Seasonal Dynamics of Growth and Secondary Metabolites Biosynthesis in *Dunaliella* sp. (ABRIINW-I1) from Urmia Lake

Robab Salami[1], Abbas Saidi[1*], Mohammad Amin Hejazi[2*], Bahman Panahi3

Received: 2024-06-12 Accepted: 2024-08-09

Abstract

Dunaliella sp. is a unicellular microorganism recognized for its ability to tolerate high salinity levels. It is a valuable source of natural pigments, lipids, and proteins and is of significant interest to various industries, including food, pharmaceuticals, cosmetics, and aquaculture. The study aimed to analysis of growth patterns and biochemical compositions throughout various seasons. For this reason, *Dunaliella* sp. ABRIINW-I1 was cultured in water samples collected from Lake Urmia during the winter, spring, summer, and autumn seasons of 2021. The salinity of the water samples was mainted at 1.5 M through diluting with demi- water and cultivating of the algae was performed at pH 7.8, with a light intensity 200 µmol photon m^{-1} s^{-1} at 28 °C in a 2L photobioreactor. The microalgae achieved a stable growth phase after an average of 10 days. Biomass production, along with protein content, chlorophyll a, chlorophyll b, and beta-carotene werequantified using spectrophotometric analysis. To assess the fatty acid composition, gas chromatography was performed on the extracted lipids utilizing the fatty acid methyl ester (FAME) profiling method. The physicochemical properties were evaluated following the guidelines established by the American Public Health Association (APHA). The findings indicate that the winter season exhibited the highest levels of protein content (50.3 %) and pigment concentration (2.88% DW), predominantly consisting of chlorophyll a, compared to the other seasons, with a significant difference. Conversly, lipid levels did not exhibit any significant variations among the different seasons. Furthermore, the ANOVA results revealed a notable difference in the data set of saturated fatty acids (SFA), polyunsaturated fatty acids (PUFA), Omega-6 fatty acids, and the ratio of unsaturated fatty acids (UFA). These findings emphasize the nutritional potential of the ABRIINW-I1 strain, particularly when cultivated during the colder months. The consistent cultivation conditions in the laboratory, along with the composition of key cations, anions, and the microbiome, likely played a significant role in shaping these outcomes. This research also demonstrates the feasibility of large-scale cultivation of *Dunaliella* microalgae in proximity to Lake Urmia. Furthermore, principal component analysis (PCA) revealed that the growth and nutritional characteristics were influenced by the ionic composition of the lake water under controlled laboratory conditions.

Keywords: *Dunaliella salina,* Growth Parameters, Profile fatty acid**,** Different seasons, Urmia Lake

1- Department of cell & molecullar Biology, Faculty of Life Sciences and Biotechnology, Shahid Beheshti University, Tehran, Iran
2- Food Biotechnology research Institute, Agricultural Biotechnology Research Institute of Iran, Agricultural Education and Extension Organization (AREEO), Tabriz, Iran
3- Department of Genomics, Branch for Northwest & West region, Agricultural Biotechnology Research Institute of Iran (ABRII), Agricultural Research, Education and Extension Organization (AREEO)
* Corresponding authors: abbas.saidi@gmail.com; aminhejazi@abrii.ac.ir
DOI: 10.48308/JPR.2024.236807.1086

Introduction

Urmia Lake, located in northwestern Iran, is renowned for its rich biodiversity and unique ecosystems, encompasses a variety of halophilic species, including a single-celled microalgae *Dunaliella* (Eimanifar and Mohebbi, 2007). The lake's water primarily comes from precipitation and river inflow. However, since 1995, precipitation rainfall has decreased (Arkian et al., 2018). Several factors have contributed to the significant decline in Lake Urmia's water levels (Schulz et al., 2020). This drawdown has led to increased salinity, alterations in ionic composition (Sima et al., 2021), and an altered salt concentration that now exceeds 300 g/l, reaching saturation levels (Parsinejad et al., 2022). The rising temperatures and salinity have caused a substantial decrease in the density of *Dunaliella* within the lake. To endure these harsh conditions, the microalgae synthesize elevated levels of β-carotene and glycerol, which serve to shield against intense light and osmotic pressure, respectively (Rad et al., 2011). The study also revealed that Lake Urmia's influence on the local climate intensifies during warmer periods, particularly in the summer, while its impact is least in the winter. Despite the ongoing decline in water levels, the lake continues to affect the local climate, albeit to a diminished extent (Dehghanipour et al., 2020).

Microalgae are a valuable source of nutrition for both humans and other organisms, owing to the richness of carbohydrates, proteins, enzymes, and fibers in their chemical composition. In addition to these essential nutrients, microalgae contain a variety of vitamins, including vitamins A, C, B1, B2, B6, and B3, as well as minerals such as iron, potassium, calcium, and magnesium (Pourkarimi et al., 2019). Microalgae are known for their beneficial pigments, such as carotenoids and chlorophyll, which have a natural antioxidant, anti-cancer, anti-diabetic, anti-hypertensive, anti-inflammatory, and, anti-obesity properties thereby playing a crucial role in human health (Sedjati et al., 2019).

Dunaliella is a unicellular and, biflagellated green microalga known for its unique morphology characterized by the absence of a rigid cell wall (Borowitzka and Siva, 2007). This genus is well-adapted to saline marine environments and is and recognized for its ability to produce valuable compounds such as polysaccharides, lipids, proteins, and pigments. As a result, *Dunaliella* has found applications in various areas including food production, aquaculture, cosmetics, wastewater treatment, and biofuels, all of which contribute significantly to its economic value (Pourkarimi et al., 2020).

The microalga *Dunaliella salina* flourishes in highly saline environments, such as salterns, where it endures intense light and temperature fluctuations. *Dunaliella salina* has developed adaptive strategies to cope with these harsh conditions; including modifications to its cell cycle and the accumulation of specialized metabolites for protection.

To date, a variety of *Dunaliella* species have been identified, many of which are flourishing in marine and brackish water habitats. One such species, *Dunaliella* sp. ABRIINW-I1, which originated from Lake

Urmia, has been isolated, purified, and cataloged. This particular strain is currently maintained in the phytotron (cell bank) of the Agricultural Biotechnology Research Institute of Iran (ABRII), Northwest and West Branch, subsequent to its collection and coding (Hejazi et al. 2018). Notably, this isolate contains a substantial amount of protein (~40% of dry weight) and exhibits a pigment concentration of 3.2%, consisting primarily of chlorophyll (1.9%) and carotenoids (1.1 %). Furthermore, it possesses a high lipid content (47%) and relatively low carbohydrate levels (4-7%), indicating a preference for energy storage primarily in the form of lipids (Gharajeh et al., 2020).

Previous studies have shown that the diversity and structure of algal communities fluctuate with the seasons and are shaped by environmental factors (Chen et al., 2023). Factors such as pH, salinity, temperature, light, and the availability of macro and micronutrients affect growth, protein production, etc. in microalgae. Optimizing cultivation conditions such as temperature, nutrient availability, pH, and illumination will enhance predictions for outdoor production. A study indicates that high light intensity can significantly impact on *Dunaliella salina,* leading to a decrease Chla and Chlb contents, while simultaneously increasing the ratio of carotenoids to total chlorophyll. Additionally, this condition enhancies the content of cellular proteins and lipids following a two -weekscultivation period (Zarandi-Miandoab et al. 2015). *Dunaliella salina*is , particularly distinguished by polar lipid content, which is approximately 41%, making it suitable for applications in both food and cosmetic industry (Vanitha et al., 2007). Furthermore, when cultivatedinhigh salinity environments, *Dunaliella salina* can accumulate over 50 % of its dry mass as glycerol. Recent studies highlightes the effectiveness of various culture conditions on *Dunaliella* sp. ABRIINW-I1. For instance, elevated NaCl concentrations and CO_2 enrichment have been found to enhance, lipid production and growth rates compared to other isolates (Gharajeh et al., 2020; Mirzahasanlou and Hejazi, 2022). Studies examining the effect of different pH and temperature on the growth and biochemical responses of *Dunaliella* showed that dry weight gain and the biochemical component enhancement occurred at pH 7.5 (Khalil et al., 2010).

The earlier results demonstrated that the Urmia lake is classified as athalassohaline, withpredominant ions being Na^+, K^+, Mg^{2+}, Ca^{2+}, SO_4^{2-}, Cl^-, HCO_3^-, and Ca^{2+} (Jookar Kashi et al. 2021 and Alkhayer et al 2023). For optimal growth, *Dunaliella* cells require sulphate along with several ions, including K^+, Ca^{2+}, Mg^{2+}, Cl^-, and, Na^+ (Barbosa et al., 2023). The ratio of $Mg^{2+}$to Ca^{2+}in *Dunaliella* cultures can be significantly effect both growth and carotene production (Hosseini Tafreshi and Shariati, 2009).

Previous research has demonstrated that the diversity and composition of algal communities vary with seasonal changes and are influenced by environmental factors (Chen et al., 2023). For example, research focusing on the seasonal distribution and population dynamics of limnic microalgae in the Noyyal River highlighted that

variations in physico-chemical parameters significantly impact microalgal populations throughout the year. Notable, during the summer, a significantproportion (29.57%) of the microalgae population was observed, attributed to stable hydrographic conditions such as temperature, salinity, and light. The highest pH was recorded in the summer (9.54 ± 0.01), while the lowest pH was noted in winter at 6.16 ± 0.005. Increased alkalinity during summer, likely due to elevated bicarbonate levels and an increased rate of photosynthsis, was associated with higher abundance of microalgae in the water samples (Kumar and Thomas, 2019). The physico-chemical parameters are crucial in shaping microalgae populations and their diversity, serving as major factors that influence population dynamics (Hulyal and Kaliwal, 2009). Seasonal variations in these parameters affect the distribution, periodicity, and abundance of microalgal populations as well as their diversity (Shekhar et al., 2008).

Bacteria play a vital role in microalgae cultures, affecting nutrient both nutrient availability and growth dynamics. They can facilitate nutrient conversion and provide essential vitamins and cofactors for microalgae (Marcilhac et al., 2015). On the other hand, negative interactions, such as contamination by fungi, bacteria, viruses, or other microalgae, and environmental pollutants, can adversely affect microalgae cultures (Patil and Gunasekera 2008). Understanding the distribution and seasonal variation of bacterial populations is essential, as they significantly influence marine ecosystems (Harley et al., 2006).

As previously mentioned, environmental conditions and water parameters play a critical role in the cultivation of microalgae. In this study, we maintained the majority most of these factors at a constant level, except for the ionic composition and microbiome of the lake water sample. This research focuses specifically on the effects of ions on the growth patterns and biochemical composition of *Dunaliella* sp. (ABRIINW-I1) inoculated in water samples throughout different seasons.

Material and methods

Sampling location

Monthly sampling of Lake Urmia, located at coordinates 37°47’45.0168”N 45°22’51.1212”E, was performed throughout the year 2021, covering all four seasons.

The water samples were collected from Lake Urmia and stored at 4 °C. The samples were analyzed promptly without any further preservation in the lab. To prepare the samples, two liters of lake water were immediately filtered under aseptic conditions using glass microfiber filters (Whatman GF/D) to remove debris and eukaryotic cells. *Dunaliella* sp. ABRIINW-I1 was then introduced into a two-liter photobioreactor (PBR) containing the filtered Lake Urmia water sample, as described by Benlloch et al. (2001).

Characterization of physicochemical properties

Electrical conductivity (EC), salt concentration, and pH were measured using EC meters (Cond 315i/SEC), by the manufacturer’s instructions. samples

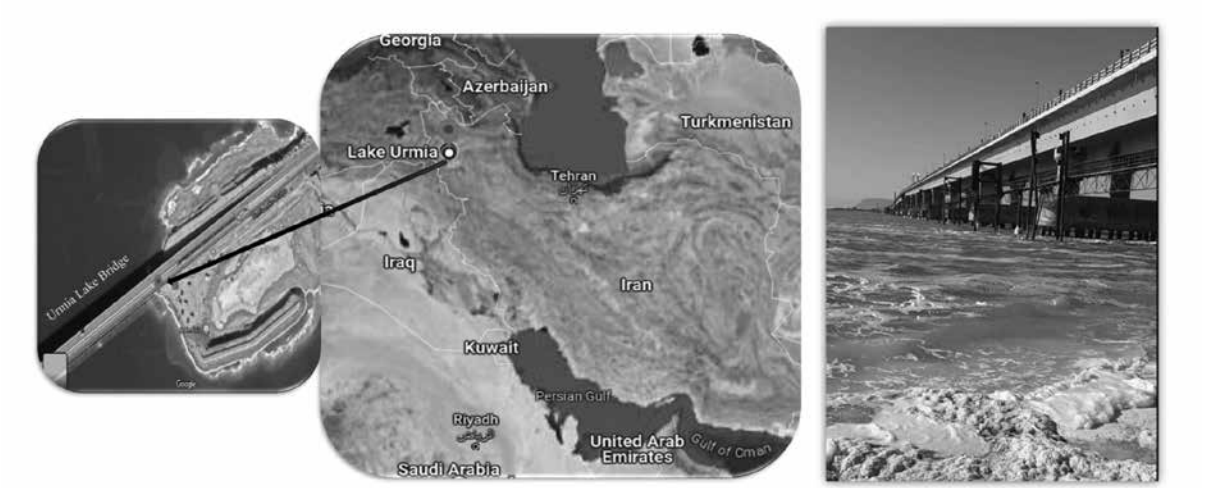

Fig. 1. This figure shows the geographical location of Lake Urmia in Iran, positioned on the right side of the mapcreated using the Google Maps online tool

were analyzed using a Flame Photometer for the analysis of potassium, and sodium concentrations, (Models PFP7). Sodium and potassium concentrations were measured using a Flame Photometer (Models PFP7) following a specific protocol. To prepare a stock sodium solution, 2.542 g dried NaCl was dissolved at 140°C for one hour until a constant weight was achieved, and then diluted to final volume of 1000 mL with water. For the preparation ofthe intermediate sodium solution, 10 ml of the stock sodium solution was diluted with water to reach to a total volume of 100.0 ml. This intermediate solution was utilized to establish a calibration curve for sodium concentrations ranging from 1 to 10 mg/l. in a subsequent step, , the standard sodium solution was prepared by diluting 10.00 ml of the intermediate sodium solution with water to a final volume of 100 ml. This sstandard olution was employed to create a calibration curve in the sodium range of 0.1 to 1.0 mg/l. Additionally, a blank and sodium calibration standards were prepared within a range of 0 to 100 mg/l (West et al., 1950).

A potassium solution and standard potassium solutions were prepared as according to the following protocol. Dried 1.907g KCl was dissolved and diluted to 1000 mL with water to prepare stock potassium solution. For the intermediate potassium solution, 10 ml of the stock potassium solution was further diluted with water toreach to the total volume of 100ml. This intermediate solution was utilized to stablish a calibration curve for potassium concentrations ranging from 1 to 10 mg/l. Moreover, a standard potassium solution was prepared by diluting 10 ml of the intermediate potassium solution to the final volume of 100 ml. This solution was employed to create a calibration curve for potassium concentrations between 0.1 to 1 mg/l. To measure the sodium and potassium of the lake water sample, after dilution, the measurements were performed by flame photometer following a standard formula (Helmke et al., 1996; Brown et al., 1988).

Additionally, other physicochemical characteristics, including calcium,

magnesium, nitrate chloride, and sulfate were determined using atomic absorption spectroscopy (Lambda 35). Initially, First, the samples were filtered to prevent suspended particles from scattering UV light, which could potentially affect the measured absorbance.. Subsequently, the samples were acidified with 1 N HCl to avoid interference from OH^- or CO_3^{2-} ions, which can absorb at 220 nm. All solutions were prepared using nitrate-free water available in the laboratory. All measurements were conducted following the guidelines set by the American Public Health Association (APHA).

Cultivation of microalgae and experimental design

The indigenous, *Dunaliella* sp. ABRIINW-I1 strain was obtained from the Iran Agricultural Biotechnology Research Institute (ABRIINW), Northwest Branch. This strain was isolated from Lake Urmia, the second-largest hypersaline lake in the world, with a salinity range of 140-220 g/L that has escalated to more than 380 g/L/L in recent years owing to a drastic decrease in the water level (Mirzahasanlou, J.P. and Hejazi, 2022). The strain was identified based on the 18S rDNA gene (1770 bp) with *Dunaliella*-specific primers and genetic variation of the gene region sequences (TIS) with an accession number MH880103 in NCBI GenBank (Hejazi et al., 2010).

Dunaliella sp. ABRIINW-I1 was initially cultured in 250 ml Erlenmeyer flasks under sterile conditions using modified Johnson's medium with a salt concentration of 1.5 M, as described by Hejazi and Wijffels (2003). After an average 15-day culture period, the algae were transferred to a two-liter photobioreactor (PBR) with a Lake Urmia water sample, and growth was sustained under continuous light using an artificial light source. The culture was continuously aerated with filtered air, following the procedure outlined by Castilla Casadiego et al. (2016). The optical density was monitored daily throughout the cultivation period.

Biomass production

Optical density, which indicates cell growth, was monitored daily using the Perkin Elmer Lambda 35-UV/V spectrophotometer at a wavelength of 730 nm. The relationship between optical density at 730 nm, biomass, and dry weight was determined experimentally as detailed by Gharajeh et al. (2020). The calculation of these parameters is based on the following equations:

$$\text{Biomass } (g.L^{-1}\ AFDW) = OD_{730} * 0.41, \quad R^2 = 0.95 \quad (1)$$

$$DW\ (g.L^{-1}) = OD_{730} * 0.8, \quad R^2 = 0.95 \quad (2)$$

Profile and pigment content

The pigment content of the isolates was extracted following the method of Sedjati et al. (2019). Three milliliters of algal biomass were centrifuged at 3000 rpm for 15 minutes, and the supernatant was removed. Three ml of 100% pure acetone solvent was then added to each falcon and vortexed until complete pigment extraction. After centrifuging the mixture again at 3000 rpm for 10 minutes, a clear supernatant was obtained. The supernatant was used to measure chlorophyll a, chlorophyll b, and beta-carotene content by the spectrophotometric method (Perkin Elmer, Lambda 35-UV-VIS) using the equations as described by Lichtenthaler and Buschmann (2001).

Chla (µg ml^{-1})= 11.24*A662-2.04*A645
Chlb (µg ml^{-1})= 20.13*A645-4.19*A662
Cart (µg ml^{-1})= (1000*A470-1.9*Chla-63.14*Chlb)/214
T Chl (µg ml^{-1})= Chla + Chlb
Chla: chlorophyll a, Chlb: chlorophyll b, Chl T: total chlorophyll, Cart: carotenoid
The concentration of pigments was determined in micrograms per milliliter (µg ml^{-1}) and then converted to a percentage of the dry-weight biomass. Chla/Chlb, Cart/Chla, and Cart/Chlb ratios were also calculated.

Protein content

The total protein content was measured using the Bradford (1976) method. Polyvinylpyrrolidone (PVP) and 50 µM potassium-phosphate buffer (pH 7) extraction buffer were added to 0.05 g of microalgae from 100 ml cultures. The mixture was then centrifuged at 1000 rpm for 30 minutes at 0°C, and the supernatant was collected for protein measurement. All extraction procedures were conducted at 0-4°C. Subsequently, 20 microliters of protein extract were mixed with 2 ml of Coomassie blue reagent and thoroughly mixed. The total protein content was determined using a spectrophotometric method at 595 nm based on a standard curve established with bovine serum albumin.

Lipid extraction and GC analysis

Lipid content was extracted according to the modified method of Bligh and Dyer described by Yang et al. (2014). gas chromatography was performed on the extracted lipids using the fatty acid methyl ester (FAME) profiling method to determine the fatty acid composition, at the Parto Bashash Danesh Gostar research institute. the direct methyl esterification method was used for Gas Chromatography (GC) analysis (Duong et al. 2015). 1 µl of the extract was injected into the GC system (Varian company, Model 4000) equipped with a flame ionization (FID) detector, the capillary column of CP-Sil 88 (CP7489) and 1079 injector. The inlet temperature of the injector and detector were set as 250 and 280 ℃, respectively. The flow rate of carrier gas was set as 1 mL min^{-1} under the application of the following program: column temperature was kept at 130 ℃ for 4 min then increased to 180 ℃ at the rate of 5 ℃ min^{-1}, then raised to 220 ℃ with the rate of 4 ℃ min^{-1} and was maintained at this temperature for 20 min. The tentative identification of fatty acids present in the samples was performed by comparing their retention time with those of the external standards in F.A.M.E mix C4-C24 (Sigma, 18919-1AMP Supelco with 37 components). Fatty acid composition in the samples was reported as the percentage of the total fatty acid content, and the relevant quantity (mg ml−1 of the extract) was measured against the internal standard C13:0 (Sigma-Aldrich, 91558) (Duong et al., 2015).

Statistical analysis

Statistical analysis was conducted using SPSS v23.0 (IBM, Armonk, NY), and a normality test was performed on the data. One-way analysis of variance (ANOVA) was utilized with a 95% confidence interval. Principal component analysis (PCA) analysis was conducted using GenStat 12th Edition.

Results and Discussion

ANOVA analysis showed no significant variation in the growth level as well as lipid and carotenoid contents in *Dunaliella* sp. ABRIINW-I1 (Table 1). However, the seasonal changes did result in significant variations in protein content, total chlorophyll (including chlorophyll a and b), saturated fatty acids, polyunsaturated fatty acids, omega-6 fatty acids, and, the ratio of unsaturated to saturated fatty acids.

Biomass yield

The isolate *Dunaliella* sp. ABRIINW-I1 displayed the highest ash-free dry weight AFDW and dry weight (DW) in the summer water sample, measuring 0.71g l^{-1} and 1.3886 g/l, respectively. As illustrated in Figure 2, the winter season came next with values of 0.68 g.l^{-1} and 1.3344 g.l^{-1}, followed by the fall season with values of 0.63 g l^{-1} and 1.23 g/L, and the spring season with the lowest quantity, 0.56 g l^{-1} and 1.1g L^{-1}(. Gharajeh et al. (2020) The Dunaliella sp. ABRIINW-I1 isolate had the lowest values, with 0.55g l-1 AFDW and 1.1 g l-1 DW, according to Gharajeh et al.'s (2020) investigation of the AFDW and DW of three isolates. This is in line with the findings found in the data observed in the spring season of the present study. Microalgal growth and its survival is influenced by habitats and seasons,where pond temperature and incident light on the pond surface fluctuates substantially in seasons in addition to normal dairy variations. During a study, *Picochlorum* sp. isolated from tropical habitat was found to be better adapted and more productive in the summer tropical simulations. Upregulation of carbon saving pathways leads to an increase in biomass accumulation in summer simulation (Manjre et al., 2022). Seasonal distribution of limnic microalgae and their correlation with physicochemical parameters of river Noyyal demonstrated that the higher pH observed in summer might have been attributed to the strong photosynthetic activity of microalgae during that season (Kumar and Thomas. 2019).

Microalgae are microorganisms that growth rapidly, in less than a day, their biomass can double (Tredici, 2010). The growth rate of microalgae was enhanced under highCO_2 conditions, according to research on the effect of varying CO_2 concentrations as a carbon source on two native *Dunaliella* strains (ABRIINW-CH2 and ABRIINW-SH33) under adjusted pH (Moghimifam et al., 2019). In addition to abiotic factors, microalgae production can be impacted by biological factors such as bacteria, fungi, viruses, and the competitive environment created by other algal species (Çelebi et al., 2021). It is crucial to note that these parameters may fluctuate with seasonal

Table 1. Statistical analysis of growth, pigment content, lipid ontent, and lipid profile of *Dunaliella* sp. ABRIINW-I1 in Lake water samples from four seasons of 2021

Source of variation	df	Mean Square														
		Biomass	Dry Weight	Lipid	Protein	Chla	Chlb	TChl	Cart	∑SFA	Omega 3	Omega 6	n3:n6	∑PUFA	∑UFA	UFA:SFA
Season	3	0.012*	0.047*	74.57ns	884.9*	0.75*	0.245*	1.834*	0.06ns	172.58*	54.30ns	79.09*	2.52ns	152**	192.6ns	0.427*
Error	8	0.049	0.18	55.62	184.5	0.16	0.044	0.256	0.33	32.28	17.58	15.67	0.9	8.55	71.55	0.67
Total	11															
CV (%)		8.52	8.53	10.32	13.56	11.14	36.46	10.37	39.3	3.72	18.83	9.13	22.7	4.35	5.71	19.61

ns = Non-significant (P>0.05); * = Significant (P<0.05); ** = highly significant (P<0.01)

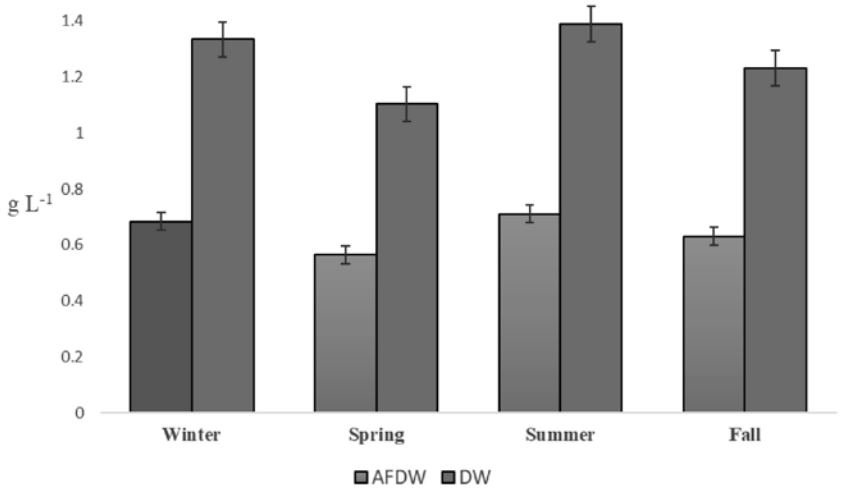

Fig. 2. The biomass concentration of *Dunaliella* sp. ABRIINW-I1 as AFDW and dry weight (DW) in different seasons of the year

variations throughout the year (Harley et al., 2006).

Microalgae productivity is largly governed by changing seasonal conditions affecting its micromorphology, physiology and metabolic activity. In this study, microalga belonging to the Picochlorum sp. that was isolated from tropical habitat was found to be better mare adaptable and twice as productive in the summertime tropical simulations.

Pigment content

Microalgae biomass cultivated in water samples of different seasons of the year showed a significant difference for Chla, Chlb, and total chlorophyll content, as indicated by variance analysis of a completely random design ($p \leq 0.05$). Specifically, the maximum chlorophyll content was observed in winter (2.88% DW), while the lowest was recorded in summer (0.98% DW). Moreover, compared to other seasons the amount of Chla, Chlb, and chlorophyll in the total biomass was significantly higher in winter. However, carotenoid content was not significantly different among different seasons ($p \leq 0.05$). According to previou study (Gharajeh et al., 2020) the Dunaliella sp. ABRIINW-I1 isolate's chlorophyll content was comparable to the quantity of chlorophyll produced by this microalgae during the winter (2.35%) in the present study (Figure 3). Unlike this study, no significant difference in the level of photosynthetic pigments was observed in different seasons of *Chaetomorpha antennina* microalgae (Vinuganesh et al. 2022).*Lipid content and Lipid profile*

Dunaliella sp. ABRIINW-I1 has been reported to produce 36% of lipid content (Gharajeh et al., 2020). In the aquaculture sector, the positive impact of lipid content on weight gain, protein deposition in muscles, disease resistance, reduced nitrogen production, increased digestibility, physiological activities, hunger tolerance, and the overall quality of aquatic animals have been recognized (Fleurence et al.

2012). Seasonal variations in the lipid content of Dunaliella sp. ABRIINW-I1 in the cells were not statistically significant. The winter samples had the highest fat concentration in the Vinuganesh et al. (2022) investigation. The fact that the loss of growth to biomass ratio in microalgae cells occurs concurrently with the hyper-production state of a metabolite is supported by the low biomass of the cells with high lipid production cultivated in spring water (Yang et al, 2018).Fatty acids with odd or fewer than 14 carbons were not found by gas chromatography, whereas fatty acids with carbons 14 to 24 were observed. . ANOVA results showed that there was a significant difference in datasets of saturated fatty acids (SFA) ($p \leq 0.05$), polyunsaturated fatty acids (PUFA) ($p \leq 0.01$), omega 6 fatty acids ($p \leq 0.05$), and the ratio of UFA: SFA ($p \leq 0.05$) with seasonal changes. In the cells cultured in fall and winter water samples, the ratio of omega 3 to omega 6 fatty acids ranged from 1.06 to 58.4. (Figure 4).

Based on the data, the fatty acid profile of microalga can be altered by modifying growth media (Rismani and Shariati, 2017). The salinity is an influential parameter in

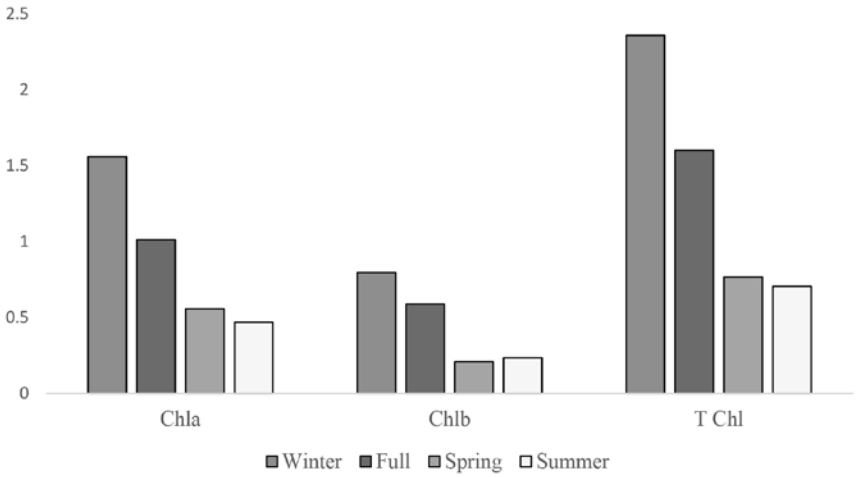

Fig. 3. Pigment content (DW%) in *Dunaliella* sp. ABRIINW-I1, Chl: chlorophyll, Chl T: total and, chlorophyll

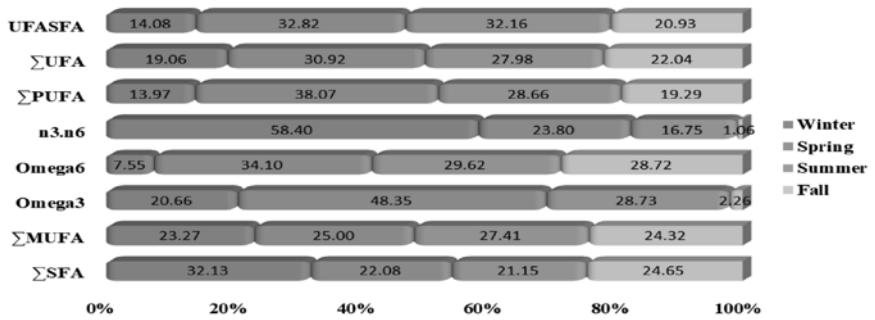

Fig. 4. Fatty acid profile of the isolate, *Dunaliella sp.* ABRIINW-I1, grown in the water samples collected in spring, summer, fall, and winter seasons. UFA: unsaturated fatty acids, SFA: saturated fatty acids, UFASFA: UFA/SFA, PUFA: Polyunsaturated fatty acid, n3: Omega3: omega 3 fatty acids, n6: Omega6: omega 6 fatty acids, n3.n6: omega 3 to omega 6 fatty acids, MUFA: mono-unsaturated fatty acids and, SFA: saturated fatty acids

the TFA concentration and fatty acid profile. Due to the high occurrence of evaporation in the spring and summer seasons, the salinity in the water increases. The high salt in both spring and summer waters has seemingly resulted in the high ratio of UFA to SFA and high production of PUFA including the major fraction of omega 6 type. Based on the studies, the TFA synthesis might be induced, unaffected, or even inhibited at different salinities depending on the microalgae origin (Adarme-Vega et al. 2012).

Microalgae, respond to changes in the external environment by adjusting the internal conditions (de Morais et al. 2015). In this study, the external factors of seasonal water samples both directly and indirectly influenced the growth and production behavior of the isolate, *Dunaliella* sp. ABRIINW-I1. Key factors such as the composition of the culture media and nutrient availability directly impact microalgal biomass and product concentration (Panahi et al. 2019., Sánchez-Bayo et al. 2020). Additionally, biomass and productivity of the microalga are also indirectly affected by the cultivation variables. A wide variety of microorganisms, such as zooplankton, protozoa, and, bacteria which coexist alongside the microalgae. Variations in microalgae behavior can result from seasonal change in the microbiota, which provides microalgae with foods(Márquez-Rocha et al. 2019). Factors such as pH, salinity, temperature, light, and the availability of macro- and micro-nutrients influence the formation and composition of the microbiome community (Steinrücken et al., 2023). Specifically, salinity and pH are crucial for the survival of certain microorganisms (Bui-Xuan et al., 2022). The light spectrum, illumination duration, and wavelength range also play a significant role in the metabolism and biomass composition of water microbiota (Sánchez-Bayo et al., 2020; Udayan et al., 2023). Furthermore, water temperature plays a substantial role in the water flora that supports microalgae, with low temperatures limiting the microbiome population (Díez-Montero et al., 2020).

Culture media serve as essential nutrient sources for the survival and proliferation of microorganisms. These media must meet the specific nutritional requirements of the microorganisms to maintain a consistent composition. The microbiome in natural water sources before collection and the microalgae after cultivation in collected water samples depend on environmental conditions that are influenced by seasonal variations. Variations in the microbiome within the culture medium are associated with the availability of essential nutrients. The presence or absence of certain nutrients and microorganisms in different cultivation media can stimulate the biosynthesis of specific compounds in microalgae (de Morais et al., 2015).

Protein content

The highest protein accumulation from the isolate was achieved in winter water samples (50.3 %) (Figure 5). It was significantly higher than the second value of 21.16 % related to the cells grown in fall samples ($p \leq 0.05$). The lowest protein content occurred in spring (1.53 %) and summer (1.34 %). Correspondingly, the seasonal variation of chemical composition in Ulva

spp. revealed the elevated protein content in winter, higher than in fall samples (Jansen et al. 2022).

In the present study, the amount of Na^+ ion was significantly higher in winter and the amount of protein content and growth by microalgae was also higher in winter. The water's phosphates, nitrates, nitrites, ammonia, and chloride levels varied significantly with the seasons' temporal climate shifts, which affected the diversity and abundance of microalgae.Nitrogen, a key variable for the growth of microalgae was provided in the form of ammonia, nitrite, and nitrate which was differently metabolized resulting in enhanced protein content and growth capacity of microalgae (Varadharajan and Soundarapandian, 2014).

Physio-chemical properties of Lake Urmia across seasonal variation

The scree plot of the PCA displays the variance explained for each component (Fig. 6). Accordingly, the first two principal components (PC) accounted for 48.48%

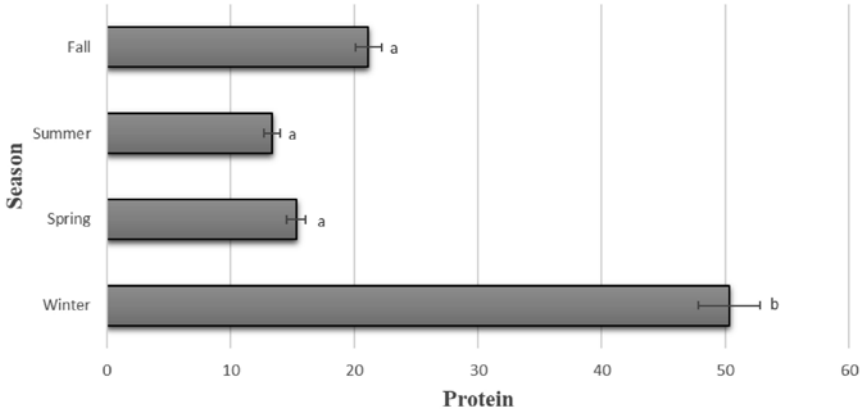

Fig. 5. The protein content of the isolate, *Dunaliella* sp. ABRIINW-I1, grown in the water samples collected in different seasons of the year

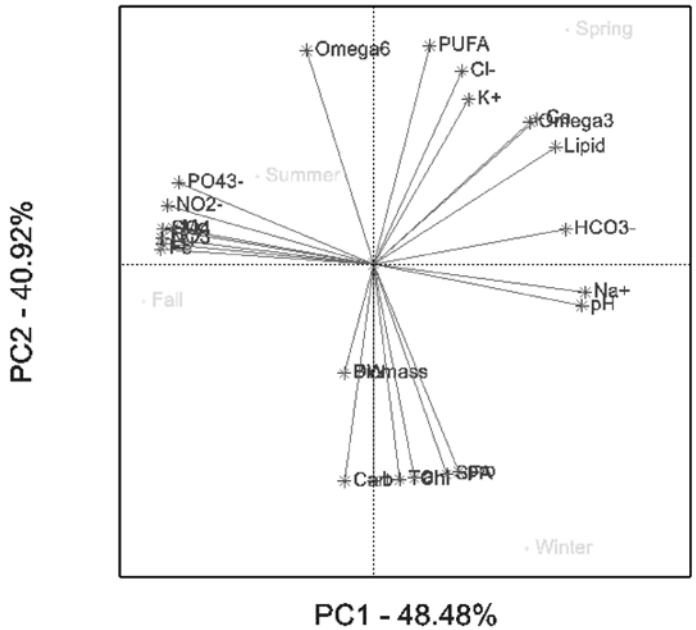

Fig. 6. Principal component analysis of *Dunaliella* microalgae variables across four seasons

and 40.92% of the variance, respectively. The first component explains the most variant. Chl b, K+, PUFA, Cl-, carotenoid, and Omega6 have the greatest impact on the first component. Mg2+, Li+, Fe2+, N+, pH, and Protein have the greatest impact on the second component of variations. In fact, results of PCA analysis showed that the Mg2+, Li+, Fe2+, N+, pH, and Protein have a similar variation pattern across seasonal change.

In general, the main dominant cations and anions in Urmia Lake are, Na^+, Mg^{2+}, K^+, Ca^{2+}, Cl^-, SO_4^{2-}, and HCO_3^-, respectively (Sharifi et al., 2018). However, due to the variation in the volume and the ionic composition of inflow water and evaporation/precipitation reactions, the chemistry of the lake water continually changes. Water quality also varies seasonally, determined by river inflows and the lake bathymetry (Lak et al., 2022).

Physico-chemical parameters are considered one of the most essential components that are capable of influencing the aquatic environment. The diversity of microalgae distribution was largly influenced by the physico-chemical parameters of the water due to seasonal variations (Kumar and Thomas, 2019). All the biochemical functions and retention of physicochemical properties of the water were prominently dependent on the pH of the surrounding environments (Jalal and Kumar, 2013). The pH of the lake is almost neutral and according to previous studies, the pH of the lake is neutral (7.2-7.5) and does not change in different places and times (Jookar Kashi et al. 2014). Our data supported these conclusions by demonstrating that, with minor variations, pH values are mostly constant around neutral throughout the seasons. variations The results showed that K^+, Mg^{2+}, and SO_4^{2-} ions were significantly high in the fall and then decreased in summer, spring, and winter. Also, it was observed that Na^+ in winter and spring was significantly higher than in summer and fall. It can be due to the decrease in rainfall, more evaporation, and the formation of salt crystals. The amount of Na^+ ions is changed under the influence of water fluctuations (Alipour 2006). Similar to the changes in ionic composition from July (wet season) to October (dry season) 2019, while the water level reduced, Na+ concentration decreased at the surface layer due to the gradual formation of halite (NaCl) at the bottom of the simulation vessel (Alkhayer et al 2023). The contribution of phosphorus and NaCl in β-carotene production in Dunaliella has been demonstrated by (Pourkarimi et al. 2020). Summer, which is regarded as a dry month and is marked by low water levels because of evaporation, which results in a concentrated level of nutrients, particularly nitrates, during these seasons, had a high variety value (Kumar and Thomas. 2019).

It was reported, that the sulfate level was elevated during the summer months, which was substantially connected with biomass and growth rate of *Dunaliella*. It has been demonstrated that sulfate deficiency in microalgae causes primarily an inhibition of cell division and accumulation of metabolites (Gimmler and Weiss, 1987). For optimal growth, Dunaliella requires sulfate concentrations of approximately 2

mmol l^{-1}(Hosseini Tafreshi and Shariati. 2009). The observations confirmed that elevated sulfate levels in summer coincided with increased biomass concentrations of Dunaliella in the water sample.Our findings align with previous studies that have shown the growth rate of *Dunaliella tertiolecta* in a high $MgSO_4$ (0.94 M) medium was lower than that in a NaCl medium of equivalent osmolality. The growth inhibition of *D. tertiolecta* in the high $MgSO_4$ medium was found to be partially alleviated by increases in extracellular Ca2+ concentrations (ranging from 1 to 11 mM) . Furthermore, measurements of intracellular cations in the current study revealed that the intracellular concentration of Ca^{2+} rose in response to the elevated extracellular Ca^{2+} concentration. These findings suggest that elevated Mg^{2+} concentrations inhibit the uptake of Ca^{2+} into the cells, which accounts for the observed growth inhibition in $MgSO_4$ media (Fujii and Hellebust, 1992).

Magnesium levels peaked during the Fall and Summer months, suggesting that seasonal evaporation might lead to a higher concentration magnesium in the lake. The observed decrease in winter could be due to increased dilution or reduced magnesium sources during the colder months. Similarly, Potassium levels exhibit a comparable seasonal pattern, being elevated in Fall and Summer while declining in winter and spring. The variations in nutrients, such as nitrate, phosphate, and sulfate, could impact primary productivity of the lake (Spiridon et al.m 2018). Overall, the data reveals significant seasonal fluctuations in the water chemistry of Lake Urmia. These variations are affected by precipitation, evaporation, runoff, and biological activity, all of which play a role in the water quality and chemistry of the lake throughout the year. Recognizing these seasonal trends is crucial for effective management and conservation of the lake's ecosystem.

The results of this study indicated that seasonal changes impact the biochemical profile and metabolic fingerprint of *Dunaliella* sp. ABRIINW-I1. This suggests that microalgae cultivation and harvesting should be aligned with the relevant seasons to optimize the production of desired metabolites. Tailoring cultivation systems to the seasonal variations in biochemical composition may enhance the productivity of target products. For example, a biotechnology study conducted in South Korea's South Sea revealed that bacterial population and diversity peaked in spring season, followed by a decrease from spring to summer, and then an increase again in autumn (Suh et al., 2015). These findings align with previous research showing that bacterial communities exhibit seasonal patterns, suggesting predictable trends in microbial diversity (Suh et al., 2015). Considering the stability of microalgae cultivation conditions in the laboratory, the composition of cations and anions and the microbiome's composition can be an effective factor in these results. Environmental factors impact the associated microbiota, which influences biomass production and metabolite yields. Each season creates unique environmental conditions and growth media that favor specific microbial communities.

The study faced limitations due to a lack

of available literature and data on related topics. However, as noted, the variation in microalga growth and production behavior is significantly influenced by the microbiome community structure (Steinrücken et al., 2023). It is essential to collect, preserve, and identify indigenous microorganisms in the lake, study their interactions, and explore various parameters associated with the lake. Future research should monitor seasonal changes in the microbiota and incorporate meta-analysis and open system cultivation studies to better understand alterations in microbiome populations during different cultivation seasons.

Acknowledgment

This work is based upon research funded by Iran National Science Foundation (INSF) under project No 99027784.

References

Adarme-Vega TC, Lim DK, Timmins M, Vernen F, Li Y, Schenk PM. (2012). Microalgal biofactories: a promising approach towards sustainable Omega-3 fatty acid production. Microbial cell factories. 11 (1): 1-10. DOI: 10.1186/1475-2859-11-96.

Alipour S. (2006). Hydrogeochemistry of seasonal variation of Urmia Salt Lake, Iran. Saline Systems. 2: 1-19. DOI: 10.1186/1746-1448-2-9.

Alkhayer M, Eghbal MK, Hamzehpour N. (2023). Brine geochemical changes and salt crust evolution of Lake Urmia in Iran. Catena. 231: 107310. DOI: 10.1016/j.catena.2023.107310.

Arkian F, Nicholson SE, Ziaie B. (2018). Meteorological factors affecting the sudden decline in Lake Urmia's water level. Theoretical and Applied Climatology. 131: 641-651. DOI: 10.1007/s00704-016-1992-6.

Barbosa M, Inácio LG, Afonso C, Maranhão P. (2023). The microalga Dunaliella and its applications: a review. Applied Phycology. 4 (1): 99-120. DOI: 10.1080/26388081.2023.2222318.

Borowitzka MA and Siva CJ. (2007). The taxonomy of the genus *Dunaliella* (*Chlorophyta, Dunaliellales*) with emphasis on the marine and halophilic species. Journal of Applied Phycology. 19: 567-590. DOI: 10.1007/s10811-007-9171-x.

Bradford MM. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry. 72 (1-2): 248-254. DOI: 10.1016/0003-2697(76)90527-3.

Brown IR and Warncke D. (1988). Recommended cation tests and measures of cation exchange capacity. Recommended chemical soil test procedures for the North Central Region. North Dakota Agricultural Bulletin. 449: 15-16.

Bui-Xuan D, Tang DYY, Chew KW, Nguyen TDP, Le Ho H, Tran TNT, Nguyen-Sy T, Dinh THT, Nguyen, PS, Dinh TMH, Nguyen TT. (2022). Green biorefinery: Microalgae-bacteria microbiome on tolerance investigations in plants. Journal of Biotechnology. 343: 120-127. DOI: 10.1016/j.jbiotec.2021.12.002.

Castilla Casadiego DA, Albis Arrieta AR, Angulo Mercado ER, Cervera Cahuana

SJ, Baquero Noriega KS, Suárez Escobar AF, Morales Avendaño ED. (2016). Evaluation of culture conditions to obtain fatty acids from saline microalgae species: *Dunaliella salina*, *Sinecosyfis* sp., and *chroomonas* SP. BioMed Research International. 2016 (1): 5081653. DOI: 10.1155/2016/5081653.

Castilla-Casadiego DA, Ramos-Avilez HV, Herrera-Posada S, Calcagno B, Loyo L, Shipmon J, Acevedo A, Quintana A, Almodovar J. (2016). Engineering of a stable collagen nanofibrous scaffold with tunable fiber diameter, alignment, and mechanical properties. Macromolecular Materials and Engineering. 301 (9): 1064-1075. DOI: 10.1002/mame.201600156.

Çelebi H, Bahadır T, Şimşek I, Tulun Ş. (2021). Use of *Dunaliella salina* in environmental applications. In Proceedings of the 1st International Electronic Conference on Biological Diversity. Ecology, and Evolution. Online, pp. 15-31. https://DOI.org/10.3390/xxxxx.

Chen C, Li P, Yin M, Wang J, Sun Y, Ju W, Liu L, Li ZH. (2023). Deciphering characterization of seasonal variations in microbial communities of marine ranching: Diversity, co-occurrence network patterns, and assembly processes. Marine Pollution Bulletin. 197: 115739. DOI: 10.1016/j.marpolbul.2023.115739.

de Morais MG, Vaz BDS, de Morais EG, Costa JAV. (2015). Biologically active metabolites synthesized by microalgae. BioMed Research international. 2015 (1): p.835761. DOI: 10.1155/2015/835761.

Dehghanipour AH, Moshir Panahi D, Mousavi H, Kalantari Z, Tajrishy M. (2020) Effects of water level decline in Lake Urmia, Iran, on local climate conditions. Water. 12 (8): 2153. DOI: 10.3390/w12082153.

Díez-Montero R, Belohlav V, Ortiz A, Uggetti E, García-Galán MJ, García J. (2020). Evaluation of daily and seasonal variations in a semi-closed photobioreactor for microalgae-based bioremediation of agricultural runoff at full-scale. Algal Research. 47: 101859. DOI: 10.1016/j.algal.2020.101859.

Duong VT, Thomas-Hall SR, Schenk PM. (2015). Growth and lipid accumulation of microalgae from fluctuating brackish and sea water locations in South East Queensland—Australia. Frontiers in Plant Science. 19 (6): 359. DOI: 10.3389/fpls.2015.00359.

Eimanifar A and Mohebbi F. (2007). Urmia Lake (northwest Iran): a brief review. Saline Systems. 3 (1): 5. DOI: 10.1186/1746-1448-3-5.

Fleurence J, Morançais M, Dumay J, Decottignies P, Turpin V, Munier M, Garcia-Bueno N, Jaouen P. (2012). What are the prospects for using seaweed in human nutrition and for marine animals raised through aquaculture? Trends in Food Science & Technology. 27 (1): 57-61. DOI: 10.1016/j.tifs.2012.03.004.

Fujii S and Hellebust JA. (1992). Effects of Ca^{2+} on the growth of *Dunaliella tertiolecta* in media with high concentrations of Mg^{2+}. Canadian Journal of Botany. 70 (7): 1331-1335. DOI: 10.1139/b92-167.

Gharajeh NH, Valizadeh M, Dorani E, Hejazi

MA. (2020). Biochemical profiling of three indigenous *Dunaliella* isolates with a main focus on fatty acid composition towards potential biotechnological application. Biotechnology Reports. 26: e00479. DOI: 10.1016/j.btre.2020.e00479.

Gimmler H and Weiss C. (1987). The effect of sulfate deficiency and excess sulfate on growth and metabolism of *Dunaliella parva*. Journal of Plant Physiology. 131 (5): 449-465. DOI: 10.1016/S0176-1617(87)80288-2.

Harley CD, Randall Hughes A, Hultgren KM, Miner BG, Sorte CJ, Thornber CS, Williams SL. (2006). The impacts of climate change in coastal marine systems. Ecology Letters. 9 (2): 228-241. DOI: 10.1111/j.1461-0248.2005.00871.x.

Hejazi MA, Barzegari A, Gharajeh NH, Hejazi MS. (2010). Introduction of a novel 18S rDNA gene arrangement along with distinct ITS region in the saline water microalga *Dunaliella*. Saline Systems. 6 (1): 1-11. DOI: 10.1186/1746-1448-6-4.

Hejazi MA., Khoshrouy R., Hosseinzadeh Gharajeh N., Etemadi MR., Madayen L., Javanmard A. (2018). Conservation and Biodiversity Analysis of the Micro alga *Dunaliella* in Shrinking Highly Saline Urmia Lake Based on Intron-sizing Method. Journal of Agricultural Science and Technology. 18 (6): 1693-1703. URL: http://hdl.handle.net/123456789/3720

Hejazi MA and Wijffels RH. (2003). Effect of light intensity on β-carotene production and extraction by *Dunaliella salina* in two-phase bioreactors. Biomolecular Engineering. 20 (4-6): 171-175. DOI: 10.1016/S1389-0344(03)00046-7.

Helmke PA and Sparks DL. (1996). Lithium, sodium, potassium, rubidium, and cesium. Methods of soil analysis: Part 3 chemical methods. 1 (5): 551-74. DOI: 10.2136/sssabookser5.3.c19.

Hosseini Tafreshi A and Shariati M. (2009). Dunaliella biotechnology: methods and applications. Journal of Applied Microbiology. 107 (1): 14-35. DOI: 10.1111/j.1365-2672.2009.04153.x.

Hulyal SB and Kaliwal BB. (2009). Dynamics of phytoplankton in relation to physico-chemical factors of Almatti reservoir of Bijapur District, Karnataka State. Environmental Monitoring and Assessment. 153: 45-59. DOI: 10.1007/s10661-008-0335-1.

Jalal FN and Kumar MS. (2013). Water quality assessment of Pamba River of Kerala, India in relation to pilgrimage season. 341-347. DOI: 10.5555/20133091512

Jansen HM, Bernard MS, Nederlof MA, van der Meer IM, van der Werf A. (2022). Seasonal variation in productivity, chemical composition, and nutrient uptake of Ulva spp. (Chlorophyta) strains. Journal of Applied Phycology. 34 (3): 1649-1660. DOI: 10.1007/s10811-022-02708-z.

Jookar Kashi F, Owlia P, Amoozegar MA, Kazemi B. (2021). Halophilic prokaryotes in Urmia Salt Lake, a hypersaline environment in Iran. Current Microbiology. 78 (8): 3230-3238. DOI: 10.1007/s00284-021-02583-w.

Jookar Kashi F, Owlia P, Amoozegar MA, Yakhchali B, Kazemi B. (2014).

Diversity of cultivable microorganisms in the eastern part of Urmia Salt Lake. Microbiology, Biotechnology and Food Sciences. 4: 36-43. DOI: 10.15414/jmbfs.2014.4.1.36-43.

Khalil ZI, Asker MM, El-Sayed S, Kobbia IA. (2010). Effect of pH on growth and biochemical responses of *Dunaliella bardawil* and *Chlorella ellipsoidea*. World Journal of Microbiology and Biotechnology. 26: 1225-1231. DOI:10.1007/s11274-009-0292-z.

Kumar PS and Thomas J. (2019) Seasonal distribution and population dynamics of limnic microalgae and their association with physico-chemical parameters of river Noyyal through multivariate statistical analysis. Scientific reports. 9(1): 15021. DOI: 10.1038/s41598-019-51542-w.

Lak R, Mohammadi A. Darvishi Khatooni, J. (2022) Lake Urmia Brine Evolution from 2007 to 2019. In: The Handbook of Environmental Chemistry. pp. 1–23. Springer, Berlin, Heidelberg.

Lichtenthaler HK and Buschmann C. (2001). Chlorophylls and carotenoids: Measurement and characterization by UV-VIS spectroscopy. Current protocols in food analytical chemistry. 1 (1): F4-3. DOI: 10.1002/0471142913.faf0403s01.

Manjre S, Paul K, Patil S, Pai P, Banerjee A, Sarkar P, Teredesai A, Shukla MR, Dasgupta S. (2022). Evaluating the effect of seasonal conditions on metabolism and photosynthetic performance of *Picochlorum* sp. and its influence on biomass productivity. Bioresource Technology Reports. 18:101029. DOI: 10.1016/j.biteb.2022.101029.

Marcilhac C, Sialve B, Pourcher AM, Ziebal C, Bernet N, Béline F. (2015). Control of nitrogen behaviour by phosphate concentration during microalgal-bacterial cultivation using digestate. Bioresource technology. 175, 224-230. DOI: 10.1016/j.biortech.2014.10.022.

Márquez-Rocha FJ, Palma-Ramírez D, García-Alamilla P, López-Hernández JF, Santiago-Morales IS, Flores-Vela AI. (2019). Microalgae cultivation for secondary metabolite production. Microalgae: From Physiology to Application. 11: 193-204.

Mirzahasanlou JP, Hejazi MA. (2022). Effect of CO_2 on Growth Parameters and Lipid Production in Dunaliella sp. ABRIINW-I1 (Chlorophyceae) Isolated from Urmia Lake, NW Iran. Plant, Algae, and Environment. 8(1):1229-1242. DOI: 10.21203/rs.3.rs-1632443/v1

Moghimifam H, Ali J, Jabbarzadeh M, Kim M, Fu HC. (2019). Bending Stiffness and Critical Forces for Polymorphic Transformations of Salmonella Flagella Measured in a Microfluidic Channel. In APS Division of Fluid Dynamics Meeting Abstracts. (pp. H31-004). Bibcode: 2019APS..DFDH31004M.

Panahi Y, Khosroushahi AY, Sahebkar A, Heidari HR. (2019). Impact of Cultivation Condition and Media Content on *Chlorella vulgaris* Composition. Advanced pharmaceutical bulletin. 9(2): 182–194. DOI: 10.15171/apb.2019.022.

Parsinejad M, Rosenberg DE, Ghale YA, Khazaei B, Null SE, Raja O, Safaie A,

Sima S, Sorooshian A, Wurtsbaugh WA. (2022) 40-years of Lake Urmia restoration research: Review, synthesis and next steps. Science of The Total Environment. 1 (832): 155055. DOI: 10.1016/j.scitotenv.2022.155055.

Patil JG and Gunasekera, RM. (2008). Tissue and sexually dimorphic expression of ovarian and brain aromatase mRNA in the Japanese medaka (Oryzias latipes): implications for their preferential roles in ovarian and neural differentiation and development. General and comparative endocrinology: 158 (1): 131-137. DOI: 10.1016/j.ygcen.2008.05.016.

Pourkarimi S, Hallajisani A, Alizadehdakhel A, Nouralishahi A, Golzary A. (2020). Factors affecting production of beta-carotene from *Dunaliella salina* microalgae. Biocatalysis and Agricultural Biotechnology. 29: 101771. DOI: 10.1016/j.bcab.2020.101771.

Pourkarimi S, Hallajisani A, Alizadehdakhel A, Nouralishahi A. (2019). Biofuel production through micro-and macroalgae pyrolysis–A review of pyrolysis methods and process parameters. Journal of Analytical and Applied Pyrolysis. 142: 104599. DOI:10.1016/j.jaap.2019.04.015.

Rad FA, Aksoz N, Hejazi MA. (2011). Effect of salinity on cell growth and β-carotene production in Dunaliella sp. isolates from Urmia Lake in northwest of Iran. African Journal of Biotechnology. 10(12):2282-2289. /www.ajol.info/index.php/ajb/article/view/93139.

Rismani S, Shariati M. (2017). Changes of the total lipid and omega-3 fatty acid contents in two microalgae *Dunaliella salina* and Chlorella vulgaris under salt stress. Brazilian Archives of Biology and Technology. 60: 01. DOI: 10.1590/1678-4324-2017160555.

Sánchez-Bayo A, Morales V, Rodríguez R, Vicente G, Bautista LF. (2020). Cultivation of microalgae and cyanobacteria: Effect of operating conditions on growth and biomass composition. Molecules. 25(12): 2834. DOI: 10.3390/molecules25122834.

Schulz S, Darehshouri S, Hassanzadeh E, Tajrishy M, Schüth C. (2020). Climate change or irrigated agriculture–what drives the water level decline of Lake Urmia. Scientific reports. 10(1): 1-10. DOI: 10.1038/s41598-019-57150-y.

Sedjati S, Santosa GW, Yudiati E, Supriyantini E, Ridlo A, Kimberly FD. (2019). Chlorophyll and carotenoid content of *Dunaliella salina* at various salinity stress and harvesting time. In IOP Conference Series: Earth and Environmental Science. 246: 012025. DOI: 10.1088/1755-1315/246/1/012025.

Sharifi A, Shah-Hosseini M, Pourmand A, Esfahaninejad M, Haeri-Ardakani O. (2018). The vanishing of Urmia Lake: a geolimnological perspective on the hydrological imbalance of the world's second largest hypersaline lake. In Lake Urmia: A Hypersaline Waterbody in a Drying Climate. pp. 41-78. DOI: 10.1007/698_2018_359.

Shekhar TS, Kiran BR, Puttaiah ET, Shivaraj Y, Mahadevan KM. (2008). Phytoplankton as index of water quality with reference to industrial pollution.

Journal of Environmental Biology. 29(2): 233.

Sima S, Rosenberg DE, Wurtsbaugh WA, Null SE, Kettenring KM. (2021). Managing Lake Urmia, Iran for diverse restoration objectives: Moving beyond a uniform target lake level. Journal of Hydrology: Regional Studies. 1(35): 100812. DOI: 10.1016/j.ejrh.2021.100812.

Spiridon C, Teodorof L, Burada A, Despina C, Seceleanu-Odor D, Tudor IM, Ibram O, Georgescu LP, Ţopa CM, Negrea BM, Tudor M (2018). Seasonal variations of nutrients concentration in aquatic ecosystems from Danube Delta Biosphere Reserve. AACL Bioflux. 11(6): 1882-1891.http://www.bioflux.com.ro/aacl.

Steinrücken P, Jackson S, Müller O, Puntervoll P, Kleinegris DM. (2023). A closer look into the microbiome of microalgal cultures. Frontiers in Microbiology. 14: 1108018. DOI: 10.3389/fmicb.2023.1108018.

Suh SS, Park M, Hwang J, Kil EJ, Jung SW, Lee S, Lee TK. (2015). Seasonal dynamics of marine microbial community in the South Sea of Korea. Plos One. 10(6): e0131633. DOI:10.1371/journal.pone.0131633.

Tredici MR. (2010). Photobiology of microalgae mass cultures: understanding the tools for the next green revolution. Biofuels. 1(1): 143-162. DOI: 10.4155/bfs.09.10

Udayan A, Pandey AK, Sirohi R, Sreekumar N, Sang BI, Sim SJ, Kim SH, Pandey A. (2023). Production of microalgae with high lipid content and their potential as sources of nutraceuticals. Phytochemistry Reviews. 22 (4): 833-860. DOI: 10.1007/s11101-021-09784-y.

Vanitha A, Narayan MS, Murthy KNC, Ravishankar GA. (2007). Comparative study of lipid composition of two halotolerant algea, *Dunaliella bardawil* and *Dunaliella salina*. International journal of food sciences and nutrition. 58 (5): 373-382. DOI: 10.1080/09637480701252252.

Varadharajan D, Soundarapandian P. (2014). Effect of physico-chemical parameters on species biodiversity with special reference to the phytoplankton from Muthupettai, South East Coast of India. Journal of Earth Science and Climatic Change. 5 (5): 1. DOI: 10.417/2157-7617.1000200.

Vinuganesh A, Kumar A, Korany SM, Alsherif EA, Selim S, Prakash S, Beemster GT, AbdElgawad H. (2022). Seasonal Changes in the Biochemical Constituents of Green Seaweed Chaetomorpha antennina from Covelong, India. Biomolecules. 12(10): 1475. DOI: 10.3390/biom12101475.

Yang F, Xiang W, Sun X, Wu H, Li T, Long L. (2014). A novel lipid extraction method from wet microalga Picochlorum sp. at room temperature. Marine Drugs. 12 (3): 1258-1270. DOI: 10.3390/md12031258.

Yang L, Chen J, Qin S, Zeng M, Jiang Y, Hu L, Lei A, Wang J. (2018). Growth and lipid accumulation by different nutrients in the microalga *Chlamydomonas reinhardtii*. Biotechnology for Biofuels. 11(1): 1-12. DOI: 10.1186/s13068-018-1041-z.

Zarandi-Miandoab L, Hejazi MA, Bagherieh-

Najjar MB, Chaparzadeh N. (2015). Light intensity effects on some molecular and biochemical characteristics of *Dunaliella salina*. Physiology. 5 (2):1311-1321. DOI: 10.30495/IJPP.2015.539659.