 0.686 µg/mL on day 4, followed by a peak on day 6. On days 8 and 10, astaxanthin concentrations plateaued at 0.932 µg/mL and 0.933 µg/mL, respectively, suggesting a possible saturation in pigment synthesis due to prolonged exposure or light intensity limits. In cultures exposed to blue LED light, astaxanthin levels were comparatively lower, with a peak value of 0.718 µg/mL on day 6 and a slight decrease to 0.682 µg/mL on day 8, indicating that while blue light supported astaxanthin synthesis to some extent, it was less effective than red light. The lowest astaxanthin concentrations were observed under green LED light, with a maximum value of only 0.462 µg/mL detected on day 6.

This outcome reflects the limited stimulatory effect of green wavelengths on carotenoid biosynthesis in *H. pluvialis*.

These findings collectively demonstrate that red LED light is the most effective spectral condition for enhancing astaxanthin accumulation in *H. pluvialis*, likely due to its role in inducing photooxidative stress and upregulating secondary metabolite pathways (Fig.5).

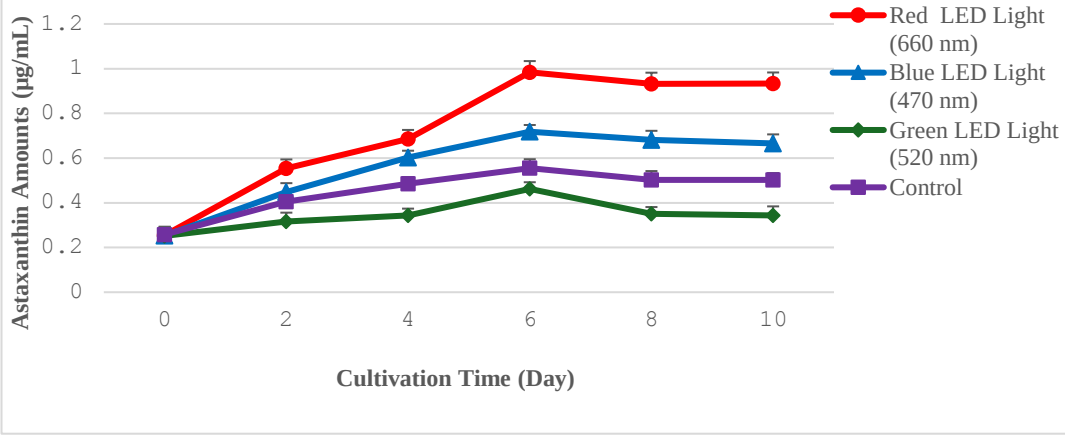


**Figure 5.** Changes in Astaxanthin Accumulation in *Haematococcus pluvialis* under Different Light Wavelengths

Xi et al. (2016) investigated the effects of different LED light wavelengths on enhancing astaxanthin production in *Haematococcus pluvialis*. In their study, red LED light (630–665 nm) was applied during the initial cultivation phase to promote cell growth, followed by a shift to blue LED light (430–465 nm) to induce astaxanthin biosynthesis.

This wavelength-switching strategy resulted in a 50–62% increase in astaxanthin accumulation compared to continuous red light exposure. Furthermore, the combined application of blue LED light with additional carbon sources, such as acetate, further enhanced astaxanthin production. Under blue light, morphological changes such as transition to the cyst stage, increased cell size, and visible red coloration were observed. These results demonstrate that a two-phase (growth + production) LED light strategy is an effective approach for maximizing astaxanthin yield.

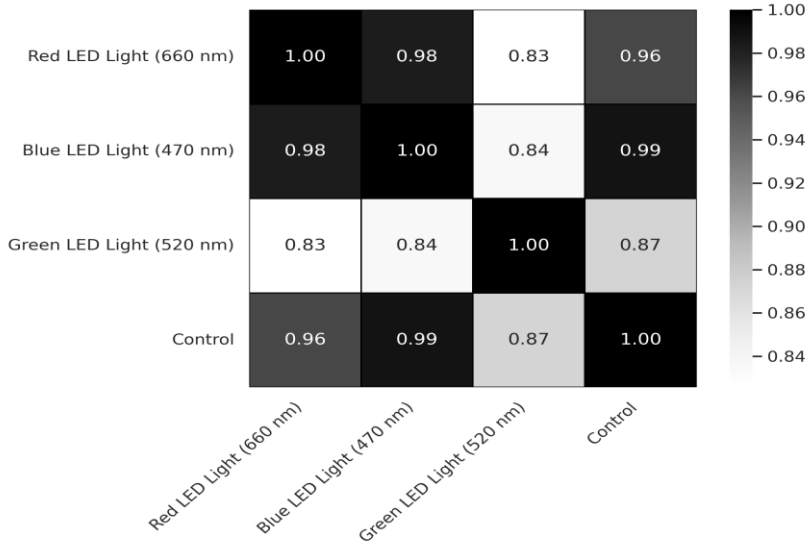


**Figure 6.** Correlation Heatmap Analysis of Astaxanthin Accumulation under Different LED Light Wavelengths

The correlation heatmap analysis revealed that red LED light (660 nm) exhibited the strongest and most consistent positive effect on astaxanthin production. Specifically, during days 6 to 10, correlation values ranged between 0.984 and 0.933. While blue light (470 nm) demonstrated a moderate level of effectiveness, green light (520 nm) and the control group showed relatively low correlations. These findings indicate that red LED light is the most suitable light source for promoting astaxanthin accumulation in *Haematococcus pluvialis* (Fig.6).

### 3.4 Effects of Different LED Light Wavelengths on Lipid Accumulation in *Haematococcus pluvialis*

In this study, lipid accumulation in *Haematococcus pluvialis* was evaluated over a 10-day cultivation period under different LED light wavelengths. The results demonstrated that light quality had a pronounced effect on lipid biosynthesis. The highest lipid accumulation was observed in cultures exposed to red LED light (660 nm), reaching a maximum of 55.85% on day 6. This was followed by cultures exposed to blue LED light (470 nm), which recorded a lipid content of 50.17% on the same day. In the control group, lipid accumulation was 42.35%, while green LED light (520 nm) resulted in the lowest accumulation at 35.14%. During the initial two days, the rate of lipid increase was more pronounced under red light, rising from 17.05% on day 0 to 28.05% on day 2. This rapid accumulation trend continued on days 4 and 6, with cultures grown under red light producing the highest values.

Although a slight decline in lipid content was observed after day 6, red LED light maintained a superior stimulatory effect compared to the other groups. Cultures treated with blue LED light also showed significant lipid accumulation, exceeding 50% by day 6, although still lower than under red light conditions. These findings clearly indicate that the light spectrum plays a critical role in modulating lipid metabolism in *H. pluvialis* (Figure 7).

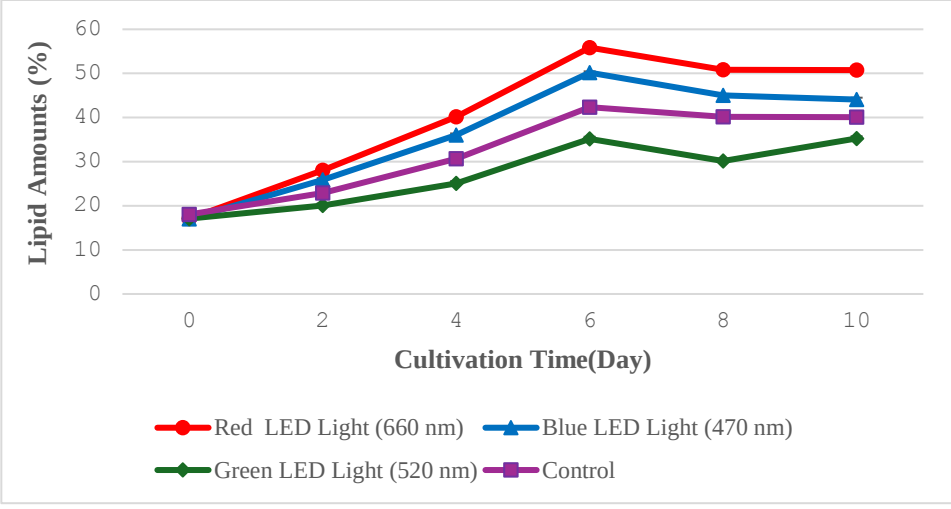


**Figure 7.** Effect of Different LED Light Wavelengths on Changes in Lipid Content of *Haematococcus pluvialis* Cultures over a 10-Day Period

These findings are consistent with previous studies in the literature. Cheirsilp & Torpee (2012) reported that combinations of red and blue light promoted lipid production in various microalgal species, with red light in particular enhancing energy conversion mechanisms and promoting lipid accumulation.

Similarly, Chen et al. (2011) observed high lipid accumulation in *Scenedesmus sp.* cultures grown under red LED light. These findings support the results of the present study and suggest that red LED light serves as a dominant inducer in the lipogenesis process. Consequently, red LED illumination can be considered the most favorable condition for maximizing lipid accumulation in *Haematococcus pluvialis* cultures.

These results provide a strategic basis for photobioreactor applications aimed at bioenergy, biodiesel production, and high-value lipid metabolite generation. Moreover, light management strategies based on such optimizations hold potential for improving the economic efficiency of commercial-scale algal cultivation systems.

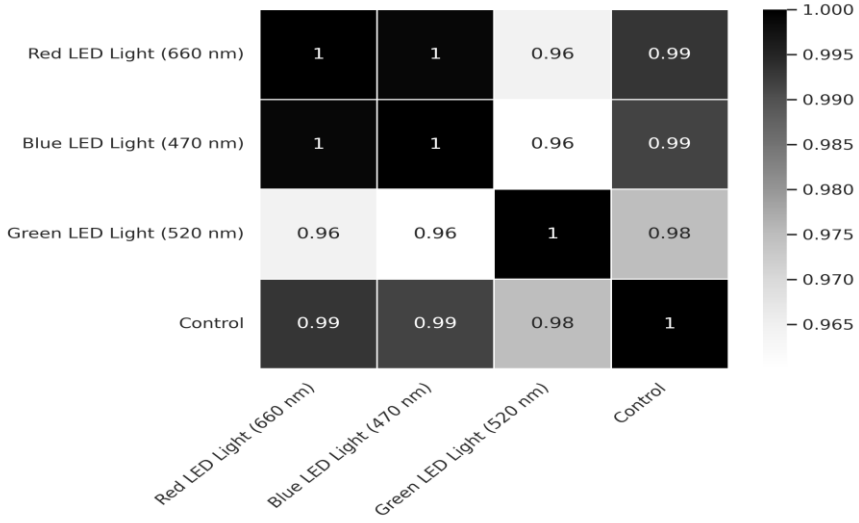


**Figure 8.** Correlation Heatmap of Lipid Content under Different LED Light Wavelengths in *Haematococcus pluvialis* Cultures

The correlation heatmap analysis of lipid content clearly reveals how the effects of different LED light wavelengths on *Haematococcus pluvialis* vary over time. The graphical data demonstrate that red LED light (660 nm) exerts a strong and consistent positive influence on lipid accumulation. From day 6 onwards, correlation coefficients ranged between 0.859 and 0.808, indicating a sustained stimulatory effect of this wavelength on lipid metabolism. Similarly, blue LED light (470 nm) also supported lipid synthesis, with correlation values ranging between 0.675 and 0.709 between days 6 and 10. These findings suggest that while blue light contributes to lipidogenesis, its effect is comparatively weaker than that of red light. In contrast, green LED light (520 nm) exhibited considerably lower correlation values, indicating a limited effect on lipid accumulation (Fig.8). The overall correlation data confirm that red LED light is the most effective light source for promoting lipid synthesis in *Haematococcus pluvialis* cultures, with its impact becoming more pronounced over time.

### 3.5 Changes in Chlorophyll a and b Content of *Haematococcus pluvialis* Cultivated Under Different Wavelengths of Light

Throughout the ten-day cultivation period, the effects of LED lights with different wavelengths on chlorophyll a accumulation in *Haematococcus pluvialis* cultures were evaluated. According to the data obtained, cultures exposed to green LED light (520 nm) exhibited a marked increase in chlorophyll a content over time, reaching a maximum of 0.682 µg/mL on day 6.

This value represented the highest chlorophyll a concentration among all experimental groups. Furthermore, under green light exposure, the chlorophyll a content was recorded as 0.663 µg/mL on day 10, indicating sustained high pigment production. In contrast, a more moderate increase in chlorophyll a content was observed under blue LED light (470 nm). Chlorophyll a reached 0.455 µg/mL on day 6 and slightly declined to 0.421 µg/mL by day 10. Cultures exposed to red LED light (660 nm), however, showed comparatively lower chlorophyll a levels. The maximum value under red light was only 0.324 µg/mL on day 6, decreasing slightly to 0.305 µg/mL by day 10. This suggests that red light may exert a limiting effect on chlorophyll a synthesis in *H. pluvialis*. Overall, the findings indicate that green LED light is the most effective spectral condition for enhancing chlorophyll a production in *Haematococcus pluvialis*, while red light appears to be less favorable for this pigment's synthesis (Fig.9).

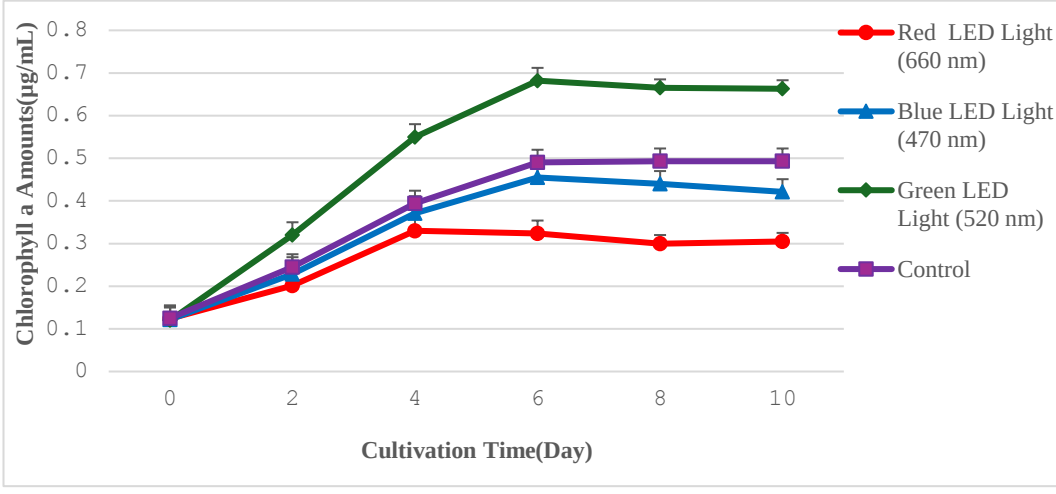

**Figure 9.** The Effect of Different LED Light Wavelengths on Chlorophyll a Accumulation (µg/mL) in *Haematococcus pluvialis* Cultures Over a 10-Day Period

Analyses conducted throughout the study revealed a pronounced increase in chlorophyll b accumulation under green LED light (520 nm). Notably, values of 0.865 µg/mL, 0.862 µg/mL, and 0.862 µg/mL were recorded on days 6, 8, and 10 of the experiment, respectively, indicating that this light condition effectively optimized photosynthetic pigment production. These results demonstrate that green light significantly enhances chlorophyll b levels in *Haematococcus pluvialis*, with a more pronounced effect compared to the other treatment groups.

Although blue LED light (470 nm) also supported pigment synthesis, the highest value obtained under this condition was 0.660 µg/mL, which remained lower than that observed in the green light group. The effect of red LED light (660 nm), on the other hand, was relatively limited, with the highest chlorophyll b concentration recorded at 0.554 µg/mL on day 6. In the control group, pigment production appeared to remain stable, reaching a peak value of 0.702 µg/mL on day 6. These findings suggest that chlorophyll b production is sensitive to the spectral properties of light and that green wavelengths may act as a stimulatory factor in its biosynthesis.

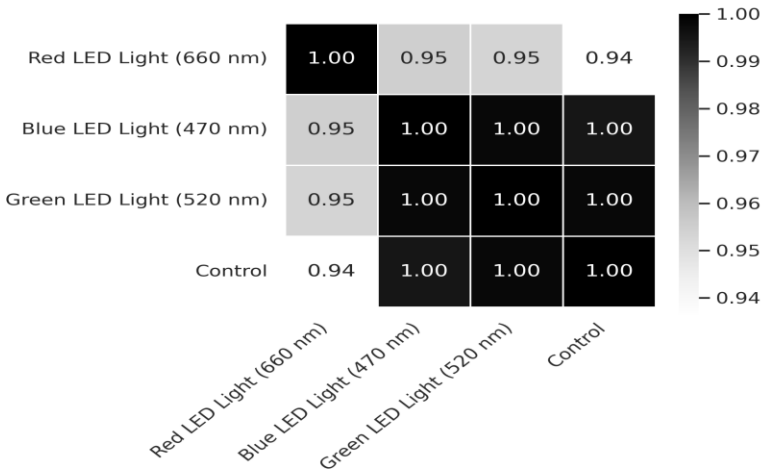


**Figure 10.** Correlation Heatmap Analysis of the Effect of Different LED Light Wavelengths on Chlorophyll a Accumulation in *Haematococcus pluvialis*

According to the correlation analysis, there is a strong positive relationship in chlorophyll a accumulation across all light groups. In particular, the perfect correlation observed among the blue, green, and control groups (r = 1.00) indicates that chlorophyll a synthesis in these groups is regulated through similar biochemical responses and that fluctuations in pigment levels follow a highly parallel trend over time depending on light quality. Although red LED light exhibits a similar trend, its relatively lower correlation values suggest that this wavelength may induce distinct effects on the chlorophyll a metabolism of microalgae( Fig.10).

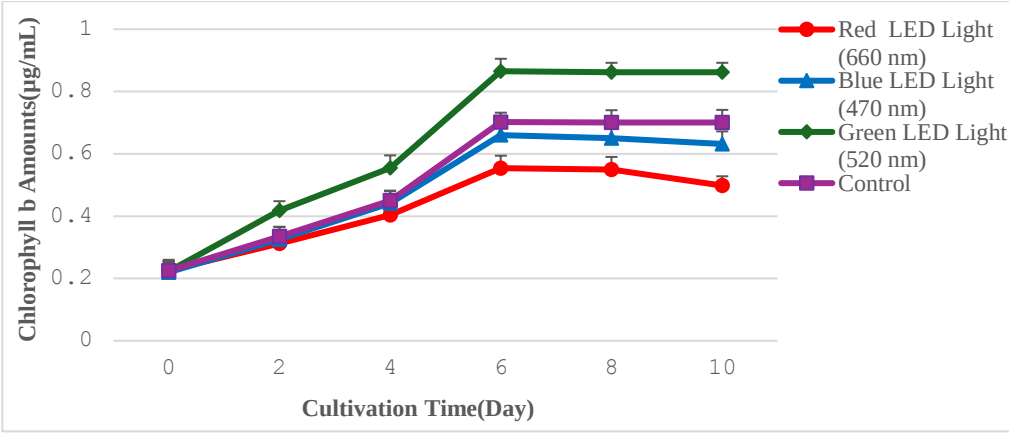


**Figure 11.** Effect of Different LED Light Wavelengths on Chlorophyll b Content in *Haematococcus pluvialis* During a 10-Day Cultivation Period

Studies evaluating the effects of green LED light on microalgal pigment accumulation have particularly focused on the stimulatory role of this wavelength in chlorophyll synthesis. Several investigations have demonstrated that green light may optimize the synthesis of chlorophyll a and b and enhance photosynthetic efficiency. Based on the analyses conducted in the present study, cultures illuminated with green LED light (520 nm) exhibited significantly higher levels of chlorophyll a and b compared to other light sources. This finding suggests that green light serves as a potent inducer specifically in the biosynthesis of chlorophyll pigments (Fig.11).

Supporting evidence from the literature aligns with these results. In a study conducted by Wang et al. (2020), green LED illumination significantly increased chlorophyll a and b content in *Chlorella vulgaris*

and *Scenedesmus obliquus*. The researchers attributed this effect to the selective stimulatory role of light spectrum characteristics on photosynthetic pigment production. Accordingly, the elevated chlorophyll levels observed under green light in this study are consistent with existing findings and further confirm the positive impact of green light on chlorophyll synthesis. In our experiment, the highest chlorophyll a and b concentrations were recorded in *Haematococcus pluvialis* cultures exposed to green LED light (520 nm), compared to other light groups. This result highlights the stimulatory influence of green light on photosynthetic pigment production. Notably, on day 6, chlorophyll a content reached 0.682 µg/mL and chlorophyll b content peaked at 0.865 µg/mL.

The obtained data indicate that green light is effective in stimulating photosynthetic mechanisms and promoting pigment biosynthesis. Supporting findings in the literature confirm this phenomenon. In a study conducted by Nazari and Moheimani (2022), green LED illumination was found to significantly increase chlorophyll a content in microalgal cultures, suggesting that the stimulatory effect of green light on chlorophyll biosynthesis can be extended to various microalgal species. Similarly, in a comprehensive review by Maltsev et al. (2021), various microalgal species subjected to green light treatments exhibited enhanced pigment accumulation, supporting the hypothesis that green spectrum illumination significantly influences photosynthetic pigment synthesis.

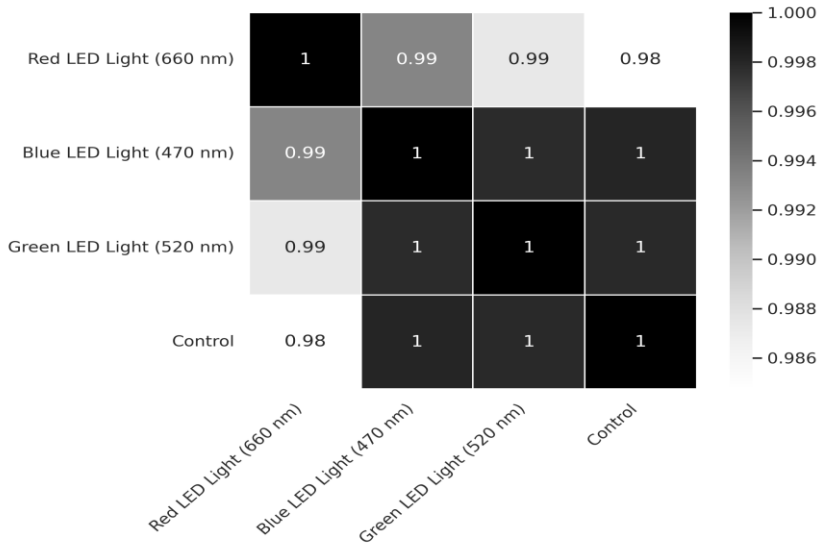


**Figure 12.** Correlation Heatmap of Chlorophyll b Levels in *Haematococcus pluvialis* Exposed to Different LED Wavelengths

An analysis of the chlorophyll b correlation heatmap revealed notable variations in the temporal accumulation patterns under different LED light sources (Fig.12). In particular, cultures exposed to green LED light (520 nm) exhibited a pronounced increasing trend starting from day 4, with strong accumulation patterns and high correlation values observed on days 6, 8, and 10. This finding indicates that green wavelength light exerts a supportive effect on chlorophyll b synthesis.

In contrast, the correlations observed under red (660 nm) and blue (470 nm) LED light conditions were of moderate intensity, and the accumulation patterns appeared relatively stable after day 6, suggesting a lower pigment synthesis potential.

The control group also demonstrated a consistent increase, though not as prominent as that observed under green light. These results highlight the superior efficacy of green light in promoting chlorophyll b production and demonstrate that its stimulatory effect becomes more pronounced over time. The observed positive correlations between light sources and chlorophyll b accumulation clearly indicate the sensitivity of photosynthetic pigment synthesis to light spectrum, underscoring the potential of green LED illumination as an optimal spectral condition for enhancing pigment production in chlorophyll b-containing microalgae.

## CONCLUSION

In this study, the effects of different LED light wavelengths (red: 660 nm, blue: 470 nm, and green: 520 nm) on *Haematococcus pluvialis* were evaluated, with comprehensive analyses performed in terms of growth performance and biochemical composition. The findings related to optical density, protein, lipid, astaxanthin, chlorophyll a, and chlorophyll b content revealed the physiological responses of the microalga to various light spectra in detail.

According to the results, red LED light led to a significant increase in growth (optical density), protein, and astaxanthin production, indicating that photosynthetic efficiency and energy conversion mechanisms reached their maximum under this wavelength. Blue LED light was particularly effective in promoting astaxanthin synthesis, likely due to the stress-inducing nature of blue light, which can stimulate carotenoid biosynthesis.

Green LED light resulted in the highest levels of chlorophyll a and b, highlighting the stimulatory effect of green spectrum on pigment metabolism. The black-and-white heatmap graphs, supported by correlation analyses, visually reinforced the relationship between light spectra and pigment as well as lipid contents. Strong positive correlations were found between green light and both lipid and pigment content, while red light showed strong correlations with growth and protein production. These findings not only contribute to fundamental scientific knowledge but also provide direct insights into strategic applications of microalgal biotechnology in fields such as health, food, agriculture, and environmental sciences. Notably, the increased astaxanthin production under red light enhances the potential of *H. pluvialis* as a potent antioxidant source for pharmaceutical and nutraceutical applications. Furthermore, the elevated protein content supports the utilization of microalgae as sustainable ingredients in animal feed and functional food formulations. The increase in chlorophyll content also supports the use of microalgae-based biological inputs such as agricultural biofertilizers and natural pesticides.

In conclusion, this study demonstrated that light spectrum can serve as a guiding tool for targeted metabolite production and that *Haematococcus pluvialis* can be optimized for growth and astaxanthin production under red light, and for chlorophyll and lipid synthesis under green light.

In this context, light-based culture optimization strategies hold significant potential in the development of sustainable biotechnological production systems. Controlled cultivation of microalgae appears to offer promising, environmentally friendly, and economically feasible solutions for health, food, and agricultural applications.

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# EFFECTS OF RED, GREEN AND BLUE LED LIGHT ON GROWTH, ASTAXANTHIN ACCUMULATION AND PROTEIN CONTENT IN HAEMATOCOCCUS PLUVIALIS