HAYATI
Journal of Biosciences

Vol. 33 No. 4, July 2026 945-961
DOI:10.4308/hjb.33.4.945-961
ISSN: 1978-3019
EISSN: 2086-4094

Research Article

# Comparative Analysis of Antioxidative Mechanisms in *Chlorella vulgaris*, *Isochrysis galbana*, and *Tetraselmis chuii* Under pH Stress

**Norhayati Yusuf[1,2]*, Nurul Shafiqa Yusof[3,4]**

[1]*Faculty of Science and Marine Environment, Universiti Malaysia Terengganu, 21030 Kuala Nerus, Terengganu, Malaysia*
[2]*Biological Security and Sustainability Research Interest Group (BIOSES), Universiti Malaysia Terengganu, 21030 Kuala Nerus, Terengganu, Malaysia*
[3]*SATREPS-COSMOS Laboratory, Centre of Research and Field Service, Universiti Malaysia Terengganu, 21030 Kuala Nerus, Terengganu, Malaysia*
[4]*Institute of Climate Adaptation and Marine Biotechnology (ICAMB), Universiti Malaysia Terengganu, 21030 Kuala Nerus, Terengganu, Malaysia*

ARTICLE INFO

*Article history:*
Received July 29, 2025
Received in revised form January 21, 2026
Accepted January 30, 2026
Available Online April 9, 2026

*KEYWORDS:*
defence mechanism,
microalgae,
oxidative stress,
pH,
reactive oxygen species

## ABSTRACT

Microalgae such as *Chlorella vulgaris*, *Isochrysis galbana*, and *Tetraselmis chui*, play a vital role in aquatic ecosystems and have considerable biotechnological applications. Medium pH strongly influences their physiological performance and oxidative balance. This study investigated the antioxidative responses of these three species grown under initial pH conditions ranging from 4 to 10, to elucidate species-specific adaptive strategies to pH-induced oxidative stress. Growth performance, enzymatic antioxidants (superoxide dismutase, SOD; catalase, CAT; guaiacol peroxidase, GPX; glutathione reductase, GR), and non-enzymatic antioxidants (ascorbate, AsA; glutathione, GSH; chlorophylls; carotenoids) were assessed. The results revealed distinct antioxidative strategies among the species. *C. vulgaris* relied predominantly on enzymatic defence mechanisms, showing marked upregulation of SOD, GPX, and GR. Compared with control conditions, SOD and GPX activities increased by 40.7% and 17.1%, respectively, at pH 4, while GR activity increased by 42.8% at pH 10. In contrast, *I. galbana* exhibited a more selective response, with strong induction of CAT activity (29.9%) at pH 4 and a substantial increase in total GSH content (79%) at pH 7, while APX activity was highest under control pH conditions. *T. chuii* demonstrated a balanced antioxidative strategy, combining moderate enhancement of enzymatic activity, particularly GR (17.2%) at pH 7, with pronounced accumulation of non-enzymatic antioxidants, including carotenoids (60.9%) and GSH (42.4%). These findings highlight the diverse adaptive capacities of microalgae to pH stress and provide valuable insights for optimising microalgal cultivation in biofuel production, aquaculture, and environmental management, as well as for developing stress-tolerant strains for sustainable biotechnological applications.

## 1. Introduction

Excessive greenhouse gas emissions from human activities have significantly amplified the effects of global warming, heightening global concern for environmental sustainability. Among these emissions, $CO_2$ accounts for three-quarters, making it the primary contributor to climate change (Huang *et al.* 2020). The ocean acts as a vital sink for atmospheric $CO_2$, which lowers the pH of seawater, a process known as ocean acidification.

---

*Corresponding Author
E-mail Address: yatiyusuf@umt.edu.my

This acidification leads to the overproduction of reactive oxygen species (ROS) in marine life, resulting in cellular failure (Vinuganesh *et al.* 2022). However, microalgae have become important biological tools for mitigating $CO_2$ as they can convert it into useful biomass (Bartley *et al.* 2014). As a result, there is increasing interest in studying how ocean acidification, including changes in $CO_2$ and pH levels, affects marine organisms, particularly microalgae, due to their potential role in supporting environmental sustainability (Yin *et al.* 2019).

The pH of the growth medium is a crucial factor that affects the physiology, growth, and accumulation of bioactive compounds in microalgae cells. pH can impact nutrient availability, enzyme activity, and overall cellular health. Microalgae often live in environments with fluctuating pH levels, necessitating mechanisms to adapt to these changes (Srinivasan *et al.* 2018; Hanachi *et al.* 2021). Understanding how pH affects microalgae is essential for optimizing their cultivation in various applications. Changes in pH can also reveal the capacity of microalgae to fix carbon through photosynthesis (Hu *et al.* 2023). Gauthier *et al.* (2022) reported that large-scale microalgae cultivation that utilizes $CO_2$ from industrial off-gas emissions increases the acidity of the culture media, lowering the pH to 3.0-4.0. Interestingly, some acid-tolerant microalgae species, such as *Dunaliella* spp., *Euglena* spp., *Chlamydomonas* spp., and *Ochromonas* spp., are often found in low-pH environments, including acid mine drainage sites, and can produce higher levels of astaxanthin, lutein, and zeaxanthin. However, adapting microalgae to changes in extracellular pH can lead to intraspecific variation (Wu *et al.* 2022).

Oxidative stress occurs when the production of ROS exceeds the organism's ability to detoxify them. ROS, such as superoxide radicals $O2^{\bullet-}$, hydrogen peroxide ($H_2O_2$), and hydroxyl radicals $OH^{\bullet-}$, can cause significant damage to cellular components, including lipids, proteins, and nucleic acids. Microalgae have developed intricate antioxidative defense mechanisms to counteract oxidative stress, including enzymatic and non-enzymatic antioxidants. Antioxidant enzymes protect cells against oxidative damage by neutralizing harmful free radicals and ROS. Superoxide dismutase (SOD) catalyzes the conversion of $O2^{\bullet-}$ into $H_2O_2$ and molecular oxygen ($O_2$), preventing oxidative damage to cells and tissues (Koletti *et al.* 2025). Similarly, catalase (CAT) facilitates the breakdown of $H_2O_2$ into water ($H_2O$) and oxygen ($O_2$), thereby reducing the buildup of $H_2O_2$ (Lee & Park 2021). Glutathione peroxidase (GPx) reduces $H_2O_2$ to $H_2O$, limiting its harmful effects, supports growth factor signalling and mitochondrial function, and maintains normal thiol redox balance (Pei *et al.* 2023). Glutathione reductase (GR) produces reduced glutathione (GSH), an antioxidant that protects cells from oxidative damage, from its oxidized form, glutathione disulfide (GSSG). Non-enzymatic antioxidants, such as ascorbate (vitamin C), GSH, and carotenoids (CAR), can directly scavenge ROS (Halliwell 2022). The effectiveness of these antioxidative mechanisms is vital for microalgae's survival and proper functioning under stress conditions, including pH fluctuations.

From an industrial perspective, precise pH control is a critical operational parameter in large-scale microalgal bioreactors, as pH fluctuations can directly affect growth performance, metabolic activity, and oxidative stress levels (Abdur Razzak *et al.* 2024). Maintaining optimal pH conditions not only enhances biomass productivity but also increases the production of valuable compounds, including chlorophylls, carotenoids, phycobiliproteins, antioxidants, omega-3 fatty acids, polysaccharides, and phenolics. These compounds are widely applied in the food, cosmetics, pharmaceutical, and nutraceutical industries due to their natural, safe, and health-promoting properties. Additionally, they contribute to microalgae's resilience in the face of varying environmental conditions (Shafiquzzaman *et al.* 2023). In open ponds and closed photobioreactors, pH variations often arise from $CO_2$ injection, nutrient uptake, and photosynthetic activity, which can impose oxidative stress if not properly regulated. The species-specific antioxidative responses observed in this study suggest that effective pH management strategies can be tailored to the physiological strengths of different microalgae, optimizing the production of high-value compounds such as pigments, antioxidants, and biofuels. Consequently, understanding pH-driven antioxidant regulation provides valuable guidance for selecting robust strains and designing cultivation protocols that enhance productivity, stress tolerance, and operational stability in industrial microalgal systems.

In this study, three microalgae species were selected: *Chlorella vulgaris*, *Isochrysis galbana*, and *Tetraselmis chuii*, which represent contrasting ecological niches, cell wall structures, and physiological traits, and metabolic profiles, making them ideal for a comparative analysis of pH induced oxidative stress responses. These fundamental biological differences influence not only their growth dynamics but also their antioxidative defense mechanisms and biotechnological potential, providing broader insights into species specific stress tolerance. *C. vulgaris* is a unicellular green alga characterized by a rigid cell wall composed of a fibrillar layer containing glucosamine, which provides structural integrity and resistance to environmental stress (Okuro *et al.* 2019). It exhibits a high growth rate and is widely studied for applications ranging from wastewater bioremediation to biofuel production, owing to its ability to generate substantial biomass and valuable bioactive compounds under diverse conditions. In addition to its rapid growth and resilience, *C. vulgaris* is recognized for its high nutritional value, particularly in proteins and lipids, and its health-promoting properties, including strong antioxidative activity (Fu *et al.* 2019). Research indicates that *C. vulgaris* can adjust its antioxidative responses to cope with changing environmental conditions, making it a suitable model for investigating how pH variations impact antioxidative mechanisms (Liu & Chen 2014). *I. galbana*, a member of the Haptophyta phylum, is rich in essential fatty acids, particularly docosahexaenoic acid (DHA), and a complex array of bioactive lipids and vitamins, which make it particularly valuable in the aquaculture and nutraceutical industries. It is distinguished by its small size and thin, flexible cell covering, which differs markedly from the rigid, cellulose-rich cell wall of *C. vulgaris*. This more delicate structure allows *I. galbana* to rapidly exchange nutrients and gases with its surrounding environment, but also makes it more sensitive to physical and chemical stressors (Ramos-Romero *et al.* 2021). Its sensitivity to environmental changes, including pH, provides a unique perspective on how marine microalgae respond to oxidative stress (Ge *et al.* 2023). *T. chuii*, a green flagellate, is known for its robustness and adaptability to various environmental conditions. It is widely used in aquaculture as feed for marine organisms and in bioremediation (Tham *et al.* 2023). It contains intermediate cell wall complexity and high adaptability, representing a species that balances stress tolerance with metabolic versatility. Its capacity to withstand and adapt to pH fluctuations makes it an excellent model for studying antioxidative responses, providing insights that can inform strategies to enhance its cultivation and stress resilience (Vijayaram *et al.* 2024; Carrillo & Anchundia 2024). The relationship between pH regulation and oxidative stress in microalgae is intricate and multifaceted. pH shifts can influence ionic balance, nutrient availability, and metabolic processes within microalgal cells. For example, acidic conditions may release of transition metals such as iron and copper, which can catalyze ROS formation via the Fenton and Haber-Weiss reactions. Conversely, alkaline conditions can impact the stability and activity of enzymes involved in antioxidative defense.

Microalgae use various strategies to reduce the effects of pH-induced oxidative stress. These include increasing the expression of antioxidative enzymes, producing non-enzymatic antioxidants, and activating signaling pathways that modulate stress responses (Mishra *et al.* 2023; Hong *et al.* 2024; Rauf *et al.* 2024). To examine how growth and metabolism adapt to pH changes, this study thoroughly investigates the antioxidative responses of *C. vulgaris*, *I. galbana*, and *T. chuii* to initial pH shifts, revealing their adaptive strategies for pH-induced oxidative stress. This research can help improve understanding of how microalgal antioxidant mechanisms respond to shifting pH conditions.

## 2. Materials and Methods

### 2.1. Culture Initiation, Cultivation, and Treatments

Axenic cultures of *Chlorella vulgaris* (UMT-M1), *Isochrysis galbana* (SWC002), and *Tetraselmis chuii* (SWC001) were obtained from the Institute of Climate Adaptation and Marine Biotechnology (ICAMB), Universiti Malaysia Terengganu. The cultures were acclimated in 500 mL conical flasks containing 450 mL of filtered, sterilized seawater at 30 ppt salinity and pH 8.0±0.2, enriched with F/2 medium (Guillard & Ryther 1962; Guillard 1975). Incubation was carried out under continuous illumination of 2,000 lux from 6,500 K LED lamps at 24°C±2°C, with aeration provided by a Hailea HAP-120 pump. All species were initially cultured from an equal inoculum of $1\times10^6$ cells/mL during the early stationary phase (Norhayati *et al.* 2023). For the treatments, *C. vulgaris*, *I. galbana*, and *T. chuii* were cultured at different initial pH levels, ranging from acidic (pH 4-6) to neutral (pH 7) and alkaline (pH 8-10), with pH 8 serving as the control.

### 2.2. Determination of Cell Density

The cell density was monitored daily for 24 days. Approximately 1 mL of the microalgae liquid culture was transferred into a 1.5 mL microcentrifuge tube. For cell counting, 200 µL of the liquid culture was diluted with 800 µL of Lugol's solution, with the diluted volume adjusted slightly (White *et al.* 2014). Ten microliters of the diluted solution were transferred into the improved

Neubauer hemocytometer cavity. The cells' density was calculated using the following formula (Sahastrabuddhe 2016).

$$\text{cell density (cell/mL)} = \frac{\text{total cells number} \times \text{dilution factor}}{\text{total squares number} \times \text{squares volume at 0.01 mm depth}}$$

### 2.3. Determination of Wet and Dry Biomass

Microalgae cultures were harvested at 8 days to determine the algal wet and dry biomass. The pellets were washed three times with distilled water to remove dissolved salts from the medium. The wet biomass was measured by weighing the pellets (g/L). Each pellet was then dried in an oven at 70±2°C for 24 hours, until it reached a constant weight for the dry biomass (g/L) (Kong *et al.* 2011). Wet and dry biomass were calculated according to Han *et al.* (2016) using the formula:

$$\text{biomass (g/L)} = \left( \frac{\text{weight of tube with pellet (g)} - \text{weight of tube (g)}}{\text{volume of sample (mL)}} \right) \times \text{1,000 mL}$$

### 2.4. Determination of Enzymatic Antioxidants

#### 2.4.1. Superoxide Dismutase (SOD)

SOD was extracted following the methods of Price *et al.* (1994) with modifications. SOD activity was assayed by adding 5 µL of supernatant to a 96-well plate, followed by 130 µL of 50 mM potassium phosphate buffer (pH 7.8) and 60 µL of SOD master mix (1:1:1 mix of 1 mM EDTA, 0.5 mM xanthine, and 1 mM cytochrome C). After adding 5 µL of xanthine oxidase, the reaction was monitored at 550 nm for 5 minutes. SOD activity was calculated as the relative inhibition of cytochrome c reduction, with 1 unit defined as 50% inhibition at 25°C.

#### 2.4.2. Catalase (CAT)

CATwas extracted using the method of Claiborne (1985) with modifications. CAT activity was assayed by adding 20 µL of supernatant to a 96-well plate, followed by 180 µL of 50 mM potassium phosphate buffer (pH 7.0) containing 15 mM $H_2O_2$. The reaction was monitored at 240 nm every 30 s for 3 minutes. CAT activity was calculated based on the decrease in absorbance at 240 nm, using a 43.6 M$^{-1}$ cm$^{-1}$ extinction coefficient.

#### 2.4.3. Ascorbate Peroxidase (APX)

APX was extracted using the method of Sairam *et al.* (1997). APX activity was assayed by adding 10 µL of supernatant to a 96-well plate, followed by 185 µL of 50 mM potassium phosphate buffer (pH 7.4) containing 0.025 mM L-ascorbic acid and 5 µL of 200 mM H2O2. The reaction was monitored at 290 nm every 30 seconds for 5 minutes, and APX activity was calculated using an extinction coefficient of 2.8 mM$^{-1}$ cm$^{-1}$.

#### 2.4.4. Guaiacol Peroxidase (GPX)

GPX was assayed by adding 10 µL of supernatant to a 96-well plate, followed by 150 µL of 100 mM potassium phosphate buffer (pH 7.0). Then, 40 µL of guaiacol-$H_2O_2$ master mix (90 mM guaiacol and 10 mM $H_2O_2$ in a 1:1 ratio) was added to start the reaction. The reaction was monitored at 436 nm every 30 seconds for 3 minutes, and the specific activity was calculated using an extinction coefficient of 26.6 mM$^{-1}$ cm$^{-1}$.

#### 2.4.5. Glutathione Reductase (GR)

GR was extracted using the method of Carlberg and Mannervik (1985) with modifications. GR activity was assayed by adding 30 µL of supernatant to a 96-well plate, followed by 150 µL of 100 mM potassium phosphate buffer (pH 7.6) containing 0.1 mM DTNB. Then, 10 µL of 12 mM β-NADPH and 10 µL of 3.25 mM GSSG were added to initiate the reaction. The reaction was monitored at 415 nm every 30 seconds for 3 minutes, and GR activity was calculated using an extinction coefficient of 6.22 mM$^{-1}$ cm$^{-1}$.

#### 2.4.6. The Protein Concentration

Protein concentration was determined using the Bradford method (1976). A standard curve using Bovine Serum Albumin (BSA) at concentrations ranging from 0 to 100 µg/mL was used to determine protein content.

### 2.5. Determination of Non-Enzymatic Antioxidants

#### 2.5.1. Chlorophylls (Chls) and Carotenoids (CAR)

Chls and CARwere extracted following the method of Torres *et al.* (2014). About 0.05 g of fresh sample was homogenized with 1.5 mL of absolute methanol for 10 minutes at 0-4°C. The homogenate was centrifuged at 10,000 rpm and 4°C for 10 minutes. 200 µL of supernatant was transferred into a flat-bottom 96-well plate. The Chls and CAR were measured at 470, 653, and 666 nm (Lichtenthaler & Wellburn 1983). The contents of Chls and CAR were calculated using the following formula:

$$\text{chlorophyll a, } C_a \text{ (mg/g FW)} = 15.65\,(A_{666}) - 7.34\,(A_{653}) \times \frac{V}{1{,}000 \times W \times d}$$

$$\text{chlorophyll a, } C_b \text{ (mg/g FW)} = 27.05\,(A_{653}) - 11.21\,(A_{666}) \times \frac{V}{1{,}000 \times W \times d}$$

$$\text{carotenoids (mg/g FW)} = \frac{1{,}000\,(A_{470}) - 2.86\,(C_a) - 129.2\,(C_b)}{245} \times \frac{V}{1{,}000 \times W \times d}$$

Where:
A is the absorbance value at a specific wavelength
V is the total methanol volume of Chls/CAR extraction
1,000 is the conversion unit from µg to mg; W is the microalgal fresh weight; and d is the light path length

### 2.5.2. Ascorbic Acid (AsA)

AsA content was determined according to the procedure of Norhayati *et al.* (2016) with minor modifications. Thirty microliters of the supernatant were diluted to 200 µL with distilled water and transferred into a flat-bottom 96-well plate. Then, 20 µL of 10% Folin-Ciocalteu reagent was added. The microplate was incubated at 25°C for 10 minutes to allow a blue color to develop. After 10 minutes, the absorbance at 760 nm ($A_{760}$) was measured. A standard curve was prepared using AsA at various concentrations from 0 to 5 µg/mL, and the amount of AsA in the sample was calculated based on this standard curve.

### 2.5.3. α-Tocopherol (TOC)

TOC determination was conducted following the method of Norhayati *et al.* (2016) with some modifications. A total volume of 25 µL of supernatant was transferred into a flat-bottom 96-well plate. Then, 20 µL of 0.1% 3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine (PDT) and 20 µL of 0.1% ferric chloride were added. Next, the volume was adjusted to 150 µL with absolute ethanol. The microplate was incubated at 25°C for 4 minutes to develop color. After 4 minutes, 10 µL of 0.2 M orthophosphoric acid was added to the mixture. The absorbance was measured at 554 nm. A standard curve was prepared using standard TOC at various concentrations from 0 to 100 µg/mL, and the amount of TOC in the sample was calculated based on the standard curve.

### 2.5.4. Total Glutathione (GSH)

Total GSH was measured following Janknegt *et al.* (2009). For the GSH assay, 10 µL of supernatant was added to a 96-well plate, followed by 150 µL of reaction mixture (100 mM potassium phosphate buffer, 100 mM EDTA, 0.11 mM DTNB, and 0.17 U/mL GR) to start the reaction. After 5 minutes, 50 µL of 0.2 mM β-NADPH was added, and the reaction rate was measured at 405 nm every 30 seconds for 10 minutes at 25°C. A standard curve with reduced GSH (0 to 0.5 nmol) was used to calculate the total GSH content in the sample. The production of 5-thio-2-nitrobenzoic acid (TNB) was calculated per 1 nmole of glutathione by extrapolation.

## 2.6. Statistical Analysis

All statistical analyses were performed using SPSS software, version 20. Results were based on means and standard deviations (SD). Normality of the results was assessed using skewness and kurtosis. One-way analysis of variance (ANOVA) was used to determine differences among treatment means. When significant differences were detected, Tukey's post hoc test was used for multiple comparisons at the 5% significance level. Differences were considered statistically significant at $p<0.05$.

# 3. Results

## 3.1. Algal Growth and Biomass Productivity

Our findings show that each species uses different strategies to cope with the pH changes, demonstrating the diversity and resilience of microalgal stress responses. *C. vulgaris*, *I. galbana*, and *T. chuii* were grown at various pH levels for 13 days, with daily monitoring of their cell densities. Based on Figure 1, the three microalgae species followed the typical pattern of microalgal development under batch culture conditions. After a short lag phase during the first 1-2 days, all species entered the exponential phase between day 2 and day 8, and maximum cell density was generally observed on day 8, indicating the peak of the exponential growth. Following this, cultures transitioned into the stationary phase, where growth plateaued. Among the tested pH levels, pH 8 produced the highest growth for *C. vulgaris* (Figure 1A) and *T. chuii* (Figure 1C), while *I. galbana* (Figure 1B) grew best at pH 7. In addition to affecting microalgal growth, changes in initial pH also influenced the wet and dry biomass of *C. vulgaris*, *I. galbana*, and *T. chuii*, as shown in Figures 2A and 2B, respectively. Among these species, *C. vulgaris* had the highest wet and dry biomass. The maximum growth for *C. vulgaris*, *I. galbana*, and *T. chuii* occurred at pH 7 and 8, except that the dry biomass of *I. galbana* remained higher across the pH range of 6 to 8.

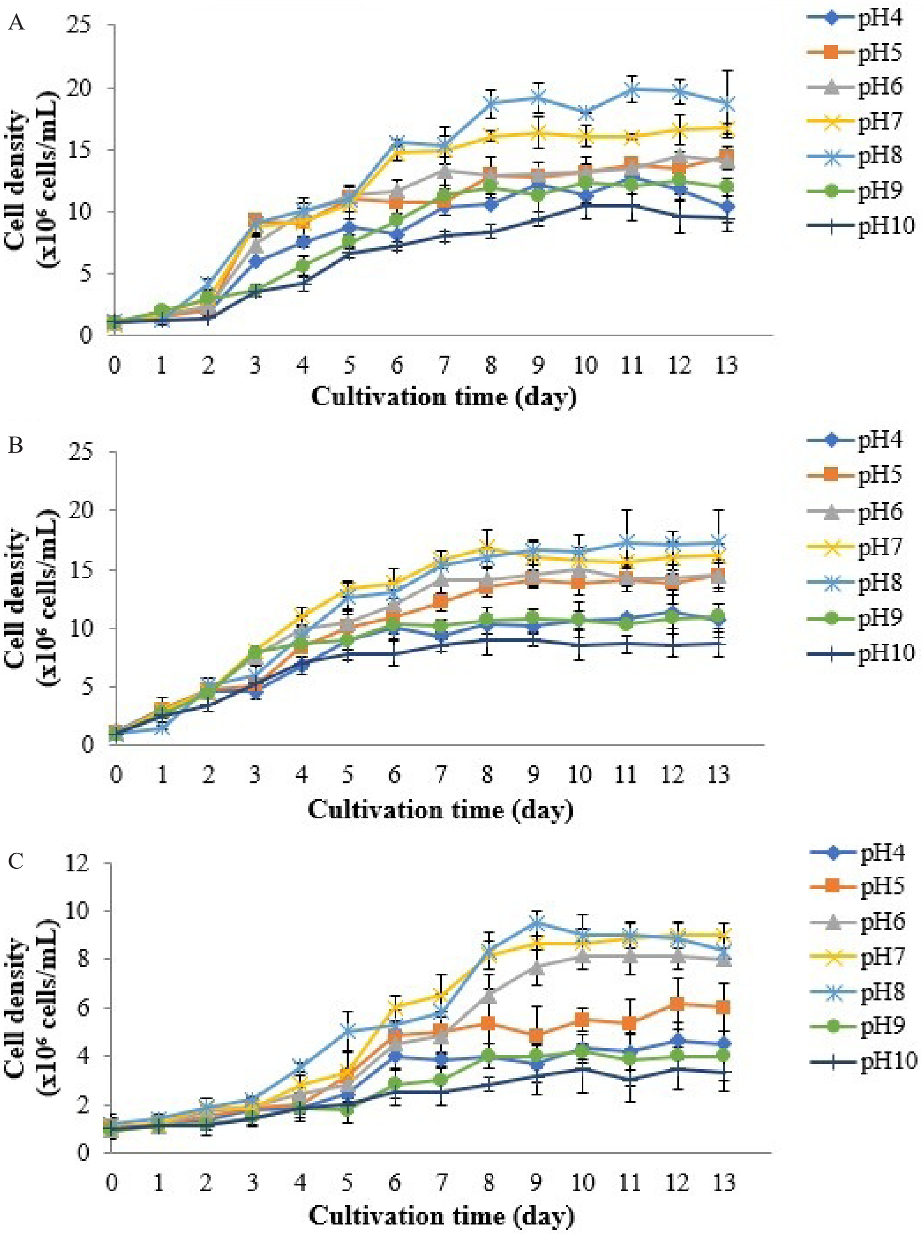

Figure 1. Growth curve of (A) *C. vulgaris*, (B) *I. galbana*, and (C) *T. chuii* at various initial pH. Data presented are means ± SD

### 3.2. Effects of Initial pH Changes on Microalgal Enzymatic Antioxidants

Various initial pH changes have a significant impact on SOD activity in *C. vulgaris*, *I. galbana*, and *T. chuii*, as shown in Figure 3A. Compared with the control, SOD activity in *C. vulgaris* was significantly induced at pH 4, followed by pH 7. Lower SOD activity was observed at pH 5-6, and it was significantly reduced at alkaline pH. For *I. galbana*, culturing at pH 10 resulted in SOD activity similar to that of the control, with no significant enhancement observed at other pH levels. However, SOD in *T. chuii* was inhibited at all treated initial pH levels compared to the control. CAT activities were generally higher in *I. galbana* than in *C. vulgaris* and *T. chuii*, except at pH 7 and 10 (Figure 3B). CAT in *I. galbana* showed comparable activities in cells treated at pH 4 and the control. In *C. vulgaris*, CAT activity slowly increased from pH 4 to 7, reached a maximum at the control pH, and then decreased thereafter. CAT activity in *T. chuii* was significantly higher in control cells than in other treatments.

Figure 3C shows that APX activity in *C. vulgaris* was inhibited at various initial pH levels, except in the control experiment, which was significantly higher than in other

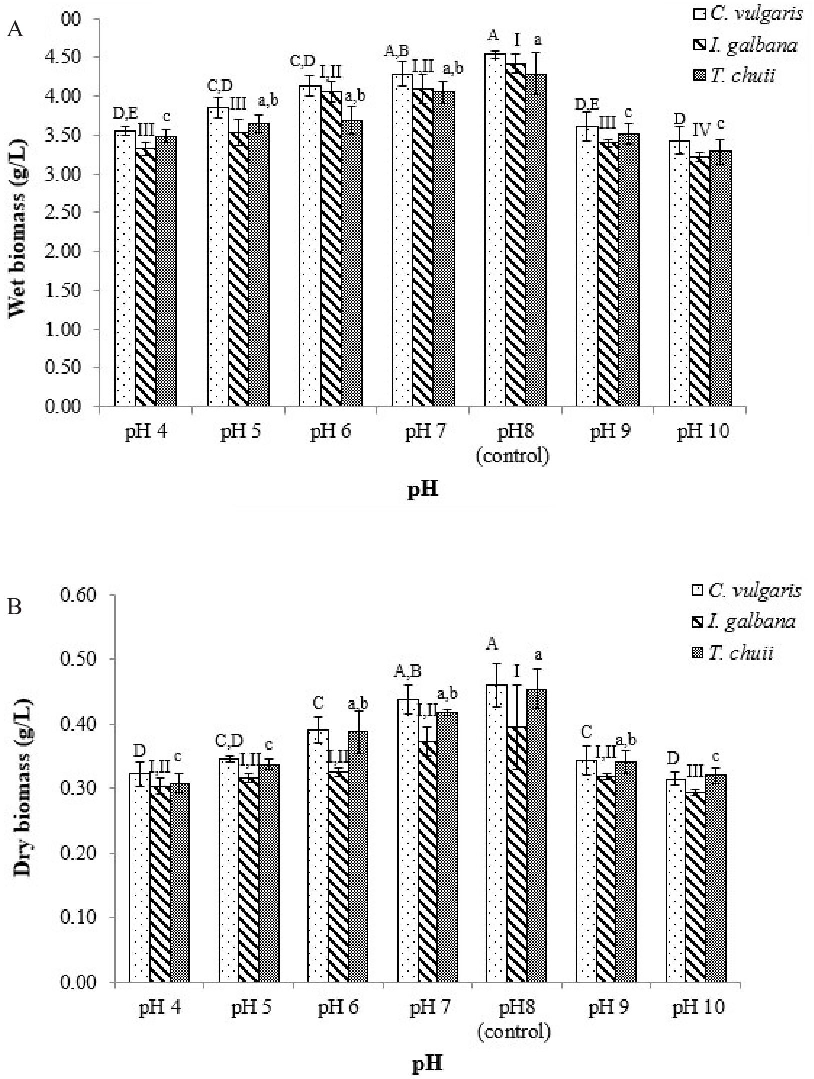

Figure 2. Wet (A) and dry biomass (B) of *C. vulgaris*, *I. galbana*, and *T. chuii* at various initial pH. Data presented are means ± SD. Significant differences between pH treatments within each species are indicated by capital letters (A-D) for *C. vulgaris*, lowercase letters (a-c) for *T. chuii*, and Roman numerals (I-III) for I. galbana ($p < 0.05$, one-way ANOVA followed by Tukey's post-hoc test). Symbols are assigned independently for each species

treatments. Lower APX activities were also observed in *I. galbana* and *T. chuii*. Based on Figure 3D, the GPX activities were significantly higher in *C. vulgaris* compared to other studied microalgae species at different initial pH values. The GPX activities were significantly enhanced in cells treated with acidic media (pH 4 to 6) and neutral media (pH 7). Higher pH led to inhibition of CAT activities. On the other hand, *I. galbana* and *T. chuii* exhibit lower activity across a range of initial pH values.

The GR activities can also be regulated by the initial pH of the culture medium, as demonstrated in Figure 3E. GR activities in the 3 selected species fluctuated at different initial pH levels. GR in *T. chuii* dominated the activities at all pH levels except pH 8 and 10. *C. vulgaris* exhibited increased growth at pH levels ranging from 5 to 7 and at pH 9, with the highest activity recorded at pH 10. In contrast, *I. galbana* showed reduced GR activity across all tested pH levels compared to the control, indicating an inhibitory effect of pH variation. On the other hand, *T. chuii* demonstrated elevated GR activity under all adjusted initial pH treatments, with the most pronounced activity observed at pH 7 and 9. The least activity was detected in control and pH 10-treated cells.

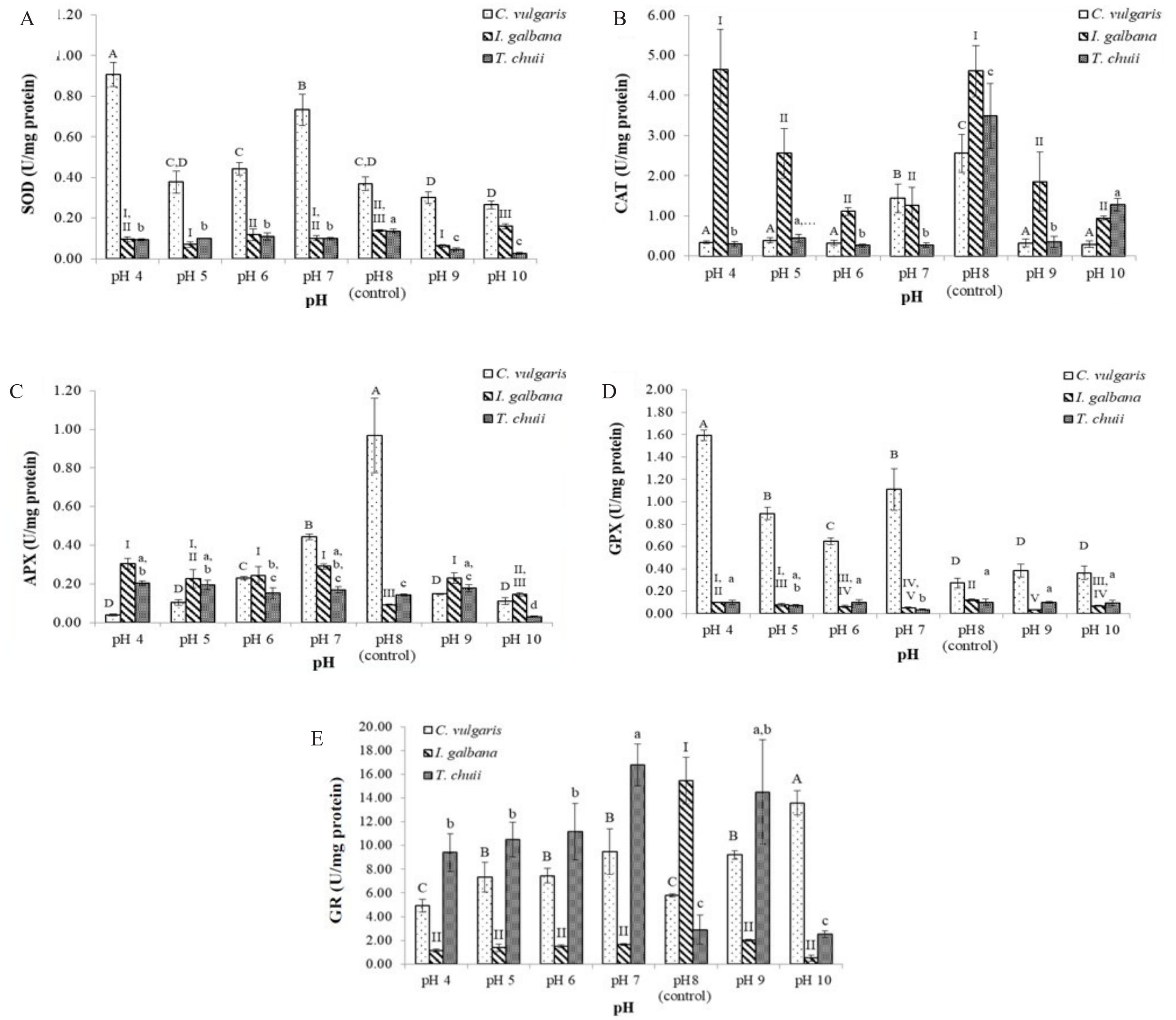

Figure 3. SOD (A), CAT (B), APX (C), GPX (D), and GR (E) specific activities of *C. vulgaris*, *I. galbana*, and *T. chuiii* at various initial pH. Data presented are means ± SD. Significant differences between pH treatments within each species are indicated by capital letters (A-D) for *C. vulgaris*, lowercase letters (a-d) for *T. chuii*, and Roman numerals (I-V) for *I. galbana* ($p<0.05$, one-way ANOVA followed by Tukey's post-hoc test). Symbols are assigned independently for each species

## 3.3. Effects of Initial pH Changes on Microalgal Pigments and Non-Enzymatic Antioxidants

As depicted in Figure 4A, higher Chl a content was generally observed in *I. galbana* compared to the other two species at pH 5-7 and pH 9. Control medium significantly enhanced chl a in *C. vulgaris*, whereas other pH levels showed significant inhibition. In contrast, Chl a accumulation in *I. galbana* was induced at pH 5, 6, 7, and 9 relative to the control. Meanwhile, *T. chuii* exhibited increased Chl a content primarily under neutral pH conditions. A contrasting trend was observed in Chl b content in *T. chuii*, which exhibited a marked increase in Chl b content at pH 4, 5, 7, and 10 compared to *C. vulgaris* and *I. galbana*. In *C. vulgaris*, the control group produced higher Chl b content than the treated groups, indicating a decline in pigment synthesis under altered pH conditions. Conversely, *I. galbana* showed a significant rise in Chl b at pH 6, 7, and 9, relative to the control. The enhanced Chl b content in *T. chuii* under pH 4, 5, and 7 further highlights its adaptive pigment response to varying pH environments. For CAR content, *C. vulgaris* demonstrated a complete reduction in CAR under various initial pH conditions, except in the control experiment. *I. galbana* showed the highest CAR at pH levels of 7 to 8, while other treated pH levels inhibited CAR accumulation. Interestingly, varying the initial pH did not significantly alter CAR production in *T. chui* throughout the experiments (Figure 4C).

In a very alkaline medium (pH 10), the AsA content of *C. vulgaris* was significantly enhanced, whereas the content remained higher at pH 6 to 8. The AsA content at pH 7 and 8 was also consistent in *I. galbana*, whereas a lower AsA content was observed at other initial pH values. Inversely, AsA content in *T. chui* fell substantially at different treated initial pH compared to the control. As illustrated in Figure 5B, a complete inhibition of TOC in *C. vulgaris* was noted at various initial pH changes compared to the control. Nevertheless, it is interesting to note that *I. galbana* cultured in pH 5 achieved the highest TOC content, while media with very acidic (pH 4), neutral (pH 7), and very alkaline (pH 10) pH levels accumulated the lowest TOC content. A significantly increased TOC content was observed in *T. chuii* when cultured at pH 7 compared to the control. Lower TOC was observed in other treated cells, with the lowest content in cells treated at pH 5.

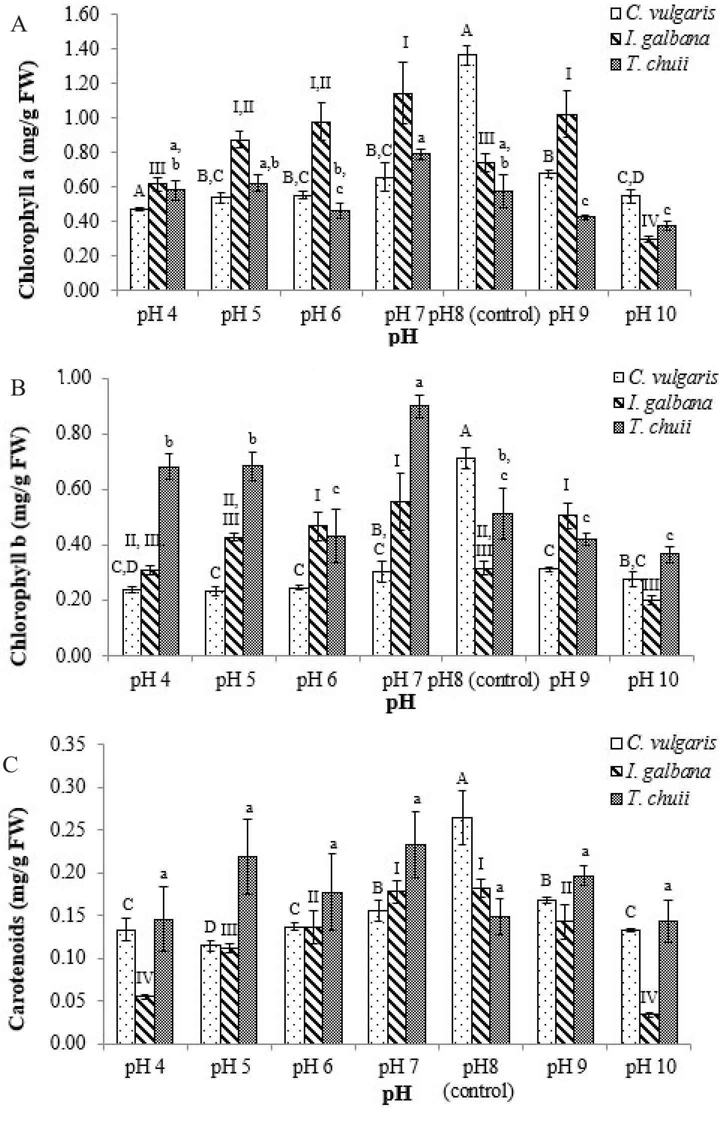

Figure 4. Chlorophyll a (A), Chlorophyll b (B), and Carotenoids (C) content of *C. vulgaris*, *I. galbana*, and *T. chuii* at various initial pH. Data presented are means ± SD. Significant differences between pH treatments within each species are indicated by capital letters (A-D) for *C. vulgaris*, lowercase letters (a-c) for *T. chuii*, and Roman numerals (I-IV) for *I. galbana* ($p<0.05$, one-way ANOVA followed by Tukey's post-hoc test). Symbols are assigned independently for each species

The total GSH content in *C. vulgaris* was significantly higher across all treated and control conditions compared to *I. galbana* and *T. chuii* (Figure 5C). However, no significant variation in GSH levels was observed among the different initial pH treatments in *C. vulgaris*, suggesting a stable antioxidant response across pH conditions. In contrast, *I. galbana* maintained relatively consistent GSH content across all pH treatments, showing no substantial difference from the control. For *T. chuii*, total GSH content increased notably at initial pH levels 5, 6, and 7, followed by a moderate rise at pH 9. Meanwhile, GSH content in cells treated at pH 4 and 10 was comparable to that in the control group.

## 4. Discussion

The cell density of all three microalgae species, *C. vulgaris*, *I. galbana*, and *T. chuii*, followed a typical growth curve pattern consisting of lag, log, stationary, and death phases (Lee *et al.* 2015), especially at pH 6-8. During the lag phase (days 0-2), cells acclimate to new environmental and nutritional conditions, showing minimal growth while initiating metabolic activity. This is followed by the exponential phase (days 2 to 8), characterized by rapid cell division, high photosynthetic activity, and efficient nutrient uptake, particularly of nitrate and phosphate. This phase is

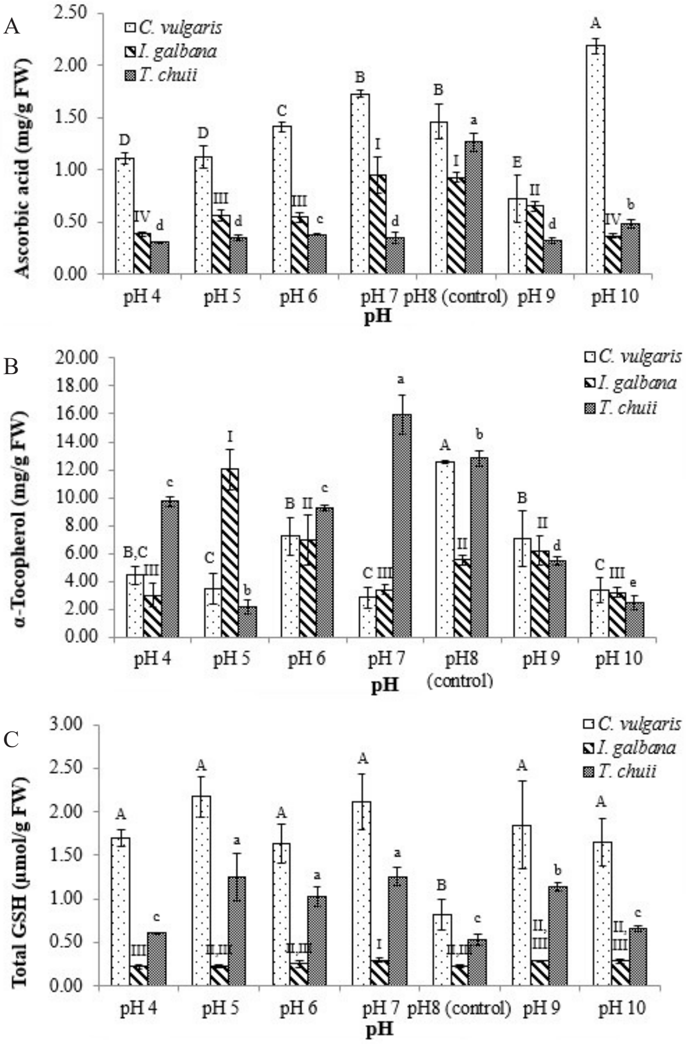

Figure 5. Ascorbic acid (A), α-tocopherol (B), and Total glutathione (C) content of *C. vulgaris, I. galbana*, and *T. chuii* at various initial pH. Data presented are means ± SD. Significant differences between pH treatments within each species are indicated by capital letters (A-D) for *C. vulgaris*, lowercase letters (a-e) for *T. chuii*, and Roman numerals (I-V) for *I. galbana* ($p < 0.05$, one-way ANOVA followed by Tukey's post-hoc test). Symbols are assigned independently for each species

often considered ideal for harvesting due to the peak in biomass accumulation (Ma'mun *et al*. 2022). After Day 8, cultures generally enter the stationary phase, where the growth rate plateaus as the cell division rate equals the death rate. This stagnation is often caused by nutrient limitation, light attenuation from self-shading, or the buildup of inhibitory metabolic byproducts. Notably, this phase is characterized by the accumulation of valuable secondary metabolites, such as pigments and antioxidants. Eventually, cultures progress into the death/decline phase, where nutrient depletion, oxidative stress, or waste accumulation leads to cell death, pigment degradation, and cellular lysis (Tasnim *et al.* 2023).

Environmental acidity and alkalinity significantly affect the growth and metabolism of microalgae. Neutral or weakly alkaline environments are suitable for the growth of most microalgae; however, the optimal pH varies by species, and deviations from this range can impact cell concentration and biomass (Gauthier *et al.* 2020). The results in Figure 2 indicated that all 3 species of microalgae studied were well adapted to the varying pH medium. Higher wet and dry weights were observed in cells grown at pH 6-8, whereas lower growth was observed in acidic (pH 4-5) and alkaline pH 9 (pH 9-10). This might be due to the protective systems and decreased photosynthetic activity, which in turn lower $CO_2$ uptake, a process essential for microalgal metabolism (Liu *et al.* 2019). Additionally, declines in growth and biomass under extreme pH levels indicate diminished capacities for photosynthesis and respiration (Abd El-Monem *et al.* 2018). Phusunti *et al.* (2017) also found that highly acidic environments can break down protein molecules by protonating nitrogen atoms in peptide bonds, forming amino acids, and ultimately reducing microalgal biomass and growth. Moreover, Gross (2000) suggests that morphological disorders in algae cells at low and high pH levels are linked to the damaging effects of $H^+$ and $OH^-$ ions. At very low pH, cells are stressed because they must maintain cytoplasmic neutrality as $H^+$ ions continually permeate the plasma membrane. Furthermore, low pH values can harm cell walls by weakening the hydrogen bonds within molecules, leading to uncontrolled cell size.

Enzymatic antioxidant activity typically peaks at neutral pH, while deviations from this optimal range, especially under highly acidic or alkaline conditions, can significantly reduce activity due to enzyme instability or denaturation (Arnnok *et al.* 2010). However, these assumptions remain theoretical, as previous studies have shown that the production of enzymatic antioxidant production can vary significantly across different pH levels, depending on the microalgal species and environmental conditions. Therefore, this study analyses the enzymatic antioxidants, including SOD, CAT, APX, GPX, and GR in *C. vulgaris*, *I. galbana*, and *T. chuii* at different initial pH levels ranging from pH 4 to 10.SOD, which catalyzes the neutralization of $O2^{\bullet-}$ by adding $H^+$ to form $H_2O_2$ and $O_2$.This reaction is crucial in protecting cells from oxidative damage caused by ROS, especially under stress conditions such as extreme pH (Jaknegt *et al.* 2009). SOD in *C. vulgaris* attained the highest activity at pH 4, because in an acidic environment, the dismutation of $O2^{\bullet-}$ is inevitable. One $O2^{\bullet-}$ passes its additional electron to another $O2^{\bullet-}$, generating peroxide radicals and eventually triggering the formation of $H_2O_2$ via the activation of SOD (Chokshi *et al.* 2017). The fluctuation of SOD activities at different initial pH levels in *I. galbana* suggests that ROS formation becomes unstable when the pH deviates from the optimal level (Peng *et al.* 2017). Additionally, *I. galbana* exhibited SOD activity at pH 10 similar to that of the control, possibly because alkaline conditions can induce oxidative, osmotic, and ionic stress in microalgae (Qu & Miao 2021). The increased production of ROS at pH levels lower or higher than the optimal can lead to enzyme denaturation, which might correlate with the inhibition of SOD activities at various initial pH values in *T. chuii* (Peng *et al.* 2017). The CAT's main function is in scavenging $H_2O_2$, a ROS, into $H_2O$ and $O_2$, thereby preventing cellular damage (Ismaiel *et al.* 2016). In this study, CAT activity in *I. galbana* was significantly enhanced in cells grown at acidic pH (pH 4) and the control, consistent with previous findings in *U. compressa* (Vinuganesh *et al.* 2022) and in *Scenedesmus* sp. (Huang *et al.* 2020). Under acidic or alkaline stress, microalgae often experience elevated levels of ROS, including $H_2O_2$. In response, CAT activity is generally upregulated to mitigate oxidative damage. Elevated CAT activity in acidic environments suggests its vital role in defending against lipid peroxidation and maintaining cellular redox homeostasis (Ismaiel *et al.* 2016). The reduction of CAT activity at different pH levels might be due to increased oxidative stress from elevated $H_2O_2$ levels, and the enzyme's structure may be impaired by denaturation, potentially reducing its activity despite higher ROS levels (Moghimifam *et al.* 2019; Samanta *et al.* 2019).

Other important enzymes, APX and GPX, are peroxidases that commonly work together with CAT to scavenge $H_2O_2$ (Giannuzzi *et al.* 2016; Fayaz *et al.* 2024). APX activities were inhibited in *C. vulgaris* compared to the control. The findings are consistent with those of Liu *et al.* (2019) for *A. platensis* and Vinuganesh *et al.* (2022) for *U. compressa*. The reduction of APX activity at different initial pHs in *C. vulgaris* can be attributed to reduced AsA generation caused by oxidative stress (Yilancioglu *et al.* 2014). In contrast to CAT, APX activity in *I. galbana* was significantly elevated at all treated initial pH levels except pH 10, compared to the control. This suggests that APX plays a more prominent role in $H_2O_2$ detoxification under pH-induced stress in this species, consistent with the finding of Gauthier *et al.* (2020). CAT is capable of decomposing large amounts of $H_2O_2$ quickly, but it operates efficiently only at high $H_2O_2$ concentrations. In contrast, APX functions effectively even at low $H_2O_2$, has a higher affinity for $H_2O_2$ than CAT, and is crucial for removing low to moderate levels of $H_2O_2$, particularly within chloroplasts, cytosol, and mitochondria. The elevated APX activity in *I. galbana* suggests its role in maintaining redox homeostasis when CAT alone may not be sufficient. Meanwhile, a slight increase in APX was observed in *T. chuii* when cultured in very acidic conditions at pH levels of 4 and 5, suggesting a lesser reliance on the ascorbate-glutathione cycle for $H_2O_2$ detoxification. Unlike *I. galbana*, which significantly upregulates APX under stress, *T. chuii* might depend more on other enzymatic antioxidants, such as GR (Figure 3E) and non-enzymatic antioxidants for ROS scavenging. On the other hand, GPX in *C. vulgaris* was substantially increased over a pH range of 4 to 7, possibly due to its greater involvement in bulk $H_2O_2$ detoxification than CAT and APX (Khataee *et al.* 2017). However, in *I. galbana* and *T. chuii*, GPX activities were consistent across various initial pH values, indicating a consistent protective role of cell membrane integrity and structure (Al-Rashed *et al.* 2016). Compared with the control experiment, GR activity in *C. vulgaris* remained unchanged in both acidic and neutral media. However, it significantly increased in the alkaline media, suggesting that the acidic or neutral culture media have a balanced ratio of GSSG and GSH forms, while the alkaline culture media contributed to the substantial increase of cell protection by the increment of the GSSG and GSH ratio (Al-Rashed *et al.* 2016). However, total degradation of GR in *I. galbana* at different initial pH levels relative to the control can be explained by the fact that GSH is consumed more quickly without being replenished, thereby disrupting redox equilibrium within cells (Samanta *et al.* 2019). Additionally, pH treatments ranging from 4 to 9 stimulated GR activity in *T. chuii*, suggesting an enhanced role of GR in the AsA-GSH pathway in significantly detoxifying $H_2O_2$ (Koletti *et al.* 2025).

Chl and CAR are major pigments that play essential roles in the photosynthetic processes and stress protection of microalgae. Together, these pigments optimize photosynthetic efficiency and enhance stress tolerance in microalgae under fluctuating environmental conditions (Angeles *et al.* 2025). Under different initial pH changes, the decline of Chl a in *C. vulgaris* was observed, resulting from damage to the pigment structure, which led to a decrease in photosynthetic efficiency and dysfunction of microalgae photosynthesis (Romero *et al.* 2020; Vinuganesh *et al.* 2022). Additionally, acidic conditions may suppress Chl a synthesis by degrading essential carbon-fixation enzymes, such as Rubisco and other Calvin cycle enzymes (Stirbet *et al.* 2020). Conversely, alkaline environments can hinder Chl a production by reducing the availability of free $CO_2$, which is vital for photosynthesis (Liu *et al.* 2019). Chl a content in *I. galbana* and *T. chuii* increased at pH 5-9 and pH 7, respectively, suggesting enhanced photosynthetic activity under these pH conditions, consistent with findings in *Desmodesmus* sp. (Buayam *et al.* 2019). The degradation of Chl b in *C. vulgaris* at all treated initial pHs may be due to excessive ROS attacking the chloroplasts, causing Chl b to be converted into functional Chl a. Additionally, shifting the initial pH also alters the Chl a/b binding protein complex, which induces the formation of ROS and causes impairment of PSII activity (Romero *et al.* 2020). In *I. galbana*, Chl b levels were notably higher at pH 6 and 7, whereas *T. chuii* exhibited peak Chl b accumulation at pH 7. These findings are consistent with Khalil *et al.* (2010), who suggested that the optimal pH for photosynthesis often falls below the pH that supports maximum growth. The variation in Chl b accumulation across different pH levels in both species could be attributed to the pH sensitivity of key photosynthetic enzymes, such as chlorophyllide a oxygenase and light-harvesting complex proteins, which regulate chlorophyll conversion and assembly within the thylakoid membranes (Liu *et al.* 2019). An optimal pH may enhance enzyme conformation and activity, improving pigment biosynthesis and energy absorption efficiency. Conversely, extreme pH levels may impair enzyme function or pigment stability, thereby reducing Chl b accumulation. Thus, the results suggest that moderately acidic to neutral pH conditions create a more favorable biochemical environment for Chl b synthesis in these microalgae.

CAR is synthesized in chloroplast membranes, which are directly involved in carbon fixation and pH regulation (Zhang *et al.* 2020).In this study, *C. vulgaris* exhibited a complete reduction in CAR content when the pH was shifted away from the control experiment (pH 8), suggesting the severity of the harmful effects of ROS (Samanta *et al.* 2019). Compared with the control, the upsurge of CAR in *I. galbana* in a neutral medium may reflect a direct microalgae response to sustain or improve photosynthetic processes, in addition to its protective function against oxidative stress (Vinuganesh *et al.* 2022). In contrast, CAR in *T. chuii* was maintained at different initial pH levels, revealing an effective role for CAR in quenching radicals and inhibiting free radical reactions (Romero *et al.* 2020).

Ascorbic acid (AsA protects microalgal cells against oxidative stress by acting as a powerful non-enzymatic antioxidant. It scavenges ROS, particularly $H_2O_2$, and regenerates other antioxidants such as vitamin E. AsA is also a key component of the AsA-GSH cycle, where it works alongside enzymes like APX to detoxify ROS and maintain redox balance, especially under environmental stress (Kováčik *et al.* 2018). The gradual increase in AsA in all studied microalgae from pH 4 to 7 is primarily due to improved cellular stability and optimal enzymatic activity under near-neutral conditions. Many biosynthetic enzymes involved in AsA production function more efficiently, and microalgae experience less oxidative stress, supporting better AsA biosynthesis. A high AsA content in *C. vulgaris* at pH 10 might be attributed to the high efficiency of the AsA-GSH cycle in alleviating oxidative stress by donating electrons to excessive ROS under a highly alkaline medium. In contrast, pH 9-10 decreases AsA levels in *I. galbana* and *T. chuii* due to several stress-related factors. The enzymes responsible for AsA biosynthesis are sensitive to alkaline conditions, thus reducing AsA production. Additionally, alkaline stress often leads to elevated levels of ROS, which can rapidly oxidize AsA, diminishing its detectable concentration. Furthermore, high pH can disrupt cellular redox balance, leading to metabolic shifts that prioritize other antioxidant pathways over AsA synthesis (Del Mondo *et al.* 2020). TOC (vitamin E) is an important non-enzymatic antioxidant involved in oxidation-reduction responses due to its aromatic ring. Based on our results, altering the initial pH harmed the production of TOC content in *C. vulgaris*, possibly due to a decline in photosynthetic pigments, which triggers the overproduction of ROS and initiates oxidative damage in the cells (Sharma *et al.* 2012). The fluctuation of TOC across different initial pH levels in *I. galbana* may be attributed to differences in ROS formation at each pH. The neutral medium significantly enhanced the production of TOC in *T. chuii* by suppressing $^1O_2$, scavenging $O2^{\bullet-}$, and terminating the lipid peroxidation reaction (Zhang *et al.* 2020), which was similar to that observed in *U. compressa* under both acidified and control conditions (Vinuganesh *et al.* 2022). Total glutathione is a key component of the cellular antioxidant defense system in microalgae, playing a vital role in maintaining redox homeostasis, detoxifying ROS, and protecting cellular components from oxidative damage. Glutathione exists in reduced (GSH) and oxidized (GSSG) forms, with a higher GSH/GSSG ratio typically indicating a healthier cellular redox state (Zhang *et al.* 2020). The total GSH content in *C. vulgaris* was induced at all treated pH levels; similar results were observed in *T. chuii* at pH 5-9, possibly related to its robust antioxidant defense system and efficient redox regulation mechanisms. Both species likely upregulate the synthesis of reduced glutathione (GSH) to counteract the increased generation of ROS. This adaptive response helps maintain cellular homeostasis and protects key biomolecules from oxidative damage. The elevated GSH across a wide pH range suggests that *C. vulgarispossesses* a more resilient glutathione-related detoxification system than the other tested species (Roy *et al.* 2021). The total GSH levels remain relatively low in *I. galbana* across different pH treatments, likely due to its limited capacity to upregulate glutathione biosynthesis under stress. *I. galbana* may prioritize other antioxidant mechanisms, such as the APX system, over glutathione-dependent responses when exposed to pH stress. Additionally, the species might have a less efficient GR activity, reducing its ability to regenerate GSH from its oxidized form (GSSG), thereby keeping total GSH levels low (Koletti *et al.* 2025). This lower GSH level may reflect reduced tolerance to oxidative stress compared to more resilient microalgae, such as *C. vulgaris*.

### 4.1. Practical Implications of pH-Dependent Antioxidant Responses in *C. vulgaris*, *I. galbana*, and *T. chui*

The species-specific antioxidative responses to pH fluctuations have important implications for industrial applications, including carbon capture, bioreactors, and biofuel production. *C. vulgaris*, with its strong upregulation of enzymatic antioxidants (SOD, GPX, GR) across acidic to alkaline conditions, demonstrates high resilience to pH stress, making it an ideal candidate for industrial systems where pH fluctuations are frequent. Therefore, the most suitable species is for systems with common pH variability. *T. chuii* exhibits a balanced response with both enzymatic (GR) and non-enzymatic mechanisms (CAR, GSH), making it adaptable to moderate pH fluctuations while maintaining pigment stability and photosynthetic efficiency, thus making it suitable for applications requiring high photosynthetic efficiency, such as carbon sequestration and biofuel production. In contrast, *I. galbana* is more sensitive to extreme pH, suggesting that it may require more tightly controlled culture conditions to maintain productivity, making it less suitable for systems with frequent pH swings but excellent for applications prioritizing high-value lipid production under stable conditions.

In Conclusion, This study demonstrates that *C. vulgaris*, *I. galbana*, and *T. chuii* employ distinct yet effective adaptive strategies to cope with pH-induced stress, reflected in their growth rates, antioxidant defense systems, and pigment analysis. All three species exhibited optimal growth and biomass accumulation under moderately acidic to neutral conditions (pH 6-8), highlighting this range as the most favorable for cellular metabolism and photosynthetic efficiency. Deviations toward extreme acidic or alkaline conditions imposed oxidative stress, disrupted photosynthetic pigments, and altered redox balance, thereby constraining growth and biomass production.

Among the species, *C. vulgaris* showed strong resilience across a wide pH range, primarily through robust activation of enzymatic (SOD, GPX, GR) and non-enzymatic (GSH, AsA) antioxidant systems, indicating a flexible, well-coordinated redox regulatory mechanism. *I. galbana* relied more on APX and CAT to mediate $H_2O_2$ detoxification, with comparatively limited involvement of the GSH system, suggesting a more specialized but less versatile antioxidant strategy under pH stress. In contrast, *T. chuii* exhibited a balanced antioxidant response, maintaining CAR and strongly activating GR and GSH-related pathways, which likely contribute to its effective ROS scavenging and membrane protection. Overall, these species-specific antioxidant and pigment-based adjustments underscore differing physiological balances and stress tolerance, positioning *C. vulgaris* as the most adaptable, *T. chuii* as moderately resilient, and *I. galbana* as more sensitive to severe pH variations.

## Acknowledgements

The authors wish to thank the research assistants and postgraduate and undergraduate students involved in this study.

## References

Abd El-Monem, A.M., Gharieb, M.M., Hussian, A.E.M., Doman, K.M., 2018. Effect of pH on phytochemical and antibacterial activities of *Spirulina platensis*. *Int J Appl Environ Sci*.13, 339-351.

Abdur Razzak, S., Bahar, K., Islam, K.O., Haniffa, A.K., Faruque, M.O., Hossain, S.Z., Hossain, M.M., 2024. Microalgae cultivation in photobioreactors: Sustainable solutions for a greener future. *Green Chemical Engineering*. 5, 418-439. https://doi.org/10.1016/j.gce.2023.10.004

Al-Rashed, S.A., Ibrahim, M.M., El-Gaaly, G.A., Al-Shehri, S., Mostafa, A., 2016. Evaluation of radical scavenging system in two microalgae in response to interactive stresses of UV-B radiation and nitrogen starvation. *Saudi J Biol Sci*. 23, 706-712. https://doi.org/10.1016/j.sjbs.2016.06.010

´Angeles, R., Carvalho, J., Hern´andez-Martínez, I., Morales-Ibarría, M., Fradinho, J.C., Reis, M.A.M., Lebrero, R., 2025.Harnessing nature's palette: Exploring photosynthetic pigments for sustainable biotechnology. *New Biotechnology*. 85, 84-102. https://doi.org/10.1016/j.nbt.2025.01.001

Arnnok, P., Ruangviriyachai, C., Mahachai, R., Techawongstien, S., Chanthai, S., 2010. Optimise and determine polyphenol oxidase and peroxidase activities in hot pepper (*Capsicum annuum* L.) pericarp. *Int Food Res J*. 17, 385-392.

Bartley, M.L., Boeing, W.J., Dungan, B.N., Holguin, F.O., Schaub, T., 2014. pH affects the growth and lipid accumulation of the biofuel microalgae *Nannochloropsis salina* and invading organisms. *J Appl. Phycol*. 26, 1431-1437. https://doi.org/10.1007/s10811-013-0177-2

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem*. 72, 248-254. https://doi.org/10.1016/0003-2697(76)90527-3

Buayam, N., Davey, M.P., Smith, A.G., Pumas, C., 2019. Effects of copper and pH on the growth and physiology of *Desmodesmus* sp. AARLG074. *Metabolites*. 9, 84. https://doi.org/10.3390/metabo9050084

Carrillo, M., Anchundia, M., 2024. Antimicrobial and antioxidant capacity of *Dunaliella salina*, *Tetraselmis chuii* and *Isochrysis galbana* and their potential use in food. A systematic review. *CABI Agric Biosci*. 5, 108. https://doi.org/10.1186/s43170-024-00316-0

Carlberg, I., Mannervik, B., 1985. Glutathione reductase. *Methods Enzymol*. 113, 484-490. https://doi.org/10.1016/S0076-6879(85)13062-4

Chokshi, K., Pancha, I., Ghosh, A., Mishra, S., 2017. Oxidative stress-induced bioprospecting of microalgae. In: Kumar, M., Ralph, P. (Eds.). *Systems Biology of Marine Ecosystems*. Cham, Switzerland: Springer. pp. 251-276.

Claiborne, A., 1985. Catalase activity. In: Greenwald, R.A. (Ed.). *CRC Handbook of Methods for Oxygen Radical Research*. Boca Raton: CRC Press. pp. 283-284.

Del Mondo, A., Smerilli, A., Sané, E., Sansone, C., Brunet, C., 2020. Challenging microalgal vitamins for human health.*MCF*. 19, 1-23. https://doi.org/10.1186/s12934-020-01459-1

Fayaz, T., Rana, S.S., Goyal, E., Ratha, S.K., Renuka, N., 2024. Harnessing the potential of microalgae-based systems for mitigating pesticide pollution and its impact on their metabolism. *J. Environ. Manag*. 357, 120723. https://doi.org/10.1016/j.jenvman.2024.120723

Fu, W., Nelson, D.R., Mystikou, A., Daakour, S., Salehi-Ashtiani, K., 2019. Advances in microalgal research and engineering development. *Curr. Opin. Biotechnol*. 59, 157-164. https://doi.org/10.1016/j.copbio.2019.05.013

Gauthier, M.R., Senhorinho, G.N.A., Scott, J.A., 2020. Microalgae under environmental stress as a source of antioxidants. *Algal Res.* 52, 102104. https://doi.org/10.1016/j.algal.2020.102104

Gauthier, M.R., Senhorinho, G.N.A., Basiliko, N,, Desjardins, S., Scott, J.A., 2022. Green photosynthetic microalgae from low pH environments associated with mining as a potential source of antioxidants. *Ind. Biotechnol*. 18. https://doi.org/10.1089/ind.2022.0013

Ge, F., Song, K., Yang, Z., Li, J., Yan, F., Zhang, M., Huang, B., Fu, G., Zheng, H., 2023. Enhancing docosahexaenoic acid production of *Isochrysis galbana* from starch-rich food processing byproducts. *Ferment*. 9, 158. https://doi.org/10.3390/fermentation9020158

Giannuzzi, L., Krock, B., Minaglia, M.C.C., Rosso, L., Houghton, C., Sedan, D., Malanga, G., Espinosa, M., Andrinolo, D., Hernando, M., 2016. Growth, toxin production, active oxygen species and catalase activity of *Microcystis aeruginosa* (Cyanophyceae) exposed to temperature stress. *Comp. Biochem. Physiol. C Toxicol. Pharmacol*. 189, 22-30. https://doi.org/10.1016/j.cbpc.2016.07.001

Gross, W., 2000. Ecophysiology of algae living in highly acidic environments. *Hydrobiologia.* 433, 31-37. https://doi.org/10.1023/A:1004054317446

Guillard, R.R., Ryther, J.H., 1962 Studies of marine planktonic diatoms: *I. Cyclotella* nana Hustedt, and *Detonula confervacea* (Cleve) Gran. *Can. J. Microbiol.* 8, 229-239. https://doi.org/10.1139/m62-029

Guillard, R.R., 1975. Culture of phytoplankton for feeding marine invertebrates. In: Smith, W.L., Chanley, M.H. (Eds.). *Culture of Marine Invertebrate Animals*. Boston, MA: Springer. pp. 29-60.

Halliwell, B., 2022. Reactive oxygen species (ROS), oxygen radicals and antioxidants: where are we now, where is the field going and where should we go? Biochem. *Biophys. Res. Commun.* 633, 17-19. https://doi.org/10.1016/j.bbrc.2022.08.098

Han, F., Pei, H., Hu, W., Jiang, L., Cheng, J., Zhang, L., 2016. Beneficial changes in biomass and lipid of microalgae *Anabaena variabilis* facing the ultrasonic stress environment. *Bioresour. Technol*. 209, 16-22. https://doi.org/10.1016/j.biortech.2016.02.103

Hanachi, P., Aghababaie, A., Noroozi, M., 2021. Effects of temperature and pH on biochemicals content of microalgae *Monoraphidium* sp. *IJMAPR.* 37, 290-299.

Hong, Y., Boiti, A., Vallone, D., Foulkes, N.S., 2024. Reactive oxygen species signaling and oxidative stress: transcriptional regulation and evolution. *Antioxidants.* 13, 312. https://doi.org/10.3390/antiox13030312

Hu, J., Meng, W., Su, Y., Qian, C., Fu, W., 2023. Emerging technologies for advancing microalgal photosynthesis and metabolism toward sustainable production. *Front. Mar. Sci*. 10, 1260709. https://doi.org/10.3389/fmars.2023.1260709

Huang, B., Shan, Y., Yi, T., Tang, T., Wei, W., Quinn, N.W., 2020. Study on high-$CO_2$ tolerant *Scenedesmus* sp. and its mechanism via comparative transcriptomic analysis. *J. $CO_2$ Util*. 42. https://doi.org/10.1016/j.jcou.2020.101331

Ismaiel, M.M.S., El-Ayouty, Y.M., Piercey-Normore, M., 2016. Role of pH on antioxidants production by Spirulina (*Arthrospira platensis*). *Braz. J Microbiol*. 47, 298-304. https://doi.org/10.1016/j.bjm.2016.01.003

Janknegt, P.J., De, Graaff, C.M., Van de Poll, W.H., Visser, R.J., Rijstenbil, J.W., Buma, A.G., 2009. Short-term antioxidative responses of 15 microalgae exposed to excessive irradiance including ultraviolet radiation. *Eur. J Phycol*. 44, 525-539. https://doi.org/10.1080/09670260902943273

Khalil, Z.I., Asker, M.M., El-Sayed, S., Kobbia, I.A., 2010. Effect of pH on growth and biochemical responses of *Dunaliella bardawil* and *Chlorella ellipsoide. World J Microbiol. Biotechnol*. 26, 1225-1231. https://doi.org/10.1007/s11274-009-0292-z

Khataee, A., Movafeghi, A., Mojaver, N., Vafaei, F., Tarrahi, R., Dadpour, M.R., 2017. Toxicity of copper oxide nanoparticles on *Spirodela polyrrhiza*: assessing physiological parameters. *Res. Chem. Intermed*. 43, 927-941. https://doi.org/10.1007/s11164-016-2674-9

Kong, W., Song, H., Cao, Y., Yang, H., Hua, S., Xia, C., 2011. The characteristics of biomass production, lipid accumulation and chlorophyll biosynthesis of *Chlorella vulgaris* under mixotrophic cultivation. *Afr. J Biotechnol*. 10, 11620-11630.

Koletti, A., Skliros, D., Dervisi, I., Roussis, A., Flemetakis, E., 2025. Oxidative stress responses in microalgae: modern insights into an old Topic. *Appl. Microbiol*. 5, 37. https://doi.org/10.3390/applmicrobiol5020037

Kováčik, J., Micalizzi, G., Dresler, S., Babula, P., Hladký, J., Chemodanov, A., Mondello, L., 2018. Metabolic responses of Ulva compressa to single and combined heavy metals. *Chemosphere*. 213, 384-394. https://doi.org/10.1016/j.chemosphere.2018.08.141

Lee, E., Jalalizadeh, M. and Zhang, Q., 2015. Growth Kinetic Models for Microalgae Cultivation: A Review. *Algal Res*. 12, 497-512. https://doi.org/10.1016/j.algal.2015.10.004

Lee, S.E., Park, Y.S., 2021. The emerging roles of antioxidant enzymes by dietary phytochemicals in vascular diseases. *Life (Basel)*. 11,199. https://doi.org/10.3390/life11030199

Lichtenthaler, H.K., Wellburn, A.R., 1983. Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. *Biochem. Soc. Trans*. 11, 591-592. https://doi.org/10.1042/bst0110591

Liu, J., Chen, F., 2014. Biology and industrial applications of Chlorella: advances and prospects. *Adv Biochem Eng Biotechnol*. 153, 1-35. https://doi.org/10.1007/10_2014_286

Liu, W., Wang, J., Liu, T., 2019. Low pH rather than high $CO_2$ concentration itself inhibits growth of Arthrospira. *Sci Total Environ*. 666, 572-580. https://doi.org/10.1016/j.scitotenv.2019.02.312

Ma'mun, S., Wahyudi, A., Raghdanesa, A.S., 2022. Growth rate measurements of *Chlorella vulgaris* in a photobioreactor by Neubauer-improved counting chamber and densitometer. *IOP Conference Series: Earth and Environment*. 963, 012015. https://doi.org/10.1088/1755-1315/963/1/012015

Mishra, N., Jiang, C., Chen, L., Paul, A., Chatterjee, A., Shen, G., 2023. Achieving abiotic stress tolerance in plants through antioxidative defense mechanisms. *Front. Plant Sci*. 14, 1110622. https://doi.org/10.3389/fpls.2023.1110622

Moghimifam, R., Niknam, V., Ebrahimzadeh, H., Hejazi, M.A., 2019. The influence of different $CO_2$ concentrations on the biochemical and molecular response of two isolates of *Dunaliella* sp. (ABRIINW-CH2 and ABRIINW-SH33). *J Appl. Phycol*. 32, 175-187. https://doi.org/10.1007/s10811-019-01914-6

Norhayati, Y., Afzan, A.W., Jannah, S.S.N., Nurul Wahidah, M.R., 2016. Antioxidative responses of *Cocos nucifera* against infestation by the Red Palm Weevil (RPW), *Rhynchophorus ferrugineus*, a new invasive coconut pest in Malaysia. *Sains Malays*. 45, 1035-1040.

Norhayati, Y., Nurul Shafiqa, Y., Malinna, J., Hazlina, A.Z., Nurfarha, M.Z., 2023. Growth, pigments production and phytochemicals variation in *Tetraselmis chui* under different cultivation parameters. *Int. Aquat. Res*. 15, 299-311. https://doi.org/10.22034/IAR.2023.1993193.1481

Okoro, V., Azimov, U., Munoz, J., Hernandez, H.H., Phan, A.N., 2019. Microalgae cultivation and harvesting: Growth performance and use of flocculants - A review. *Renew. Sustain. Energy Rev*. 115, 109364. https://doi.org/10.1016/j.rser.2019.109364

Pei, J., Pan, X., Wei, G., Hua, Y., 2023. Research progress of glutathione peroxidase family (GPX) in redoxidation. *Front. Pharmacol*. 14, 1147414. https://doi.org/10.3389/fphar.2023.1147414

Peng, L., Zhang, Z., Lan, C.Q., Basak, A., Bond, N., Ding, X., Du, J., 2017. Alleviation of oxygen stress on *Neochloris oleoabundans*: effects of bicarbonate and pH. *J Appl. Phycol*. 29, 143-152. https://doi.org/10.1007/s10811-016-0931-3

Phusunti, N., Phetwarotai, W., Tirapanampai, C., Tekasakul, S., 2017. Subcritical water hydrolysis of microalgal biomass for protein and pyrolytic bio-oil recovery. *Bioenergy Res*. 10, 1005-1017. https://doi.org/10.1007/s12155-017-9859-y

Price, A.H., Taylor, A., Ripley, S.J., Griffiths, A., Trewavas, A.J., Knight, M.R., 1994. Oxidative signals in tobacco increase cytosolic calcium. *The Plant Cell*. 6, 1301-1310. https://doi.org/10.1105/tpc.6.9.1301

Qu, D., Miao, X., 2021. Carbon flow conversion induces alkali resistance and lipid accumulation under alkaline conditions based on transcriptome analysis in *Chlorella* sp. BLD. *Chemosphere*. 265, 129046. https://doi.org/10.1016/j.chemosphere.2020.129046

Ramos-Romero, S., Torrella, J. R., Pagès, T., Viscor, G., Torres, J. L., 2021. Edible microalgae and their bioactive compounds in the prevention and treatment of metabolic alterations. *Nutrients*. 13, 563. https://doi.org/10.3390/nu13020563

Rauf, A., Ahmed Khalil, A., Awadallah, S., Ali Khan, S., Abu-Izneid, T., Kamran, M., Hemeg, H.A., Mubarak, M.S., Khalid, A., Wilairatana, P., 2024. Reactive oxygen species in biological systems: Pathways, associated diseases, and potential inhibitors-A review. *FSN*. 12, 639-1363. https://doi.org/10.1002/fsn3.3784

Romero, N., Visentini, F.F., Márquez, V.E., Santiago, L.G., Castro, G.R., Gagneten, A.M., 2020. Physiological and morphological responses of green microalgae *Chlorella vulgaris* to silver nanoparticles. *Environ. Res*. 189, 109857. https://doi.org/10.1016/j.envres.2020.109857

Roy, U.K., Nielsen, B.V., Milledge, J.J., 2021. Antioxidant production in *Dunaliella*. *Appl. Sci*. 11, 3959. https://doi.org/10.3390/app11093959

Sahastrabuddhe, A.P., 2016. Counting of RBC and WBC using image processing: a review. *Int. J Res. Eng. Technol*. 5, 356-360. https://doi.org/10.15623/ijret.2016.0505067

Sairam, R.K., Shukla, D.S., Saxena, D.C., 1997. Stress induced injury and antioxidant enzymes in relation to drought tolerance in wheat genotypes. *Biol. Plant*. 40, 357-364. https://doi.org/10.1023/A:1001009812864

Samanta, P., Jang, S., Shin, S., Kim, J.K., 2019. Effects of pH on growth and biochemical responses in *Agarophyton vermiculophyllum* under different temperature conditions. *J Appl. Phycol*. 32,1-11. https://doi.org/10.1007/s10811-019-01933-3

Shafiquzzaman, M., Hasan, M.M., Haider, H., Ahmed, A.T., Razzak, S.A., 2023. Comparative evaluation of low-cost ceramic membrane and polymeric micro membrane in algal membrane photobioreactor for wastewater treatment. *J. Environ. Manag*. 345, 118894. https://doi.org/10.1016/j.jenvman.2023.118894

Sharma, P., Jha, A.B., Dubey, R.S., Pessarakli, M., 2012. Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions. *J Bot*. 2012, 217037. https://doi.org/10.1155/2012/217037

Srinivasan, R., Mageswari, A., Subramanian, P., Suganthi, C., Chaitanyakumar, A., Aswini, V.,Gothandam, K.M., 2018. Bicarbonate supplementation enhances growth and biochemical composition of *Dunaliella salina* V-101 by reducing oxidative stress induced during macronutrient deficit conditions. *Sci. Rep*. 8, 1-14. https://doi.org/10.1038/s41598-018-25417-5

Stirbet, A., Laz´ar, D., Guo, Y., Govindjee, G., 2020. Photosynthesis: basics, history and modelling. *Annals Bot*. 126, 511-37. https://doi.org/10.1093/aob/mcz171

Tasnim, N., Karmakar, D., Hasan, R., Islam, R., Hossain, S., Shaikh, A., Karim, R., 2023. Effect of light intensity and pH on cell density assessed by spectrophotometry for the unicellular algae *Chlorella vulgaris*. *Am. J. Plant Sci*. 14, 472-481. doi: 10.4236/ajps.2023.144031

Tham, P.E., Lim, H.R., Khoo, K.S., Chew, K.W., Yap, Y.J., Munawaroh, H.S.H., Show, P.L., 2023. Insights of microalgae-based aquaculture feed: A review on circular bioeconomy and perspectives. *Algal Res*. 28, 103186. https://doi.org/10.1016/j.algal.2023.103186

Torres, P.B., Chow, F., Furlan, C.M., Mandelli, F., Mercadante, A., Santos, D.Y.A.C.D., 2014. Standardization of a protocol to extract and analyze chlorophyll a and carotenoids in *Gracilaria tenuistipitata* Var. Liui. Zhang and Xia (Rhodophyta). *Braz. J. Oceanogr*. 62, 57-63. https://doi.org/10.1590/s1679-87592014068106201

Vijayaram, S., Ringø, E., Ghafarifarsani, H., Hoseinifar, S.H., Ahani, S., Chou, C.C., 2024. Use of Algae in Aquaculture: A Review. *Fishes* 9, 63. https://doi.org/10.3390/fishes9020063

Vinuganesh, A., Kumar, A., Prakash, S., Alotaibi, M.O., Saleh, A.M., Mohammed, A.E., Beemster, G., T.S., Abd Elgawad, H., 2022. Influence of seawater acidification on biochemical composition and oxidative status of green algae Ulva compressa. *Sci. Total Environ*. 806, 150445. https://doi.org/10.1016/j.scitotenv.2021.150445

White, L.H., Martin, D.W., Witt, K.K., Vogt, F., 2014. Impacts of nutrient competition on microalgae biomass production. *J. Chemom* 28, 448-461. https://doi.org/10.1002/cem.2534

Wu, M., Wu, G., Lu, F., Wang, H., Lei, A., Wang, J., 2022. Microalgal photoautotrophic growth induces pH decrease in the aquatic environment by acidic metabolites secretion. Biotechnol. *Biofuels.* 15, 115. https://doi.org/10.1186/s13068-022-02212-z

Yilancioglu, K., Cokol, M., Pastirmaci, I., Erman, B., Cetiner, S., 2014. Oxidative stress is a mediator for increased lipid accumulation in a newly isolated *Dunaliella salina* strain. *PLoS One*. 9, e91957. https://doi.org/10.1371/journal.pone.0091957

Yin, D., Wang, Z., Wen, X., Ding, Y., Hou, X., Geng, Y., Li, Y., 2019. Effects of carbon concentration, pH, and bubbling depth on carbon dioxide absorption ratio in microalgae medium. *Environ. Sci. Pollut. Res. Int*. 26, 32902-32910. https://doi.org/10.1007/s11356-019-06287-4

Zhang, S., He, Y., Sen, B., Wang, G., 2020. Reactive oxygen species and their applications toward enhanced lipid accumulation in oleaginous microorganisms. *Bioresour. Technol.* 307, 123234. https://doi.org/10.1016/j.biortech.2020.123234