



## **Different salinity effects on the mass cultivation of *Spirulina* (*Arthrospira platensis*) under sheltered outdoor conditions in Oman and Malaysia**

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### **ABSTRACT**

Mother earth is facing multitude of problems such as desertification, diminishing arable land and malnutrition. One way to overcome these problems is through the cultivation of *Spirulina*, *Arthrospira platensis*. *Spirulina* was mass cultivated in land-based tanks in Oman and Malaysia. The objective of this study is to determine the different effects of salinity and comparative climate patterns to the mass production of *A. platensis* under sheltered outdoor conditions in both Oman and Malaysia. With extremely contrasting environments, *A. platensis* has unique ability to grow in both tropical (Malaysia) and arid (Oman) outdoor conditions. Mass cultivation has been carried out at different salinity (5, 15, 25 and 35 ppt) over a period of 10 days with triplicates for each treatment in both countries. For the 10 days of cultivation in Oman, the highest average means of optical density measured at 620nm (ABS) was  $1.691 \pm 0.099$  at salinity of 5 ppt which was significantly higher ( $p < 0.05$ ) than those grown at salinity of 25 and 35 ppt. Though, highest average means of biomass ( $\text{g L}^{-1}$ ) dry weight achieved with 35ppt,  $0.848 \pm 0.039$  was not significantly different from other salinity concentrations. While in Malaysia, the highest optical density, 620nm (ABS) was recorded from *Spirulina* culture treatment with 5 ppt,  $0.974 \pm 0.052$  which was not significantly different from other salinity treatments and the dry weight at  $0.575 \pm 0.032 \text{ g L}^{-1}$  was significantly higher than 25 and 35 ppt. Although in this study salinity has shown variability in term of dry weight and productivity, overall productivity showed promising potential for further development of commercial *Spirulina* farms using seawater medium.

**Keywords:** *Spirulina*, *Arthrospira platensis*, salinity, outdoor, climate pattern

### **INTRODUCTION**

*Spirulina* is a spiral microalga in size from 0.2 to 0.3mm. It has a unique ability of growing naturally in some alkaline lakes, in both tropical and subtropical parts of the world. The first attempt of mass production was carried out by Sosa Texcoco Co. in Mexico, supported by the technology of Institute Francais du Petrole (IFP) (Durand-Chastel, 1980; Ciferi, 1983; Ciferi & Tiboni, 1985). *Spirulina* can be cultivated and grown in both freshwater and seawater medium (Mary Leema et al., 2010). The commercial production of *Spirulina* used growth medium with sodium bicarbonate as a carbon source (Kebede, 1997) which counts for at least 60% of all nutrient costs. Sodium bicarbonate is added to replenish the amount of  $\text{CO}_2$  depleted from the medium due to the algal growth. Adaptation to salinity and temperature are vital to the cultivation of *Spirulina*. Natural seawater has a highly variable chemical composition (Gagneux- Moreaux et al., 2007), containing more than 50 known elements and a large number of organic compounds. Economic viability of *Spirulina* depends on the quantities of biomass produced. Large volumes of media cultures are required in order to operate and maintain commercial farm scale which is done usually using outdoor pond systems (Richmond, 1999; Tredici, 2004). Since seawater contains numerous minerals (Moisander et al., 2002), it can often cover some of the fertilizer cost as well as being a source of water. Especially in countries with arid environment that have been facing foremost issues with freshwater shortage for daily usage of residential, industrial and agriculture areas (Naim, 2003). Though, it is encouraged and proven cost effective to utilize seawater in agriculture sectors particularly for microalgae cultivations (Mary Leema et al., 2010). Even though Malaysia has a tropical weather climate, fresh water supply is still as critical issue as diminishing lands for agriculture. Alternatively, utilizing seawater rather than freshwater for commercial *Spirulina* production would be practical and cost effective.

For the moment, major disturbing crisis in Oman are soil salinization and lands degradation. Excessive ground water pumping and improper farming practices have gradually increases salinity in soil. The fact that these happened, soil salinization occurrences were accelerated even more by scanty rainfall, extreme temperature, evaporation, sea water intrusion and evapotranspiration (Robert & James, 1992; Hussain et al., 2006). Researches are still in progress to determine the best methods to deal with the soil salinization occurrence. Different approaches are studied to investigate best way encountering and solving issues on agriculture industries. Scientists in Pakistan have been conducting studies and have shortlisted some plants that have

natural inherent ability to adapt in extreme environments and their salt tolerance level (Malik, 2002). However, only a small number of food producing plants could withstand and tolerate high salinity level in soil. Land deteriorations, vegetation loss due to desertification occurrences and soil degradation have hugely reduced land usage for agricultural sectors. Therefore, few countries have started to shift more towards commercial urban farming and specifically into microalgae cultivation.

Salinity issues can either provide a better opportunity or be a risk for some countries. From most microalgae, *Spirulina* has shown good tolerability through wide range of salinity and one of the ways to maintain good quality of *Spirulina* culture is by regulating salinity concentration. *A. platensis* (*Spirulina*) was confirmed having high endurance to different salinity level but salt has also been documented to be main growth inhibiting substance and is responsible for inhibiting the electron transport activities of PSI and PSII (Boyer, 1982; Zeng & Vonshak, 1998; Pitman and Lauchli, 2002; Zhang et al., 2010). Other freshwater invasive microalgal species are incapable to grow under saline conditions. It is stated that by manipulating environmental parameters like salinity, the growth of competitors can be limited while improving the specific growth rate of the domain culture species (Roessler, 1990; Rocha et al., 2003; Bartley et al., 2013). However in outdoor condition, temperature control of the culture remains another challenge affecting the growth of *Spirulina*.

More studies on the effect of various stress factors such as salinity and temperature in the growth of *Spirulina* by Vonshak et al. (1996), Rafiqul et al. (2003), Koru and Cirik (2003). Salinity has been suggested as a controlling factor for blooms of cyanobacteria in estuaries and is considered as one of the major constraints on species diversity and productivity of natural population of both fresh and marine algae. In a view of environmental fluctuations in which natural population of *Spirulina* occur and the commercial importance of the species, there is a need to study its growth response to variations in salinity, climate patterns and ionic components. Direct use of natural seawater without addition of nutrients, trace elements and vitamins for algal cultures is seldom recommended (Harrison & Berges, 2005, Gagneux-Moreaux et al., 2007). Macronutrients such as nitrogen and phosphorus, or trace metals, may be limiting in seawater (Harrison & Berges, 2005). This is rectified by adding specific nutrients like carbon, nitrogen and iron. Successful cultivation requires a good understanding of the physiology and ecology of the specific species of interest so that the setup meets the specific requirements of the target species (Borowitzka, 2005).

The initial costs to start off commercial *A. platensis* farm are hugely expensive for some third world countries. Therefore, more studies were developed in order to break through current market prices close to affordable worth. Interests on cost reduction were peculiarly more towards modifying nutrient medium using alternative and cheap sources for microalgae growth (Madkour et al., 2012). High humidity levels could lead to mass contamination for open cultivation systems thus it might affect the purity and cleanliness of the culture (Wang et al., 2012). Under sheltered condition, *A.platensis* grown in the land based tanks are expected to be secluded from any exterior element interference. Maintaining the growth and purity of *Spirulina* culture would be the most vital from the whole cultivation systems (Naqqiuddin et al., 2014). In Oman, sheltering provides cover from dust, insects or any other contaminant agents and for heat or light intense reduction. While in Malaysia, sheltered tanks were enclosed in order to prevent *Spirulina* culture diluted with rainwater and to maintain cultivation in hygiene condition from any other organisms.

This paper presents our investigation and findings on the productivity of *A. platensis* grown under sheltered outdoor condition in different salinity level and geographical location of both Oman which has arid conditions and in Malaysia which has tropical climate conditions. This study is exceptionally important to indirectly offer some ideas of *Spirulina* cultivation completed in Oman and Malaysia based on the yield and specific growth rate. Either with intense light and low humid in Oman outdoor condition or with average light and high humid condition in Malaysia, this paper has drawn out generally which location would be preferable for *Spirulina* commercial cultivation. Noticeably, there are inadequate studies conducted in comparing cost reduced system in growing microalgae. Majority studies on monoculture cultivation system were conducted in laboratory conditions rather than to be experimented directly under outdoor conditions.

## MATERIALS AND METHODS

### Preparation of Reference Growth Medium

Kosaric medium was prepared (Tompkins et al., 1995) with minor modification using commercial fertilizer (g L<sup>-1</sup>): 5.0 NaHCO<sub>3</sub>, 0.25 NaCl, 0.1 CaCl<sub>2</sub>, 0.2 MgSO<sub>4</sub>.7H<sub>2</sub>O, 0.221 Urea, 0.07 H<sub>3</sub>PO<sub>4</sub>, 0.242 KOH, 0.02 FeSO<sub>4</sub>.7H<sub>2</sub>O and 0.5 mL/L of trace metals solution composed of following elements (g L<sup>-1</sup>): 2.86 H<sub>3</sub>BO<sub>3</sub>, 1.81 MnCl<sub>2</sub>.4H<sub>2</sub>O, 0.22 ZnSO<sub>4</sub>.7H<sub>2</sub>O, 0.08 CuSO<sub>4</sub>.5H<sub>2</sub>O, 0.01 MoO<sub>3</sub>, and 0.01 COCl<sub>2</sub>.6H<sub>2</sub>O (Sukumaran et al., 2014). Urea was added to culture medium by using fed-batch methods (pulse fertilization) (Danesi et al., 2002).

## Treatments

Each salinity treatments (5, 15, 25 and 35 ppt) were prepared in triplicate. The proportion of common salts were dissolved slowly in prepared growth medium, adjusted until salinity level required achieved using salinometer (YSI Model 33, S-C-T Meter). Once the required salinity growth medium was prepared, 5% of the total culture volume was inoculated with *A. platensis*. This procedure is necessary to adapt *A. platensis* growing in particular salinity.

## Culture parameter and Growth Measurement

Light intensity in ( $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ): (Licor Li-250), culture temperature ( $^{\circ}\text{C}$ ) (Fisher Scientific), salinity ‰ (ppt), conductivity ( $\mu\text{S}/\text{m}$ ): (YSI Model 33, S-C-T Meter) in Malaysia; (ATAGO MASTER-S/Mill  $\alpha$ ) in Oman and pH of the culture medium: (Mettler Toledo Model 330) in Malaysia; (twinpH) in Oman were recorded daily. Growth performance parameter determination based on the following methods: Optical density (absorbance: at spectral range of 620nm) (Naqqiuddin et al., 2014): (Hitachi U-1900) in Malaysia; (JENWAY 6300) in Oman. Dry weight following method in Sorokin, (1973), was analyzed daily. Data analysis for productivity and specific growth rate were analyzed using SPSS software (Version 21) through One-way ANOVA, Tukey HSD and bivariate correlation for comparison between growth parameters readings.

## Productivity

Productivity was calculated using the following equation according to Danesi et al. (2011):

$$P_x = (X_m - X_i)(T_c)^{-1}$$

where:  $P_x$  = productivity ( $\text{g L}^{-1} \text{ day}^{-1}$ )

$X_i$  = initial biomass concentration ( $\text{g L}^{-1}$ )

$X_m$  = maximum biomass concentration ( $\text{g L}^{-1}$ )

$T_c$  = cultivation time related to the maximum biomass concentration (days)

## Specific growth rate

Specific growth rate ( $\mu$ ) was calculated by the following formula according to Markou et al (2012):

$$\mu = (\ln X_m - \ln X_i)(T_c)^{-1}$$

where:  $X_i$  = initial biomass concentration ( $\text{g L}^{-1}$ )

$X_m$  = maximum biomass concentration ( $\text{g L}^{-1}$ )

$T_c$  = cultivation time related to the maximum biomass concentration (days)

Specific culture protocol in Oman and Malaysia were as follow:

The experiment in Oman was conducted in the Agricultural Experimental Station of Sultan Qaboos University, Muscat, Oman while in Malaysia; experiment was conducted at the sheltered courtyard, Biology Department, Faculty of Science, Universiti Putra Malaysia. Strain of *Arthrospira platensis* was brought from the algae culture collection at Plant Physiology Lab, Biology Department, Faculty of Science, Universiti Putra Malaysia. Pure *A. platensis* culture was maintained in closed container and placed in a temperature controlled room at  $25^{\circ}\text{C}$ . Procedures for maintaining the culture purity were carried out following

Vonshak et al. (1983). Stock cultures of the *A. platensis* were adapted to 4 different salinity (5, 15, 25 and 35 ppt) and were maintained as separate stock cultures. Cultivation was carried out under sheltered polyethylene sheet (60% -70 % of transparency) in outdoor condition and the light intensity range was as shown in **Fig. 7**. Land-based tanks were prepared in similar design in Malaysia and Oman (with capacity of 50L). Ten percent stock culture (5L) were used and inoculated into 45 L of mixture of prepared media with filtered tap water. The specimen culture was also been aerated with diaphragm air pump and placed in outdoor conditions for direct light sources. The land-based tanks (with capacity of 50 L: 50 cm x 50 cm x 30 cm) were arranged and located at the sheltered courtyards of Biology Department. Land based tanks were covered with 50 cm x 50 cm hard plastic sheet and aerated through air tubes and air stone. After 3 cycles of acclimatization phase, prepared *A. platensis* attained sufficient adaptation for stable growth during real experiment.

## RESULTS AND DISCUSSION

### Growth of *A. platensis* in different salinity

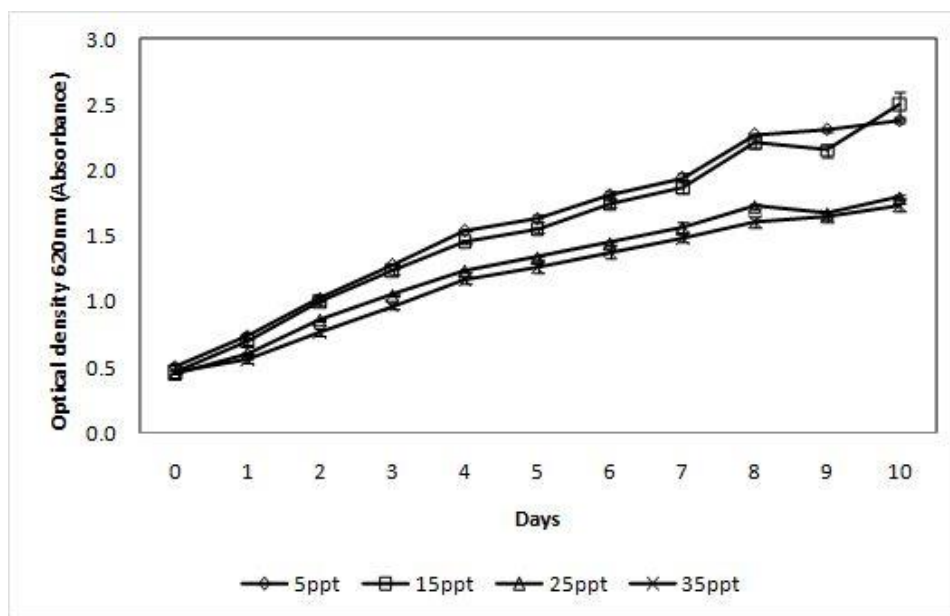
#### OPTICAL DENSITY

##### (i) In Oman

The effect of salinity on growth of *A. platensis* in terms of optical density in Oman and Malaysia were presented in **Fig. 1** and **Fig. 2** respectively. Initial stocking density ABS (optical density) for all salinity tested were  $\pm 0.5$ . All salinity tested showed linear increase in optical density up to 10 days. Maximum optical density (values are presented as Mean  $\pm$  SE) on the Day 10 were  $2.498 \pm 0.096$  for 15 ppt,  $2.376 \pm 0.014$  for 5 ppt,  $1.791 \pm 0.019$  for 25 ppt and  $1.735 \pm 0.052$  for 35 ppt. However, there are two distinct growth pattern; 5 and 15 ppt groups which had significantly higher optical density ( $p < 0.05$ ) compared to groups treated with 25 and 35 ppt and no significant difference ( $p > 0.05$ ) within group (5 and 15 ppt) and (25 and 35 ppt). The highest average mean  $\pm$  SE of the optical density was 5 ppt,  $1.691 \pm 0.099$  was significantly higher than 25 and 35 ppt, ( $p < 0.05$ ).

##### (ii) In Malaysia

The initial mean  $\pm$  SE density recorded on the first day for salinity treatments of 5, 15, 25 and 35 ppt were at  $0.323 \pm 0.019$ ,  $0.370 \pm 0.031$ ,  $0.402 \pm 0.041$  and  $0.372 \pm 0.003$  (ABS) respectively (**Fig. 2**). At Day 10, 5 ppt gave the highest optical density mean reading  $\pm$  SE (620 nm) was achieved at  $1.443 \pm 0.032$  and the lowest mean  $\pm$  SE of optical density observed in 25 ppt at  $1.016 \pm 0.005$ . The mean  $\pm$  SE of the optical density was recorded highest with 5ppt,  $0.974 \pm 0.052$  was not significantly different than 15, 25 and 35 ppt, ( $p > 0.05$ ).



**Figure 1:** Optical density of *A. platensis* grown in different salinity concentrations (5, 15, 25 and 35) ppt for 10 days in Oman. Values are presented as Mean  $\pm$  SE (n = 3).

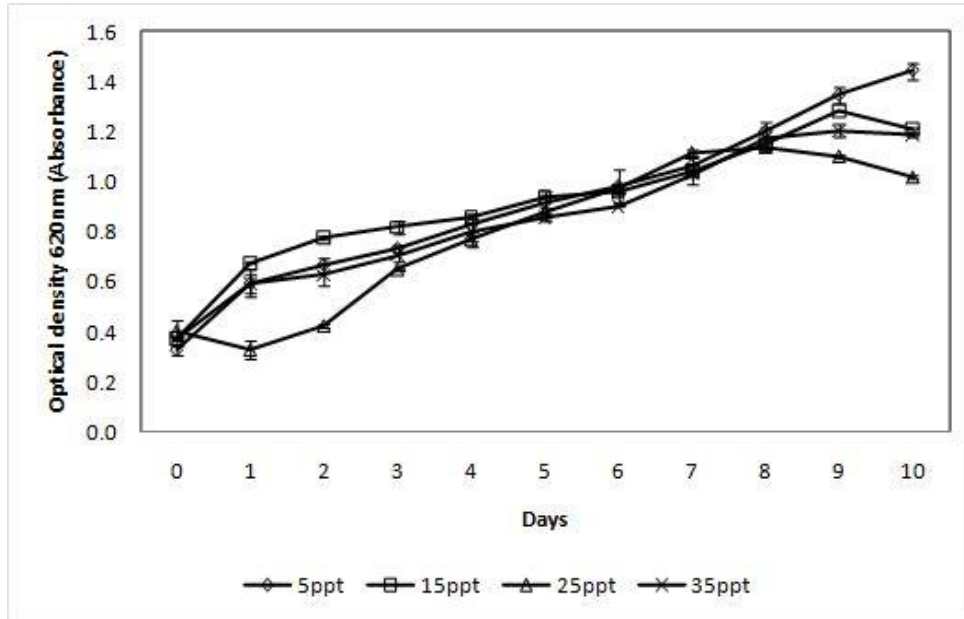


Figure 2: Optical density (ABS) for different salinity (5, 15, 25 and 35) ppt for 10 days in Malaysia.

Values are presented as Mean  $\pm$  SE (n = 3).

#### DRY WEIGHT

##### (i) In Oman

For the dry weight ( $\text{g L}^{-1}$ ), the highest mean  $\pm$  SE achieved on the Day 10 was obtained with 15 ppt salinity concentration,  $1.203 \pm 0.066$  and the lowest was obtained at 25 ppt,  $1.132 \pm 0.030$  (Fig. 3). The highest average mean  $\pm$  SE of dry weight ( $\text{g L}^{-1}$ ) was observed with 35ppt,  $0.848 \pm 0.039$  was not significantly different compared to other salinity treatments (5, 15 and 25 ppt), ( $p > 0.05$ ).

##### (ii) In Malaysia

The mean  $\pm$  SE of dry weight ( $\text{g L}^{-1}$ ) on first day was started at  $0.163 \pm 0.005$ ,  $0.144 \pm 0.014$ ,  $0.264 \pm 0.009$  and  $0.165 \pm 0.002 \text{ g L}^{-1}$  respectively for all triplicates of (5, 15, 25 and 35 ppt) (Fig. 4). On the Day 10, the highest mean  $\pm$  SE of biomass dry weight were obtained from 5 ppt with  $0.830 \pm 0.009$ . The second highest recorded on dry weight ( $\text{g L}^{-1}$ ) was from *A. platensis* cultured with 15 ppt salinity concentration,  $0.710 \pm 0.008$ . The lowest recorded dry weight ( $\text{g L}^{-1}$ ) shown by *A. platensis* culture treatment with 25 ppt,  $0.535 \pm 0.017$ . The average mean  $\pm$  SE of dry weight ( $\text{g L}^{-1}$ ) were achieved with 5ppt,  $0.575 \pm 0.032$  was significantly different and higher than 25 and 35ppt, ( $p < 0.05$ ). However, the average mean  $\pm$  SE of dry weight of 5ppt was not significantly different from 15 ppt, ( $p > 0.05$ ).

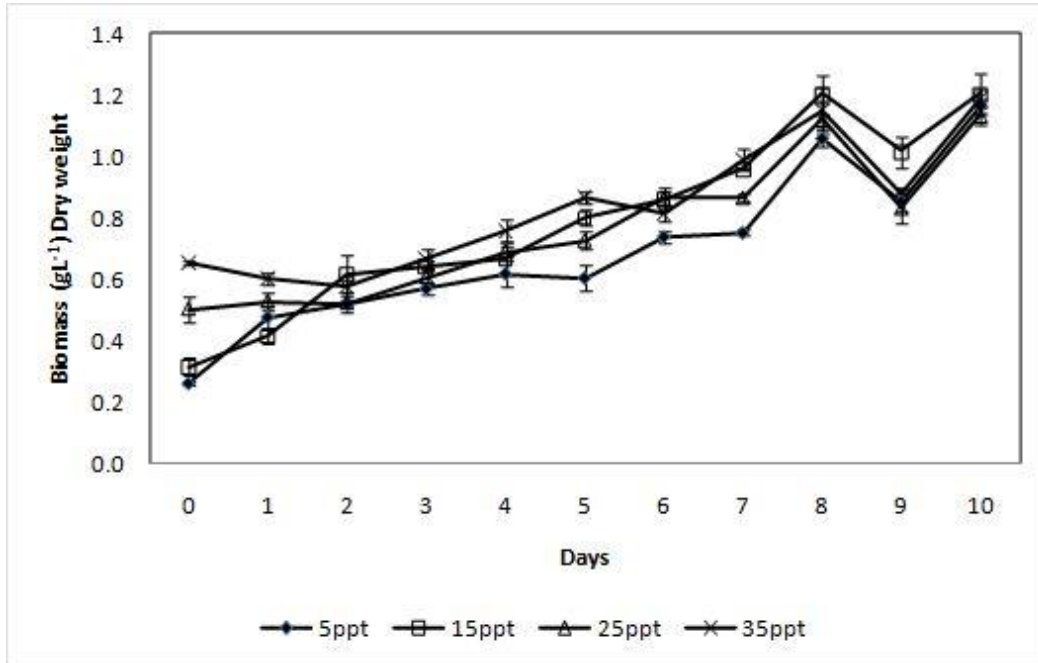


Figure 3: Dry weight ( $\text{g L}^{-1}$ ) at different salinity (5, 15, 25 and 35) ppt for 10 days in Oman. Values are presented as Mean  $\pm$  SE (n = 3).

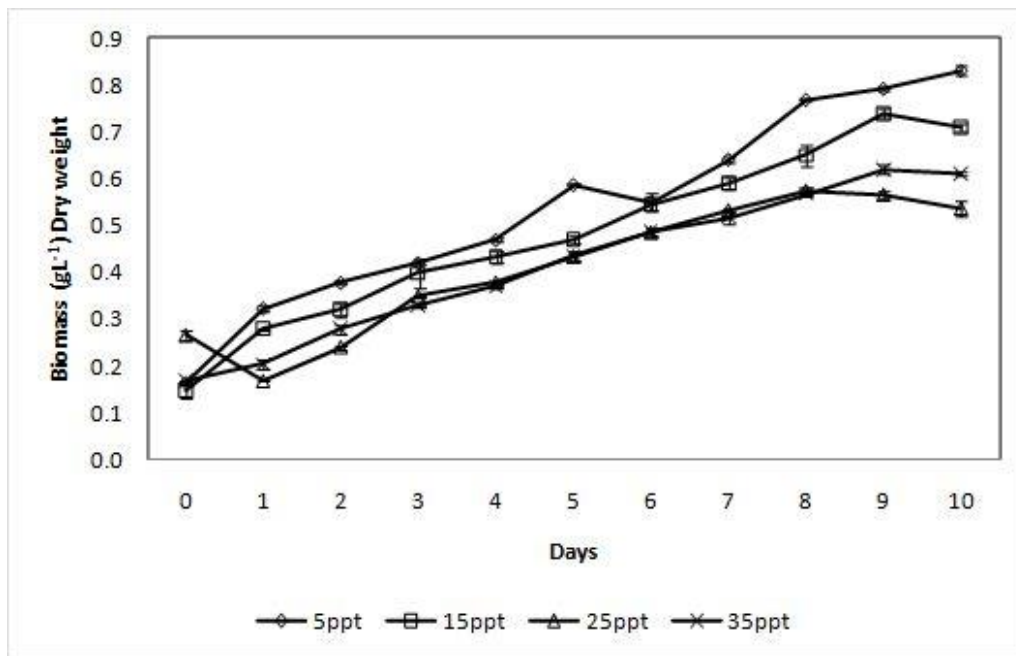


Figure 4: Dry weight ( $\text{g L}^{-1}$ ) at different salinity (5, 15, 25 and 35) ppt for 10 days in Malaysia. Values are presented as Mean  $\pm$  SE (n = 3).

Based on Table 1, growth parameters between optical density and biomass dry weight between 2 country location (Oman and Malaysia) are significantly ( $p < 0.01$ ) correlated. It can be concluded that each groups of (biomass dry weight vs optical density), (optical density in Oman and in Malaysia) and (biomass dry weight in Oman and in Malaysia) are related ( $p < 0.01$ ) respectively.

**Table 1: Results of correlation between growth parameters in Oman and in Malaysia.**

Treatments	Biomass & OD in Oman (pearson correlation, r)	Biomass & OD in Malaysia (pearson correlation, r)	OD in Oman & Malaysia (pearson correlation, r)	Biomass in Oman & Malaysia (pearson correlation, r)
5ppt	0.889*	0.963*	0.949*	0.904*
15ppt	0.948*	0.974*	0.947*	0.907*
25ppt	0.898*	0.987*	0.965*	0.848*
35ppt	0.876*	0.962*	0.950*	0.844*

\* Correlation is significant at the 0.01 level (2-tailed).

**Table 2: Results of productivity (g L<sup>-1</sup> d<sup>-1</sup>) and specific growth rate (μ d<sup>-1</sup>) of *A. platensis* grown in different salinity in Oman and Malaysia.**

Treatments	Productivity in Oman (g L <sup>-1</sup> d <sup>-1</sup> )	Specific growth rate in Oman (μ d <sup>-1</sup> )	Productivity in Malaysia (g L <sup>-1</sup> d <sup>-1</sup> )	Specific growth rate in Malaysia (μ d <sup>-1</sup> )
5ppt	0.090 ± 0.002 <sup>a</sup>	0.150 ± 0.001 <sup>a</sup>	0.067 ± 0.000 <sup>a</sup>	0.163 ± 0.002 <sup>a</sup>
15ppt	0.089 ± 0.005 <sup>a</sup>	0.135 ± 0.007 <sup>a</sup>	0.057 ± 0.001 <sup>a</sup>	0.160 ± 0.008 <sup>a</sup>
25ppt	0.063 ± 0.001 <sup>b</sup>	0.082 ± 0.009 <sup>b</sup>	0.027 ± 0.001 <sup>b</sup>	0.071 ± 0.001 <sup>b</sup>
35ppt	0.054 ± 0.003 <sup>b</sup>	0.060 ± 0.005 <sup>c</sup>	0.031 ± 0.007 <sup>b</sup>	0.131 ± 0.001 <sup>c</sup>

\* Each value is presented as Mean ± SE (n = 3). Means within each column with different letter (a-c) differs significantly ( $p < 0.05$ ).

**Table 3: Results of correlation between growth parameters (optical density & biomass vs light intensity) in Oman and in Malaysia.**

Treatments	OD vs Light in Malaysia (pearson correlation, r)	OD vs Light in Oman (pearson correlation, r)	Biomass vs Light in Malaysia (pearson correlation, r)	Biomass vs Light in Oman (pearson correlation, r)
5ppt	0.083	0.142	0.093	0.352**
15ppt	0.155	0.162	0.135	0.209*
25ppt	0.128	0.089	0.156	0.243*
35ppt	0.033	0.080	0.174	0.148

\*\* Correlation is significant at the 0.01 level (2-tailed).

\* Correlation is significant at the 0.05 level (2-tailed).

The pH values observed for all salinity concentration in Oman and Malaysia were presented in mean ± SE (n = 3) in **Fig. 5** and **Fig. 6** respectively. In Oman, average pH were initially recorded for (5, 15, 25 & 35 ppt) were 9.45 ± 0.050, 9.33 ± 0.067, 9.23 ± 0.033 and 9.10 ± 0.00. The highest recorded of pH was 15 ppt, 11.4 ± 0.00, while the lowest of pH observed on 35 ppt, 10.6 ± 0.00. In Malaysia, 15 ppt had the highest pH values, 10.42 ± 0.060, whereas the lowest pH recorded on 35 ppt, 10.20 ± 0.129. The pH of all treatments increased throughout the experiment till the final day. This indicated that pH values were not affected by different salinity treatments under sheltered condition of Oman and Malaysia.

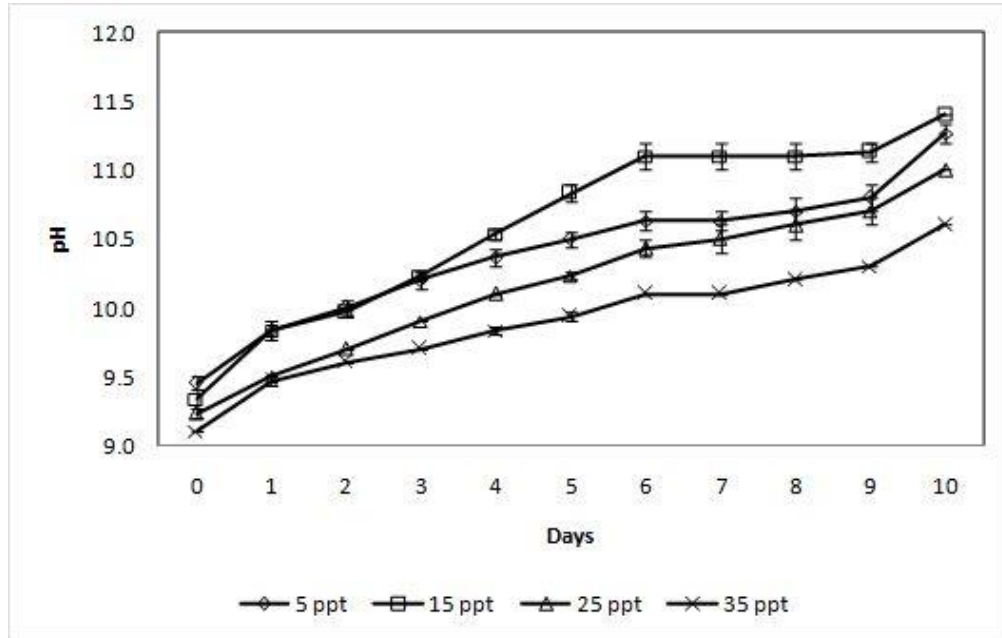


Figure 5: pH of grown *A. platensis* in different salinity concentration (5, 15, 25 & 35 ppt) in Oman. Values are presented as Mean  $\pm$  SE (n = 3).

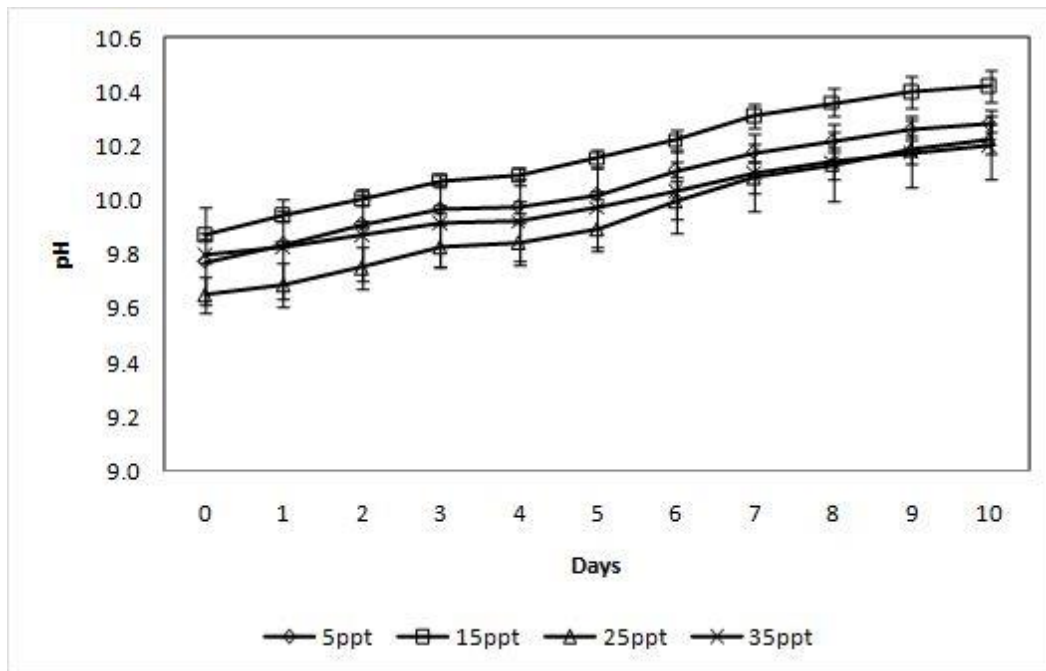


Figure 6: pH of grown *A. platensis* in different salinity concentration (5, 15, 25 & 35 ppt) in Malaysia. Values are presented as Mean  $\pm$  SE (n = 3).



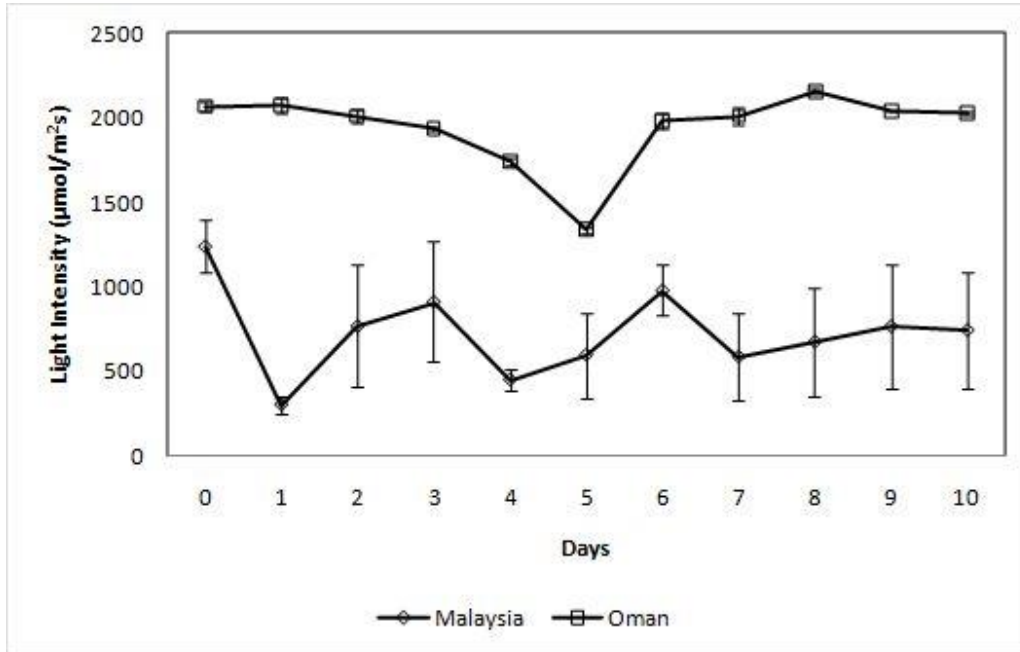


Figure 7: Average light intensity ( $\mu\text{mol}/\text{m}^2\text{s}$ ) for the 10 days of cultivation duration in Oman and in Malaysia. Values are presented as Mean  $\pm$  SE (n = 3).

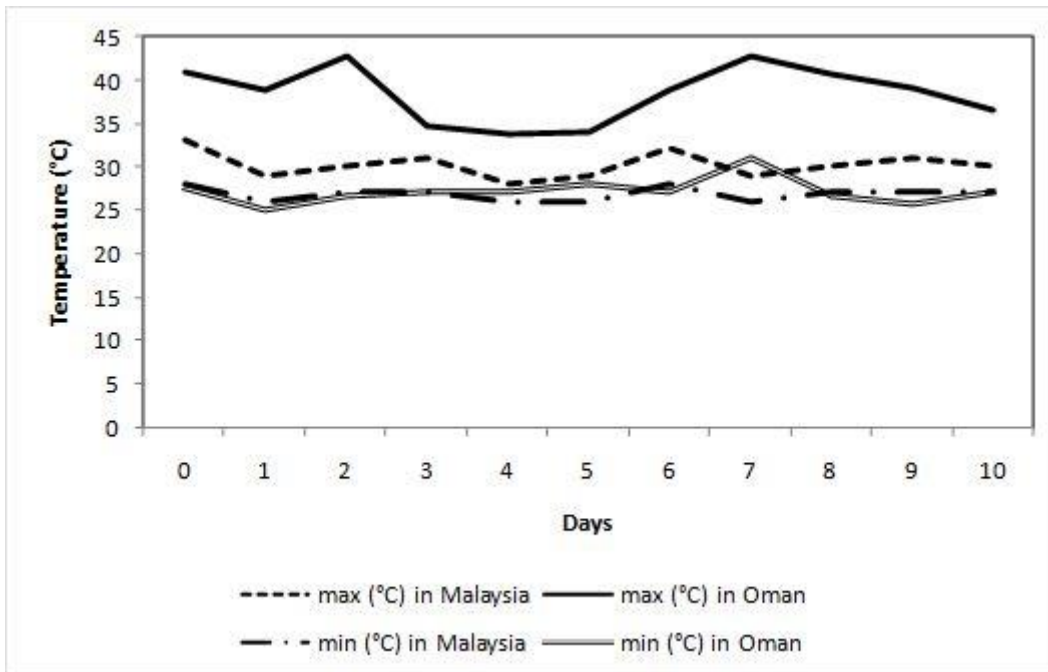


Figure 8: Minimum and maximum range of temperature (°C) in Malaysia and Oman during cultivation periods.

In this study, there has been no attempt to directly compare the difference of *Arthrospira platensis* (*Spirulina*) production in Oman and in Malaysia. What is more, this paper gives general idea on location and weather differences with salinity effects on *A. platensis* cultivation under outdoor sheltered condition in both countries. Observation concluded upon general trend in both countries taking into account dissimilarities in culture management, time difference and water properties. Malaysia and Oman not only differ geographically but also differ in term of water quality and weather pattern. Oman is an arid land, experiencing water

scarcity but with stable weather and little cloud cover. On the other hand, Malaysia receives numerous rains throughout a year, high incident of cloud cover and unpredictable weather. Although the country of Oman and Malaysia differs in terms of geographical locations and weather conditions, the growth of *A. platensis* under different salinity treatments based on optical density (ABS) and biomass dry weight ( $\text{g L}^{-1}$ ) had similar pattern. However, high incidence of cloud covers are probable factor affecting the growth productivity of *A. platensis* in Malaysia at 5 ppt,  $0.067 \pm 0.000 \text{ g L}^{-1} \text{ d}^{-1}$  (for comparison, the values are equivalents to  $73.22 \pm 0.567 \text{ g m}^{-2} \text{ d}^{-1}$ ) which is significantly lower ( $p < 0.05$ ) than in Oman,  $0.090 \pm 0.002 \text{ g L}^{-1} \text{ d}^{-1}$  (equivalents to  $90.33 \pm 1.683 \text{ g m}^{-2} \text{ d}^{-1}$ ) (**Table 2**).

Weather difference in Malaysia with mix weather patterns, high amount of rainfall and dense of cloud covers has significantly influenced growth parameters like light intensity and temperature. Cloud cover reduces light availability (photo inhibition effects) reaching *A. platensis* culture for photosynthesis process and overall affecting the productivity rate of *A. platensis* (Reboloso Fuentes et al., 1999). In this study, light intensity was significantly higher in Oman than in Malaysia, which has resulted higher productivity in terms of dry weight productivity in all salinity tested (**Table 2**).

Between days of cultivation, light intensity ( $\mu\text{molm}^{-2}\text{s}^{-1}$ ) in Oman was significantly higher ( $p < 0.05$ ) than the light intensity in Malaysia. However, light intensity between days in Oman was invariable compared to Malaysia which has been unpredictable ( $p < 0.05$ ). This explained the results of light intensity (**Fig. 7**) having high standard errors in Malaysia due to inconsistency of high light intensity and temperature reflected from dense cloud cover in Malaysia. Light intensity is closely related with temperature as infrared which is the component of sunlight are responsible for the high temperature (Vonshak et al., 1982) as occurred in Oman; high temperature was reflected by high light intensity illuminations (**Fig. 7 & 8**). Temperature in Oman was observed few degrees higher than in Malaysia. Based on **Fig. 8**, the average min and max of 10 days temperature ( $^{\circ}\text{C}$ ) reached in Malaysia was  $\sim 26.8$  and  $\sim 30.2$  respectively. Meanwhile in Oman, the average min and max temperature ( $^{\circ}\text{C}$ ) during the cultivation period was  $\sim 27.2$  and  $\sim 38.4$  respectively. The same outcome was analyzed for the temperature showed significant higher between days of cultivation in Oman. Past researches have confirmed high tolerance to light and temperature can increase microalgae productivity than in suboptimal condition (Vonshak et al., 1982; Renaud et al., 2002; Danesi et al., 2004).

Reports from high cloudiness outdoor conditions area on the yield of *A. platensis* were achieved at  $8\text{-}12 \text{ g m}^{-2}\text{d}^{-1}$  by Becker & Venkataraman in India (1984) and  $8.9\text{-}12.4 \text{ g m}^{-2}\text{d}^{-1}$  by Seshadri & Thomas (1979) in India respectively. There has been large variation of sea surface temperatures (SSTs) observed since during the late 1970s which has contributed to the increasing of moist convection and cloudiness by adding moisture and reduces the atmospheric stability at a warm state of the Indian Ocean (Rajeevan et al., 2000). As in arid or semi-arid conditions, lower growth rates might be related to the light limitation in dense cultures for optimal conditions in the summer, whereas during winter the low daytime temperature caused big limitations to the growth of *A. platensis* (Vonshak et al., 1982).

While in Peninsular of Malaysia, the experiment area has recorded rainfall amount between 30%-70% above average (MMD, 2013). The highest recorded amount of rainfall was in Kuantan (270 km to the west coast from our experiment area) with amount of 1075.2 mm. However, weather informations collected from local meteorological department are less precised due to the weather sampling stations that were located distantly. The cloud cover density over specific location varies within short time and caused extreme changes of weather through the day. Results shown signified that the productivity of *A. platensis* were influenced by the growth parameters such as light intensity and surface temperature as effects from climate pattern from two different geographical location of Oman and Malaysia. Weather pattern in Oman has arid condition with less than 0.1 mm amount of rainfall during the 10 days of the experiment. The Arab Peninsula can be categorized into arid and semi arid areas excluding the high terrains in the southwest and little range bordering the Gulf of Oman (Meigs, 1953).

Cultivated *Spirulina* under sheltered outdoor condition in Oman with the lowest average of light intensity recorded,  $1334 \pm 15.62 \mu\text{molm}^{-2}\text{s}^{-1}$  had higher yield than the highest average light intensity in Malaysia outdoor condition,  $1238.7 \pm 153.33 \mu\text{molm}^{-2}\text{s}^{-1}$ . Wang et al., (2007) carried out a series test of different light wavelengths and illumination intensities using light emitting diodes (LEDs) effects on *A. platensis* achieved highest specific growth rate of 0.40 (day<sup>-1</sup>) under the condition of 3000 ( $\mu\text{molm}^{-2}\text{s}^{-1}$ ). Hu et al., (1998) obtained highest output rate of  $16.8 \text{ g dry weight m}^{-2}\text{h}^{-1}$  under direct beam radiation, photon flux density (PFD) of 8000  $\mu\text{molm}^{-2}\text{s}^{-1}$ . Another experiment presented best cellular growth at 5klux compared to 2klux inferred that zero photo inhibition may occur with highest light intensity used on *Spirulina* cultivation (Danesi et al., 2004). According to Materassi et al., (1984), under illumination of (light/dark cycles of 12/12h)  $100 \mu \text{ Einstein.m}^{-2}.\text{s}^{-1}$  (PAR) showed constant productivity during light periods but slightly higher in seawater than control medium (mean values of 70.9 and 67.4 mg dry wt.  $\text{L}^{-1} \cdot 12\text{h}^{-1}$  respectively). However under the dark periods of respiration, the loss of biomass was significantly higher in seawater culture than in control. Based on **Table 3**, the correlations between (optical density and dry weight) to the light intensity ( $\mu\text{mol.m}^{-2}.\text{s}^{-1}$ ) were calculated for both countries. Light was not significantly correlated ( $p > 0.05$ ) to any variables except to biomass in Oman with salinity treatments of 5, 15 and 25 ppt, which is at 0.352, 0.209 and 0.243 respectively.

In an experiment of salinity stress conducted indoor, *A. platensis* cells grown were higher tolerance to lower photon flux density (PFD) compared to higher PFD suggesting that salinity-stress enhanced photoinhibition of photosynthesis (Zeng & Vonshak, 1998). Whereas in this experiment with addition of sheltering to the cultivation tanks from higher light intense in outdoor condition, there was no significant different ( $p > 0.05$ ) of dry weight biomass between different salinity treatments. It indicated that sheltering the cultivated *A. platensis* tanks played significant roles of reducing photoinhibition of photosynthesis even with salinity stress. Other growth parameters such as pH, aeration, growth medium and salinity in Oman and in Malaysia shown similar trend have not differ significantly ( $p > 0.05$ ) since identical culture protocol was adopted (Richmond & Grobbelaar, 1986; Belay, 1997).

Data from this study suggested that salinity do influence productivity of *A. platensis*. Distinct subset group of the salinity treatments are (5 and 15 ppt) and (25-35 ppt). At higher salinity, osmotic pressure of cell membranes takes place where the cell metabolic activities and responses decline immediately once exposed salts stress. For that reason, this explained the growth of *A. platensis* in 25 and 35 ppt which are significantly lower ( $p < 0.05$ ) compared to 5 and 15 ppt grown *A. platensis*. *Spirulina* (*A. platensis*) was reported did not stop growing even under extremely high salinity at 88 g L<sup>-1</sup> though the lowest salinity gave highest growth (Reed et al., 1985; Kebede, 1997; Moisaner et al., 2002). According to Ravelonandro et al., (2011), the optimal growth of *A. platensis* was obtained with salinity of 13g L<sup>-1</sup> compared to other different medium of salinities with 20, 25, 30 and 35 g L<sup>-1</sup> respectively. Seeing that salinity helps inhibiting invasive species, *A. platensis* should be grown in high saline at commercial stage especially under high humid outdoor condition regardless of contrast lower growth at higher salinity (Kebede, 1997).

Underground waters in Oman have been facing major devastating problem of increasing salinity greater than 6000 µS/cm in 2005 (Al Barwani & Helmi, 2006). In these arid areas, the shortage of the surface water makes absolute reliance upon the groundwater withdrawal from wells. Frequent extraction of groundwater sources for agriculture purposes has resulted in severe salinization (Stanger, 1984). Soil degradation affected from salinity, improper irrigation and desertification are reasons of increasing discarded lands. Salinity functions to control invasive species during monoculture cultivation especially for *A. platensis* which has unique ability of high salinity tolerance.

More studies of increasing growth of *A. platensis* from decreasing salinity have been reported (Rosales et al., 2005; Vonshak et al., 1988). The effects of salinity on productivity of *A. platensis* from previous studies have been seen to be conflicting. However, some studies declared positive remarks on salinity stress. Vonshak (1997) has stated that different strains of the microalgae species would give different indication on the changes in growth rate and doubling time after adaptation as which of the strains have higher resistance towards salt stress. Growth of *A. platensis* by Mary Leema et al., (2010) showed highest specific growth rate calculated with seawater media, SW2 at 0.26 µ<sub>max</sub> (d<sup>-1</sup>) compared to the control growth using Zarrouk media. Though, the biomass dry weight (g L<sup>-1</sup>) between SW2, 2.99 ± 0.06 and Zarrouk media, 3.11 ± 0.09 were found not significantly different ( $p > 0.05$ ) and were slightly significantly different ( $p < 0.05$ ) to the rest of seawater media. Salinity stress on *A. platensis* also has led to enhancement or induction of biologically active compounds (Shalaby et al., 2010).

Despite the unique tolerance of *A. platensis* to survive higher salinity, the acclimatization phase is still crucial in order to accumulate better results. Without inadequate acclimatization period, the culture of *A. platensis* may either collapse earlier or show less productivity all through the experiment period (Vonshak et al., 1996; Belay, 1997; Vonshak, 1997). Repeated acclimatization over a period of several months or cycles is necessary to have stable and good growth (Mary Leema et al., 2010). From this study, despite different geographical location, contrasting weather, exposure to different salinity and grown under sheltered outdoor conditions, the production of *A. platensis* showed similar trend and has performed better than other crops (Tripler et al., 2007). Hence *A. platensis* offer better alternative where other crops failed to perform better under this circumstances. With adequate acclimatization phase, *A. platensis* strains that were originated from the same sources have successfully grown using the same pattern of growing even under different climate of Oman and Malaysia. This finding offers opportunity for both Oman and Malaysia; in Oman by having sparse water usage and abundance of unused lands; the saline water can be used for production of *A. platensis*; in Malaysia, the extent of freshwater usage is more for daily utilization, thus sheltered tanks using seawater growth medium can be used for mass cultivation of *A. platensis* economically.

## CONCLUSION

The difference in weather pattern may not give terrible consequences to the cultivation of *A. platensis*. Salinity has thus far not shown any implications on the cultivation of *Spirulina* (*A. platensis*) under sheltered outdoor condition. For better productivity under sheltered outdoor condition, it is highly recommended to grow and precede *A. platensis* farms in Oman with greater constant amount of light though out a day replacing discarded unusable agricultural lands. Whereas in Malaysia, lands and freshwater are gradually limited due to expanding population, instead, *Spirulina* farms could be deployed in coastal areas.

## ACKNOWLEDGEMENTS

We acknowledged the support of Sultan Qaboos University in providing the facilities and Universiti Putra Malaysia assistances for the experimental work.

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