Open Access
J. Glob. Innov. Agric. Sci., 2024, 12(3):685-692
ISSN (Online): 2788-4546; ISSN (Print):2788-4538
DOI: https://doi.org/10.22194/JGIAS/24.1378
http://www.jgiass.com

The Impact of pH and Growth Phases on Photosynthetic Pigments and Carotene of *Coelastrella saipanensis* N. Hanagata (Scenedsmacese, Shaerophleales)

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The present study aimed to investigate the effects of level pH and the growth phases of *Coelastrella saipanensis* on Chlorophyll a,b, total, and Carotene. The algae were cultured in BG11 media and grown at different pH levels. We measured chlorophyll a, b, total chlorophyll, growth phases, and carotene concentrations. The results showed that at pH 8.5, the measurements of photosynthetic pigments-chlorophyll a, Chlorophyll b, and the total chlorophyll (0.183, 0.268, and 0.433 mg L^{-1}, respectively). The highest values of chlorophyll a (0.185 mg L^{-1}), and b (0.339 mg L^{-1}), and the total chlorophyll (0.492 mg L^{-1}) were recorded in the stationary phase. In addition, the study found that at pH 8.5 and the beginning of the stationary phase, the highest levels of chlorophyll a, b, and total chlorophyll were 0.216, 0.398, and 0.603 mg L^{-1}, respectively, in correlation with pH-cell harvesting phase interaction. Similarly, carotene concentrations were increased as photosynthetic pigments and recorded the highest values (2.533 mg L^{-1}) at pH 8.5, and 3.275 mg L^{-1} at the stationary phase. Therefore, the algal *C. saipanensis* is a vital source of carotene to support nutrition and health It is used as an antioxidant.

Keywords: *Coelastrella saipanensis*, pH, Chlorophyll, Carotene, Growth phases, Nutritional support.

INTRODUCTION

Algae have important roles in the processes of the environmental system and the water quality Its importance at the industrial and therapeutic levels is because it possesses many different active metabolic compounds, which are secondary metabolites (Rimet, 2012). They have species-specific responses, and they are highly sensitive to a number of chemical, physiological, and biological variables of aquatic environments (Schneider *et al*., 2011). Green algae are classified as one of the eukaryotic organisms having evolutionary diverse groups. In addition, they have the ability to adapt to different habitats through different growth averages (De Clerck *et al*., 2013). Lutzu and Dunford (2018) reported the importance of algae as a source of many bioactive compounds. *Coelastrella* was identified first by Chodat in 1922. It was described as a unicellular algae or a few-celled groups of relatively tiny spherical to oval cells. The cell diameter is 6-15 micrometers. Also, the plastids are cup-shaped and wall-positioned (Kaufnerov and Elias, 2013). These algae can adapt to different terrestrial or aquatic environments. The majority of this algae was founded in the terrestrial environment attached to the soil, rocks, and wood barks (Wang *et al*., 2019; Nayana *et al*., 2022). In Iraq, this alga is considered one of the modern-specified genera in Iraqi freshwaters belonging to Spaeropleales and Scenedesmaceae (Abed *et al*., 2018; Al-Rawi *et al*., 2018). *Coelastrella* has a great economic importance in energy sector, food, and pharmaceutical industries (AL-Rawi *et al*.,2020). Many of its types can produce lipids and carotenoids under different stress conditions; such as density of light, diffrent of pH, nutrition and salinity, etc. (Aburai *et al*., 2013). *Coelastrella* sp. is one of the green microalgae which is a source of biologically active substances having anti-tumor and antioxidant effects. In addition, it has antibacterial activity against *E. coli* UPEC, *P. aeruginosa*, and *K. pneumonia*, and antifungal activity against *Candida albicans*. Furthermore, it was reported that algae have an anti-effect against Hela cancer cells depending on the dose and exposure time (Yotova *et al*., 2022). Photosynthetic pigments are regarded as basic components used in the food industry it is natural colors in foods. The demand for these pigments increases because consumers prefer biological products than manufacturing products. Besides, they play a role in the efficiency of the photosynthetic process. The world demand for natural food pigments and antioxidants is constantly rising due to increase

Fadeel, Z.G., F.M. Hassan and M.M.I.A. Mahdawe. 2024. The Impact of pH and Growth Phases on Photosynthetic Pigments and Carotene of *Coelastrella saipanensis* N.Hanagata (Scenedsmacese, Shaerophleales). Journal of Global Innovations in Agricultural Sciences 12:685-692.
[Received 2 May 2024; Accepted 10 July 2024; Published 13 Aug 2024]

in living standards and the production of natural pigments and antioxidants via microalgae (Agocs and Deli, 2011). Many types that belong to the green algae genus of *Coelastrella* are largely able to produce natural pigments. However, the structural, functional, molecular aspects of many green chloroplasts are unillustrated. Many species of this genus exert a strong capacity of accumulating carotenoids so they have recently attracted researchers' attention since can be applied to food and energy industries (Wang *et al*., 2018; Wang *et al*., 2019). Algae can largely increase the production of highly valuable pigments such as carotenes. These pigments are regarded as secondary photosynthesis pigments which absorb light energy. In addition, they have an important role in protecting chlorophylls a and b from photo-oxidization, besides their role as anti-oxididant. These components are as important for the human as they are precursors for vitamin A, and contribute in the mitigating the incidence of having cancer and heart diseases and stimulating the immune system (Gupta *et al*., 2021). Zittelli *et al*. (2023) stated that microalgae can produce carotene having the capacity to deal with oxidative stress because of their characteristics of getting rid of the Reactive Oxygen Species (ROS). This makes them suitable as a natural source of antioxidants. Many studies have focused on selecting promising strains of algae that produce carotenes and these studies have worked to extract and purify them. Natural carotenes extracted from green algae showed useful effects on treating diseases related to health Such as nutritional supplements and pharmaceutical preparations. This is attributed to its anti-oxidant activity, and being the most common compounds in nature; they present commonly in many photosynthetic organisms (Mekinić *et al*., 2023).

The Research aims at the effect of abiotic stress in the growth medium on photosynthetic pigments and carotene of *C. saipanensis* is enhancement the production of bioactive compounds (carotene).

MATERIALS AND METHODS

***Algal material*:** The alga *Coelastrella* was obtained from the Collection Algae Lab at the College of Sciences-University of Baghdad. Its purity was validated by the microscopic examination. All the study experiments were conducted in the Laboratory of Cell Planting and Tissue Agriculture / Department of Biology/ College of Education for Pure Sciences/ University of Diyala.

***Morphological characterization*:** The samples were examined by light microscope (Novel /Holland). The shape and size of the cell were measured in micrometer (Figure 1).

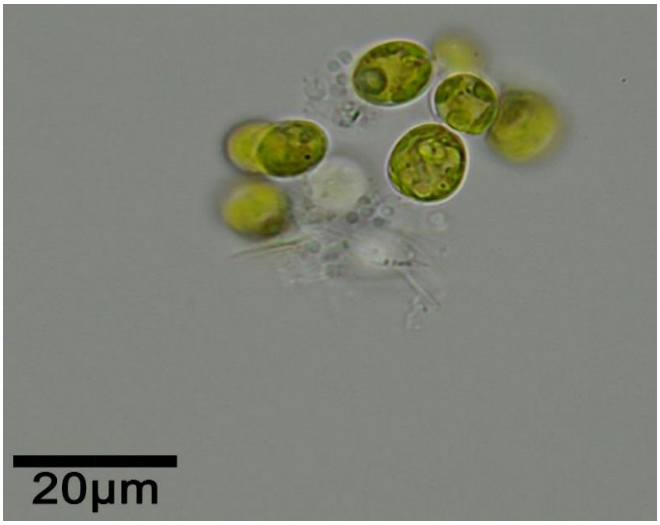


Figure 1. The Phenotypic Shape of *C. saipanensis* under the Microscope.

***Media and culture conditions*:** The sample was growth to the cultural BG-11 media (Table 1). The alga was cultivated in sterilized conditions. The algal samples were kept in a growth room at 25±2 ºC within light-dark alternating system-16/8-hour light/dark and specific light intensity 3000 Lux (Figure 2).

Table 1. Components of the growing medium BG-11.

No.	Components of medium	Concentration
1	Sodium nitrate	1.5000 %
2	Dipotassium hydrogen phosphate	0.0314 %
3	Magnesium sulphate	0.0360 %
4	Calcium chloride dihydrate	0.0367 %
5	Sodium carbonate	0.0200 %
6	Magnesium Disodium EDTA	0.0010 %
7	Citric acid	0.0056 %
8	Ferric ammonium citrate	0.0060 %

Figure 2. Algal manipulation from sampling to experiments step.

***Molecular Diagnosis*:** The DNA of the sample was extracted according to ABIO pure extraction protocol provided by the American company ABIO pure USA. Quantus Fluorometer was used for detecting the concentration of gDNA. One microliter of gDNA was mixed with 200 microliters of diluted pigment of Quantifluor. The mixture of gDNA and pigment was incubated for 5 minutes at room temperature to determine gDNA concentration.

***Primer Preparation, PCR, and Agarose Electrophoresis*:** ITS1-FWD primer pair (3´-TCCGTAGGTGAACCTGCGG-5´), and ITS4-Rev (3´-CCTCCGCTTATTGATATGC-5´) were used to amplify 18S rRNA to detecting the genetic level of algae (Hadi *et al*., 2019).

The PCR mixture was prepared and the PCR products of amplified 18S rRNA were electrophoresed on (1.5%) agarose gel. The gene of interest was visualized by ethidium bromide and the photo was captured by the Get imaging system as (Hadi *et al*., 2019).

Chlorophyll A and Chlorophyll B, and the total chlorophyll pigments were estimated according to Arnon, (1949). The extraction of pigments was performed using 3 ml of (80%) ice-cold acetone with 10 ml of algal isolate, the mixture was centrifuged at 3500 rpm for 10 minutes, and 1 ml of the filtrate was placed in a Cuvette. The absorbance of chlorophyll A and B was measured at Ab_{645} and Ab_{663} nm by UV Spectrophotometer. The pigments were estimated according to the following equations:

$$Chlorophyll\ a = 12.7xA\ 663nm - 2.69 \times A645\ nm$$

$$Chlorophyll\ b = 22.9xA\ 645nm - 4.68 \times A663\ nm$$

$$Total\ Chlorophyll = 20.2xA\ 645nm + 8.02 \times A663\ nm$$

A= Absorbance at particular wavelength

***Carotene estimation*:** The estimation of carotene was done followed the method of Eijckelhoff and Dekker (1997). The extraction of chlorophyll was measured at Ab_{4810}nm and the following equation was used to calculate the concentration.

$$Carotene = 200xA\ 480nm$$

A= Absorbance at particular wavelength

***Experimental design*:** The growth of *C. saipanensis* cells was monitored during the growth phases. The cells were harvested at the last two days of the logarithmic phase, and at the first two days of the stationary phase. The final harvesting was carried out in the last two days in the stationary phase. The algal cells were filtered via filter paper, and the filtrate was left to dry at room temperature to obtain a dried powder. Only 1 gm of the dried powder was obtained (Jawad, 1982). Each treatment is done with three replicates. Three different pH values were measured (4.5, 6.5, and 8.5), and the relative value of pH 7 was evaluated as a control

***Algal extraction*:** The algae were extracted by hot method. This was done by placing of 1 gm of dried powder of *C. Saipanensis* in a thimble of the soxhlet devise plus 150cm^3 ethanol solvent in 250 ml conical flask. The device was connected to a condenser. The process was conducted for 6-8 hours in 7 cycles and, then left to cool, transferred to dark bottles, and kept at -20 °C for further analyses (Sandhya, 2013).

***pH Treatments*:** For pH experiments, 1 M solution of NaOH and HCl was prepared to change pH from basic to acidic and vice versa. Only 100 ml of the algal isolate was added to 900 ml of BG11 media in a 1000 ml conical flask with gentle shaking. The pH values were changed to 4.5, 6.5, 7, and 8.5 by the gradual addition of HCl and NaOH drops to the culture using a pH meter.

Statistical Analysis: The study experiment was conducted via a completely randomized design (CRD) in 3 replicates for each treatment. The differences between the means were compared by using the statistic program JASP depending on the programming language R for treating data via Tukey test at probability level 0.001.

RESULTS AND DISCUSSION

The results of phenotypic diagnosis explained that the *C. saipanensis* species oval shape. The length was 9-10 micrometers, and width was 7-8 micrometers. The autospores were observed as groups of 2 or 4 spores. It appeared that there was a great similarity between this species and *Chlorella* spp. due to the phenotypic features (Altaf *et al*., 2018). Therefore, the molecular analysis is important for algal diagnosis. The evolutional relationships of the classification were known. The evolutional history was concluded via UPGMA method (Sneath and Sokal, 1973). The results of the current study showed that algae *C. saipanensis* has gen 18 s RNA a molecular weight (700 pb) as in the figure (3). The analysis is based on 9 successive nucleotides and all the sites containing missing data were left out. The total sum was 281 sites of the final data. The evolutionary analyses were done according to MEGA6 method (Tamura *et al*., 2013). The results indicated that the analysis sequences. 18S rRNA of the algae under study was 100% correspondent in NCBI site with the database of the algae *C. saipanensis* under accession numbers MT375484.1, MH176093.1, MF407353.1, ON561825.1, ON454286.1, MW929195.1 and HG328355.1.

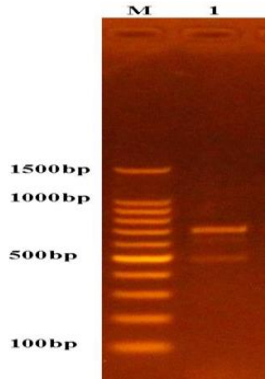


Figure 3. The Results of Gene Amplifying of the Algae Sample.

***Photosynthetic pigments estimation*:** The sample was registered in NCBI as *C. saipanensis* under accession no. LC752948.1 (Figure 4).

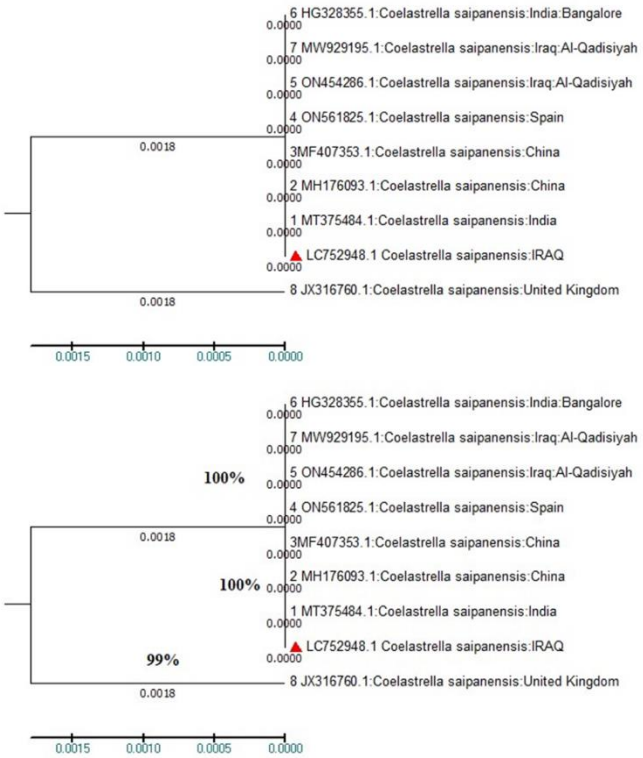


Figure 4. The Evolutionary Genetic Tree Recorded in NCBI.

***The effect of pH on photosynthetic pigments of C. saipanensis during growth phases and their interaction*:** The results showed a high significant increase in the mean values of chlorophyll a and b, and the total chlorophyll were 0.183, 0.268 and 0.433 mg L^{-1}, respectively at the pH 8.5 (Figure 5). In contrast, all the values were decreased at pH 4.5 to be 0.098, 0.198 and 0.297 mg L^{-1}, respectively. This decrease in values of photosynthetic pigments at pH of 4.5 was attributed to the decrease of magnesium occurred in decreased pH, and to the important role of magnesium atom in the centre of chlorophyll molecule (Al-Janaby and Qasim, 2015). In addition, Amin *et al*. (2023) stated that the acetic culture enhance the complete decomposition of chlorophyll A and B, consequently, affect the photosynthesis. It was obvious from Figure (6) that the highest means of chlorophyll pigments were 0.185, 0.339 and 0.492 mg L^{-1} for chlorophyll a and b, and the total chlorophyll respectively in the first two days of stationary phase. In this stationary phase, there was no increase in the numbers and size of algal cells. This accompanied with metabolic activity which was attributed to the increase in producing these compounds at this phase, and the gradual decrease at the end of the phase which accompanied with a deterioration of the culture quality and the depletion of nutrients (Andersen and Kawachi, 2005). Concerning the interaction between different concentrations of pH with different harvesting phases of algal cells, the results appeared in Figure (7) showed that the highest values of means in each of chlorophylls A and B, and total chlorophyll were in the second phase, that is, in the first two days of the stationary phase at the pH 8.5. These values were 0.216, 0.398 and 0.603 mg L^{-1} respectively. In contrast, they decrease to be their lowest at the end of stationary phase, all concentrations were decreased, that is, when the algal cells reached the deterioration or death.

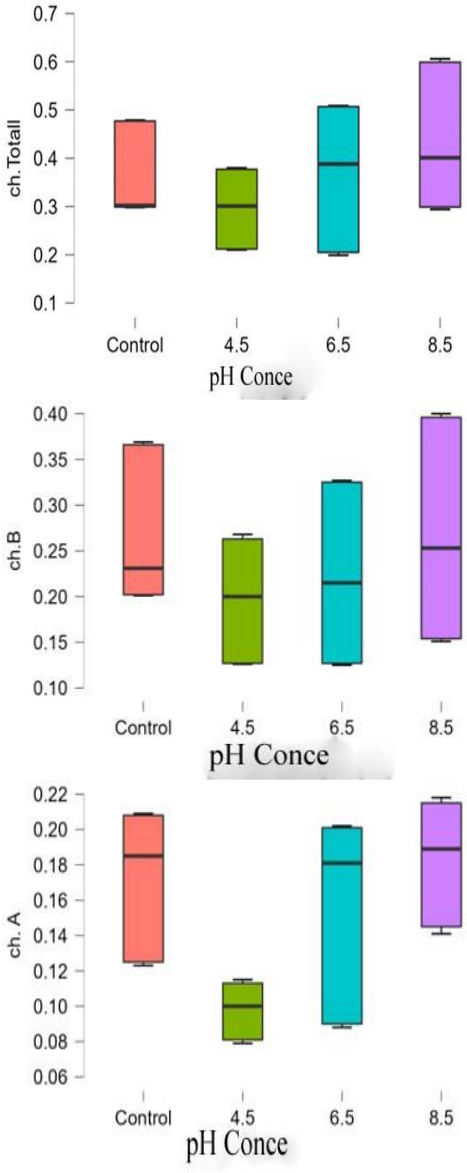
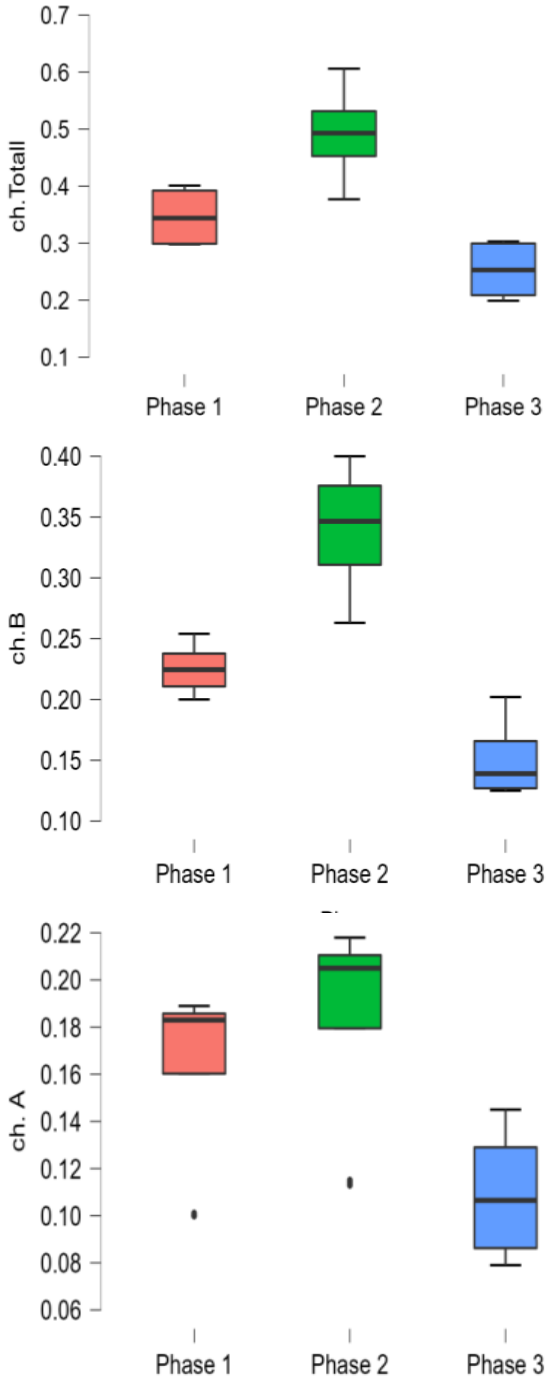


Figure 5. Chlorophyll pigments in culture of *C. sapanensis* at different Levels of pH.



Phase 1= the last two days of logarithmic phase; Phase 2= the first two days of the stationary phase; Phase 3= the last two days of the stationary phase

Figure 6. Photosynthetic pigments concentrations during the growth phases.

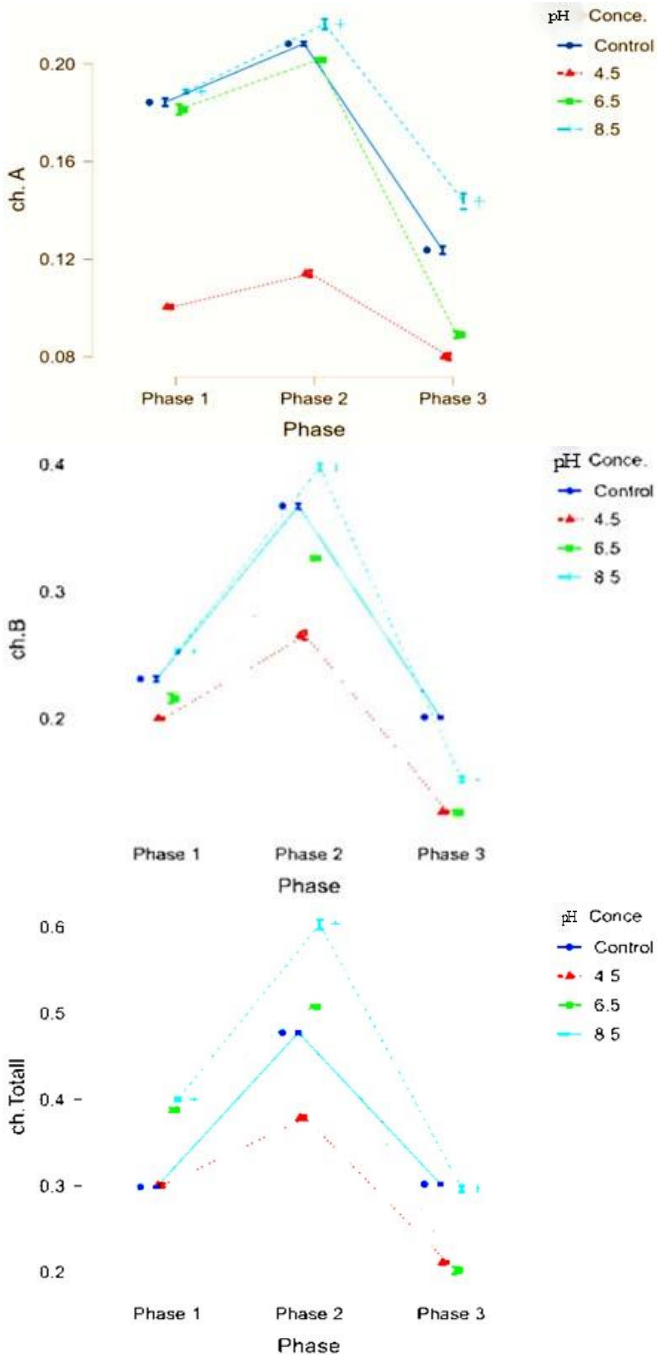


Figure 7. The effect of pH levels during the growth phases on photosynthetic pigments.

***The effect of pH on Carotene pigments of C. saipanensis during growth phases and their interaction*:** The results of carotene shown in Figure (8) indicated that the highest value (2.533mg L^{-1}) was at the pH 8.5, whereas, the lowest value was recorded at pH 4.5 (1.369 mg L^{-1}). Liu and Lee (2000), reported a high carotenoid yield was at pH 8, and low yield at

pH 9. The results indicated that there was a high significant increase in the mean of carotene values (Figure 9), where the concentration reached to 3.275 mg L^{-1} during the stationary phase. In contrast, it was decreased to 1.608 mg L^{-1} at the last two days of this phase. This is attributed to that the algal growth phases passed affect largely the production of metabolic compounds; there is abundance of these compounds at the stationary phase and the biological synthesis of these compounds is tightly related to the growth stages (Zhou *et al*., 2023). An increase in the mean values of carotene was noticed at the second phase in all pH levels (4.5, 6.5, 8.5), were 3.10, 3.33, 3.70 and 3.50 mg L^{-1}, respectively (Figure 10). In contrast, they decreased under all pH levels during the end of the stationary phase. This is in turn emphasize the role of growth role in determine the average of produced compounds.

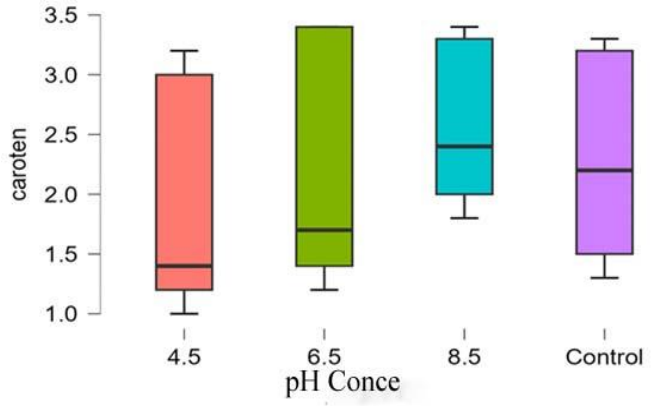
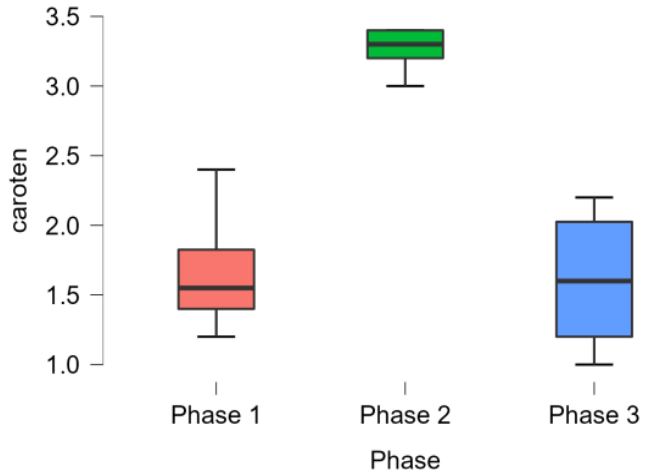


Figure 8. Carotene of *C. sapanensis* at different Levels of pH.



Phase 1= the last two days of logarithmic phase; Phase 2= the first two days of the stationary phase; Phase 3= the last two days of the stationary phase

Figure 9. Carotene concentration during algal growth phase.

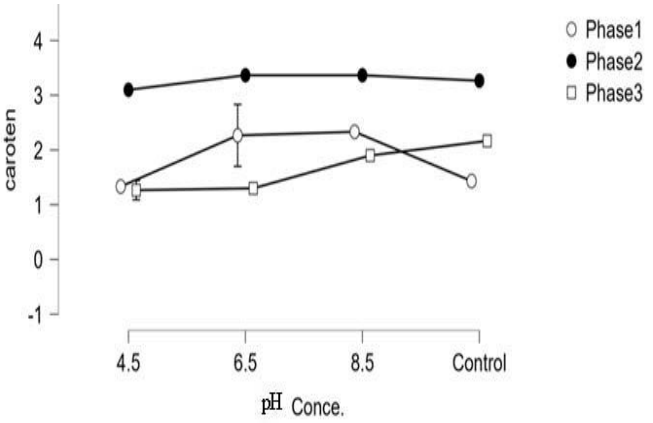


Figure 10. Carotene concentration at different pH level during algal growth phase.

***Conclusions*:** High concentration of carotene yield from *C. sapanensis* at pH 8. Different concentrations were obtained during the growth phases, and an increase in photosynthesis pigments and carotenes was noticed in the present study. Furthermore, the harvest of cells at the beginning of the stationary phase is the best for getting the highest concentrations of these compounds.

***Authors' contributions statement*:** Muthana M.I. Al-Mahdawe designed, completed the experiments; Zeina Gany Fadeel prepared the draft; Fikrat M. Hassan reviewed and finalized the draft.

***Conflict of interest*:** The authors declare no conflict of interest.

***Funding*:** No finding

***Ethical statement*:** This article does not contain any studies regarding human or

Animal.

***Availability of data and material*:** We declare that the submitted manuscript is our work, which has not been published before and is not currently being considered for publication elsewhere?

***Code availability*:** Not applicable

***Consent to participate*:** All authors participated in this research study.

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***Consent for publication*:** All authors submitted consent to publish this research article in JGIAS.

***SDG's Addressed*:** No

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