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EFFECT OF NaCl CONCENTRATION ON THE PRODUCTION OF *Arthrospira maxima* CULTIVATED IN THE HUMID TROPICS

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ABSTRACT

The production of microbial biomass can be affected by the composition of the culture medium, including the content of salts and there is little information in terms of the NaCl concentration. Therefore, in this study, the effect of the addition of different NaCl concentrations (0.0, 2.0, 2.5, and 3.0 g L^{-1}) in a Jourdan medium was evaluated on cell growth and physical and chemical properties of *Arthrospira maxima* cultivated in the region of Cordoba, Veracruz, Mexico. The experiment was established under a completely randomized design with a factorial arrangement (AxB). During the production stage, cell growth and morphology of filaments were evaluated, and after harvesting, the physical and chemical analysis (moisture, crude fiber, total protein and lipid content, mineral composition, photosynthetic compounds, antioxidant activity, and structural composition through FTIR-Spectroscopy analysis) of *A. maxima* was performed. Results obtained show that the addition of NaCl did not affect cell development, the morphology of the filaments, or the content of photosynthetic compounds. The maximum rate of biomass production in all treatments was at 6 d, without significant differences (p > 0.05) among treatments. In all treatments, *A. maxima* showed adequate concentrations of protein and minerals, as well as antioxidant activity. FTIR spectra showed absorption bands associated with proteins, carbohydrates, lipids, and nucleic acids. A decrease in the amide I and amide II bands of the proteins was observed with the addition of NaCl. *A. maxima* cultivated in these tropical climatic conditions had excellent properties to be used as a functional ingredient in the development of innovative and biotechnological food products for human consumption.

Keywords: antioxidant, *Arthrospira maxima*, cyanobacteria, NaCl, production, protein.

INTRODUCTION

Spirulina is the name by which a group of species of the *Arthrospira* genus is known, consisting of photosynthetic cyanobacteria with spiral-shaped filaments (Wan *et al.*,

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2016). This group of cyanobacteria contributes around 55 - 70 % of the protein in dry weight; in addition to containing other nutrients such as carbohydrates (15 - 25 %), lipids (5 - 6 %), vitamins (pro-vitamin A, ascorbic acid, tocopherol, and B-complex vitamins), nucleic acids (6 - 13 %), pigments (phycocyanin, chlorophyll, and carotenoids), and minerals (calcium, magnesium, potassium, iron, phosphorus, manganese, copper, sodium, and selenium) (Wan *et al*., 2016). Due to their properties and characteristics, different production areas have focused on making use of these cyanobacteria in order to develop new innovative products for social welfare (Mehar *et al*., 2019).

The growth of these microorganisms is influenced by factors such as the nutrient solution, the amount of initial strain, pH, temperature, and sunlight (Ogbonda *et al*., 2007). Naturally, the species of the *Arthrospira* genus grow in highly alkaline waters (< 30 g L^{-1}) with a pH of around 8.5 – 11.0, the higher the pH and the conductivity of the water, the greater the survival of these organisms (Wan *et al*., 2016). For their cultivation, the nutrient solutions must contain an adequate level of salts to reach optimal values of pH and electrical conductivity (Mutawie, 2015). In the current market, there are different nutrient media formulated according to the culture growth conditions (Soni *et al*., 2017). However, an imbalance in the optimal concentration of mineral salts can negatively affect production, nitrates and chlorides have the greatest impact on the induction of osmotic stress (Vonshak *et al*., 1983).

Due to different mechanisms developed through evolution, cyanobacteria of the *Arthrospira* genus can adapt to stress, through morphological, physiological, and biochemical adjustments that cause saturation of organic solutes and metabolites (Sujatha and Nagarajan, 2014). All these osmotic adjustments cause the subcellular structures to be protected and some antioxidant enzymes such as superoxide dismutase (SOD), polyphenol oxidase (PPO), peroxidase (POD), and catalase (CAT) to reduce the oxidative damage caused by those free radicals that originated by high salinity levels (Kebeish *et al*., 2014).

Within the *Arthrospira* genus, about 40 species are described, one of which is *A. maxima*, which has been cultivated for hundreds of years in the basin of Lake Texcoco, Mexico, in temperate climate conditions, but few studies have been developed to evaluate performance in tropical climates. The objective of this study is to evaluate the effect of adding different NaCl concentrations on the physical and chemical properties of *A. maxima* cultivated in the tropical region of Córdoba, Veracruz, Mexico. The hypothesis to be tested is that this species can be cultivated in the tropical climate conditions of Cordoba, Veracruz, and that the salinity induced by the addition of NaCl would modify metabolism and the concentration of biomolecules and bioactive compounds.

MATERIALS AND METHODS

Description of the photobioreactor

To evaluate the growth and biomass production of *A. maxima* under humid tropical climate conditions, a low-cost photobioreactor of our design was implemented. This photobioreactor is made up of: 1) a translucent plastic container, 25.5 cm high x 13.3 cm long x 11.5 cm wide (with a capacity of 3 L), which allows sunlight to fall on the cells and thus facilitate photosynthesis; and 2) an aeration system, using an air pump (Hagen, Elite 802; China) with a capacity of 75 L h^{-1} for the oxygenation of the culture medium.

Growing conditions

The strain of *A. maxima* used in the experiment was donated by the Faculty of Chemical Engineering of the Universidad Iberoamericana de Puebla, and the Universidad Autónoma Metropolitana Campus Iztapalapa, Mexico. The cultivation of *A. maxima* was established outdoors in a shade mesh greenhouse, with average temperature and solar radiation during the year of 30 °C and 759 w m^{-2}, located in the experimental field of the Colegio de Postgraduados Campus Cordoba, Veracruz, Mexico, located at 18° 51′ 24″ N, 96° 51′ 47″ W, at 645 m.

The cells were inoculated in a Jourdan alkaline medium at a concentration of 2.16×10^3 cells mL^{-1} (with a previous cell count using the Sedgewick Rafter chamber) under the following composition (per liter of purified water): 2 g KNO_3, 8 g $NaHCO_3$, 0.2 g $MgSO_4$, 0.1 g NaH_2PO_4, and 0.1 g $FeSO_4$. All the components were mixed in dechlorinated water. To evaluate the effect of NaCl, culture batches in triplicate were established at different salt concentrations: 2.0 (T2), 2.5 (T3), and 3 (T4) g L^{-1} NaCl, and a control culture (T1) without the addition of NaCl. Each 3 L experimental unit was inoculated with a 2 L stock solution of *A. maxima* cells, previously adapted.

Biomass production

Cell growth and morphology of *A. maxima* were evaluated according to the method established by Huarachi-Olivera *et al*. (2015), using an optical microscope (Carl Zeiss, Primo Star Fixed-Köhler HAL/LED; White Plains, NY, USA) with a 10X objective lens. A sample of each treatment was extracted every 72 h for 12 d. *A. maxima* cells were preserved with Lugol's solution for 24 h, following the method by Rosińska *et al*. (2017), and then the cell count was done with the use of the Sedgewick Rafter chamber. Cell density (D) was calculated according to the following equation.

$$D\ (\text{cel mL}^{-1}) = \frac{\text{number of counted cells}}{\text{number of counted field}} \times \frac{1000\ \text{fields}}{1\ \text{mL}} \qquad (1)$$

Obtaining biomass

Biomass collection was carried out at 12 d, using the filtering method (40-micron fabric, 200 threads cm^{-3}) of 1/3 of the volume of the culture medium. The biomass was rinsed with distilled water to remove the excess of nutrient salts. The fabric was dehydrated in a hot air dryer (Excalibur, 2900ECB; Sacramento, CA, USA) at 45 °C for 4 h to preserve the most labile components, according to the method described by Desmorieux *et al*. (2010). The resulting material was ground in a food processor (Nutribullet, NB-101B;

Hong Kong, China). The dried samples were packed in hermetically sealed bags and stored at room temperature until analysis.

Physical and chemical analysis

The moisture and crude fibre content of the *A. maxima* powder were determined based respectively on the Official Mexican Standard NOM-F-083-1986 (https://studylib.es/doc/5465578/nmx-f-083- 1986-alimentos-determinaci%C3%B3n-de-humedad-en) and the Mexican Standard NMX-F-090-S-1978 (http://www.dof.gob.mx/nota_detalle.php?codigo=4799842&date=03/27/1979).

The protein content was determined based on the provisions of the Official Mexican Standard NOM-F-068-S-1980 Kjeldahl method (http://dof.gob.mx/nota_detalle.php?codigo=4858024&fecha=04/08/1980). The percentage of protein was obtained by multiplying the percentage of nitrogen by the factor 6.25. The total lipid content was evaluated according to the NMX-F-089-S-1978 standard (http://www.dof.gob.mx/nota_detalle.php?codigo=678206&fecha=21/05/2004) and the Soxhlet technique model EXTRACTION-06C, using petroleum ether for extraction.

For the determination of the mineral concentration of *A. maxima*, the obtained powder was dried at 72 °C for 48 h in a forced-air oven to remove moisture from the sample (Riossa HCF-125; Guadalajara, Mexico), and ground to a particle size of 2 mm. The total nitrogen (N) content was determined by the Kjeldahl method (Bremner, 1965). The sample was placed in a digester, with a solution of $C_7H_6O_3$ in H_2SO_4 at a concentration of 3.3 %. To determine the concentrations of P, K, Ca, Mg, Fe, Cu, Zn, Mn, and B, acid digestion of the dry and ground sample was done with HNO_3:$HClO_4$ (2:1, v/v), according to the method described by Alcántar and Sandoval (1999), and an inductively coupled plasma optical emission spectrophotometer (ICP-OES 725-ES; Agilent; Santa Clara, CA, USA) was used for determination.

Determination of photosynthetic compounds

Chlorophyll-*a* was evaluated according to the method described by Harborne (1973) with some modifications. A 0.25 g sample of powdered *A. maxima* was homogenized with 10 mL of 80 % acetone, vortexed for 1 min, and incubated at 4 °C for 24 h in the dark. Of the total volume, 5 mL were taken and centrifuged at 9391 x*g* for 10 min. Then, 0.5 mL of the supernatant was taken and made up to 10 mL with distilled water to be measured at wavelengths of 663 nm and 645 nm with a UV-Visible spectrophotometer (Thermo Scientific, GENESYS 10S; Madison, WI, USA). Chlorophyll-*a* concentration was calculated with equation 2.

$$\text{Chlorophyll } a \text{ (mg g}^{-1}\text{)} = \frac{12.70\ (\text{A663}) - 2.59\ (\text{A645})}{\text{Dry weight (g)}} \quad (2)$$

Phycocyanin (PC) was determined following the methodology established by Bryant *et al*. (1979) with some modifications. A 0.25 g sample of powdered *A. maxima* was homogenized with 10 mL of potassium phosphate buffer (50 mM, pH 6.8). Subsequently, it was frozen at -80 °C for 48 h, then thawed to be centrifuged at 9391 x*g* for 15 min. Finally, 0.5 mL of the supernatant were taken, made up to 10 mL with distilled water, and the absorbance at 615 and 652 nm were measured in a UV-Visible spectrophotometer (Thermo Scientific, GENESYS 10S; Madison, WI, USA). The PC concentration was calculated with equation 3.

$$PC\ (mg\ g^{-1}) = \frac{(A615 - 0474\ (A652)}{5.34} \quad (3)$$

FTIR spectroscopy

The structural composition of *A. maxima* was determined with a Fourier Transform Infrared Spectrophotometer (FTIR) (Bruker, Vertex; Billerica, MA, USA) by the Attenuated Total Reflectance (ATR) method. The spectrums of each treatment were obtained in triplicate and 64 scans averaged with a resolution of 4 cm^{-1} in the spectral region of 4000-400 cm^{-1} were used. For the analysis of the spectra, Origin 6.1 (OriginLab Corporation; Northampton, MA, USA) was used.

Antioxidant Activity

The capturing activity of the aqueous extract of *A. maxima* and 2,2-diphenyl-1-picrylhydrazyl (DPPH) was determined based on the method described by Brand-Williams *et al*. (1995). The aqueous extract of each treatment of the powdered *A. maxima* was prepared according to the method described by Velázquez *et al*. (2014) with some modifications. A 0.25 g sample of powdered *A. maxima* was mixed with 10 mL distilled water, incubated for 30 min at room temperature, and homogenized with a vortex every 10 min. From the mother extract, 0.25 mL was taken and made up with 10 mL distilled water. 2 mL of the solution was taken, mixed with 2 mL of the DPPH solution (0.0039 g 100 mL^{-1} ethanol), stirred vigorously for 10 s, kept in the dark for 20 min and measured at 517 nm with a UV-Visible spectrophotometer (Thermo Scientific, GENESYS 10S; Madison, WI, USA). The antioxidant activity was calculated using equation 4:

$$I\ (\%) = \frac{1-As}{Ac} \times 100 \quad (4)$$

where *I* is the inhibition (%); As, the absorbance of the sample, and Ac, the absorbance of the blank, sample solution, and water without DPPH.

Statistical analysis

For the statistical analysis of the data, a completely randomized design with an A×B factorial arrangement was used; factor A was the NaCl concentration added to the culture medium with four levels (0, 2.0, 2.5, and 3.0 g L^{-1}) and factor B was the growth time with five levels (0, 3, 6, 9, and 12 d). The analysis was done with SAS® v.9.4. Mean comparisons were done using Tukey's test, $p \leq 0.05$. The selection of this test responded to the multiple comparisons among all treatments, looking for the test that allows a better control in the decision errors. Likewise, the comparison of means of the chemical analyses of powdered *A. maxima* was done using Student's t-test, $p \leq 0.05$. The study was based on this test since the number of observations (n < 30) best fit a *t*-distribution for statistical analysis. In both tests, the interest was the paired comparison of the means among all treatments.

RESULTS AND DISCUSSION

Biomass production

The cell growth and morphology of *A. maxima* changed under the different NaCl concentrations in the culture medium at the time of harvest (Figure 1). The cells developed in all treatments, filaments with three to six coils were observed, which coincides with the morphological characteristics of *Arthrospira* described by Huarachi-Olivera *et al*. (2015). Furthermore, the morphological features of *A. maxima* were not affected by the effects of sunlight, since no straight filaments were seen (Huarachi-Olivera *et al*., 2015).

Exponential growth was observed from the first to the third day during the development of the culture (Figure 2) in all the treatments. A stationary stage is then observed from the fourth to the sixth day; on this day, the maximum production of biomass was reached (3.916×10^4 cells mL^{-1}), which indicates that from this point on, the biomass can be harvested. Finally, a stage of cell decline, or death was observed. The mean values showed no significant differences ($p < 0.0001$) among treatments, except for T1 on day 3. In studies carried out with Zarrouk medium for *A. platensis*, a gradual growth was observed from day 5 to day 15, and a decline from this day to day 20 at concentrations of 0.73, 1.41, 2.19, 2.92, and 3.65 g L^{-1} NaCl (Mutawie, 2015). This behaviour differs from that reported in this study, since no latency or adaptation stage of the cells was observed, possibly as a consequence of the tolerance capacity of the strain to the culture medium. Furthermore, the experimental conditions were

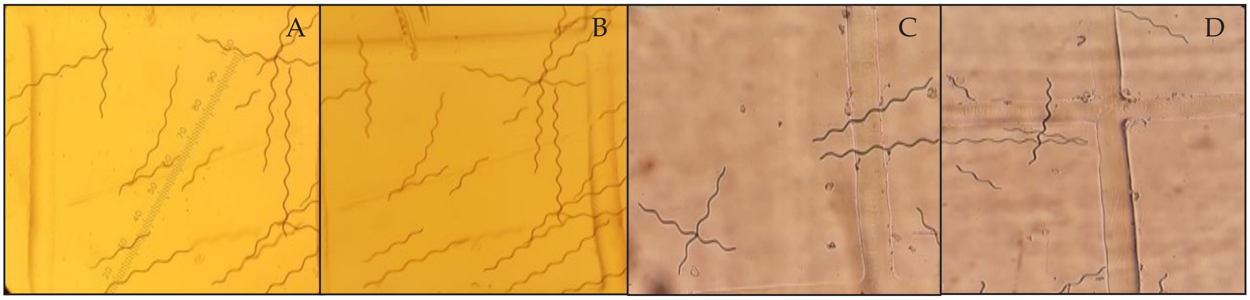

Figure 1. Filaments of *Arthrospira maxima* cultivated under different NaCl concentrations in tropical climate conditions in the region of Cordoba, Veracruz, Mexico. Images taken during the harvest. A: T1, control; B: T2, 2.0 g L^{-1} NaCl; C: T3, 2.5 g L^{-1} NaCl; and D: T4, 3.0 g L^{-1} NaCl.

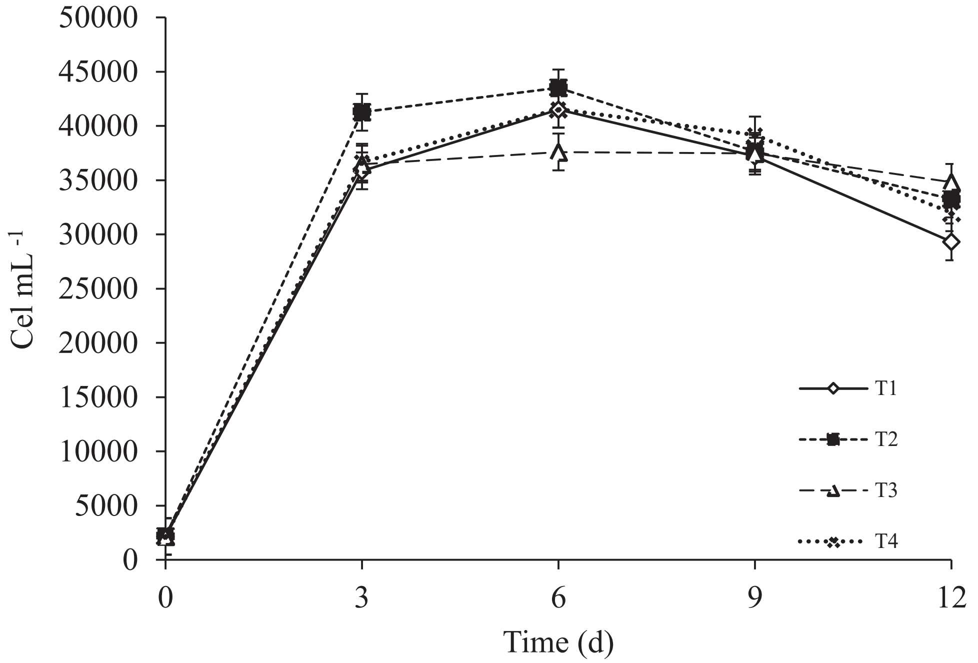

Figure 2. Growth curve of *Arthrospira maxima* cultivated in different NaCl concentrations under tropical conditions in the region of Cordoba, Veracruz, Mexico. T1: control; T2: 2.0 g L^{-1} NaCl; T3: 2.5 g L^{-1} NaCl; and T4: 3.0 g L^{-1} NaCl.

different, since Mutawie (2015) established the experiment in a culture chamber installed in the laboratory at controlled temperature and light. In the case of this study, the experiment was outdoors under a shade-mesh greenhouse in tropical conditions, with temperatures warmer than 18 °C in the region of Cordoba, Veracruz.

Under controlled laboratory conditions, the *A. platensis* species was capable of growing at concentrations of 23.37 and 35.06 g L^{-1} NaCl, showing potential to be produced with seawater (Liu *et al*., 2016). When growing *A. platensis* under controlled laboratory conditions in Zarrouk medium with up to 35.06 g L^{-1} NaCl, changes in the biochemical composition were observed in terms of biomass content, total chlorophyll, phycocyanin, and lipids (Sujatha and Nagarajan, 2014).

Additionally, the behaviour of the growth rate and the metabolic processes of the cyanobacteria could be influenced by factors such as the amount of inoculated strain, light intensity, pH, temperature, type of culture system (photobioreactors under greenhouse conditions), and not only by the concentration of nutrient salts (Ogbonda *et al*., 2007).

Physical and chemical analysis

The physical and chemical variables analized showed significant differences among treatments ($p \leq 0.05$), (Table 1). The humidity in all the treatments showed significant

Table 1. Physical and chemical characterization of powdered *Arthrospira maxima* cultivated in different NaCl concentrations under tropical conditions in Cordoba, Veracruz, Mexico.

Treatment	Humidity %	Crude fibre %	Protein %	Fat %
T1	12.60 ± 0.11 a	0.27 ± 0.03 c	60.66 ± 0.45 a	1.47 ± 0.07 a
T2	9.72 ± 0.11 d	0.62 ± 0.03 a	53.78 ± 0.45 b	0.92 ± 0.07 c
T3	11.94 ± 0.11 b	0.48 ± 0.03 b	46.82 ± 0.45 d	1.13 ± 0.07 bc
T4	10.84 ± 0.13 c	0.22 ± 0.03 c	50.31 ± 0.45 c	1.24 ± 0.07 ab

* Data are expressed as mean ± standard deviation. Significant differences ($p \leq 0.05$) among treatments for the parameters measured are indicated with different letters. T1: control; T2: 2.0 g L^{-1} NaCl; T3: 2.5 g L^{-1} NaCl; and T4: 3.0 g L^{-1} NaCl.

differences. T1 was the one that obtained the highest levels ($p \leq 0.0001$), compared to the rest of the treatments. This variation in the humidity of the samples could be attributed to external factors, such as the relative humidity in the environment, and by the drying method. Biomass treated with hot air drying is known to exhibit rapid surface dehydration, but under the surface, it forms layers that generate moisture (Stramarkou *et al*., 2017). However, the Mexican Standard NMX-F-508-1988 specifications for Spirulina (http://www.dof.gob.mx/nota_detalle.php?codigo=4779957&fecha=14/10/1988) establish a maximum humidity value of 10 %, being T2 the one that is equal to or below that value.

The crude fibre analysis of the powdered *A. maxima* showed significant differences ($p \leq 0.05$) among treatments (Table 1). T2 and T3 showed the highest percentages of crude fibre, with significant differences among themselves ($p \leq 0.0001$) and the rest of the treatments. The treatments T1 and T4 obtained the lowest crude fibre content and showed no significant differences among them ($p > 0.05$), (Table 1). The Mexican Standard NMX-F-508-1988 specifications for *A. maxima* establishes that the maximum allowed crude fibre content is 0.9 %, thus the values obtained in this study are favorable and within the established reference value. Therefore, *A. maxima* may be considered a good source of dietary fibre.

The maximum protein concentration of 60.66 % was recorded in T1 at a pH range of 10.2 – 10.5 for 12 d. The total protein content decreased significantly in the treatments to which NaCl was added to the nutrient medium. The lowest protein content was obtained in T3. In *A. platensis* cultivated in Zarrouk medium, the amount of total protein gradually decreased as NaCl levels increased to 0.73, 1.41, 2.19, 2.92, and 3.65 g L^{-1}, with the control treatment reaching 69.33 % protein (Mutawie, 2015). Similarly, in *A. platensis* cultivated under standard conditions in Zarrouk medium, the protein content was 65 % in samples at pH 9.5, compared to those that received a carbon source and a pH control (7.5 and 8.5) with 18.75 % and 64 % protein, respectively (Mehar *et al*., 2019).

In this study, the performance in the treatments is attributed to the adaptive capacity of *A. maxima* to grow under saline conditions (Mehar *et al*., 2019). Consequently, the treatments tested here have an excellent protein content, and the prevailing tropical climatic conditions in the region of Cordoba, Veracruz are suitable for their cultivation; therefore, it meets the protein value described by Wan *et al*. (2016).

In the case of lipids, the values of *A. maxima* under the established culture conditions were found in a range of 0.92-1.47 %. T1 showed the highest lipid value (1.47 %) and showed significant differences with T2 and T3. In *A. platensis*, 0.65 g of lipids per 100 g of dry biomass have been recorded (da Silva *et al*., 2019), a percentage lower than that reported in this research. In *A. fusiformis* subjected to saline stress, a direct relationship was found between lipid content and salinity level (Rafiqul *et al*., 2003). In this study, the lipid content is lower than the 5-10 % reported in the literature (Michael *et al*., 2019).

Cyanobacteria stand out for their content of macro and microelements. In this study, it was observed that the incorporation of NaCl into the culture medium had a significant influence ($p \leq 0.05$) on the concentration of minerals (Table 2). It was observed that the higher the concentration of NaCl in the nutrient medium, the lower the concentrations of P and K. Contrarily, the concentration of Ca increased as the dose of NaCl in the nutrient medium increased. The Mg concentration increased in T2 but decreased to the level of the control in T3 and T4. S was similar in the control and T2 but decreased in T3 and T4.

Regarding microelements, the concentration of Fe also increased in the same proportion as the level of NaCl increased in the culture medium. The highest concentration of B was observed in T4, followed by T2 and T3; T1 presented the lowest levels of this element. The highest concentration of Mg was observed in T4, followed by T3; T2

Table 2. Elemental composition of *Arthrospira maxima* powder cultivated in different NaCl concentrations under tropical conditions in the region of Cordoba, Veracruz, Mexico.

Elements	Treatment			
	T1	T2	T3	T4
Phosphorus (P)	9.99 ± 0.16 a	9.23 ± 0.16 b	9.04 ± 0.16 b	8.35 ± 0.16c
Potassium (K)	6.52 ± 0.11 a	5.25 ± 0.11 b	5.41 ± 0.11 b	5.05 ± 0.11 b
Calcium (Ca)	6.77 ± 0.11 d	7.74 ± 0.11 c	12.45 ± 0.11 b	19.68 ± 0.11 a
Magnesium (Mg)	3.28 ± 0.05 b	3.67 ± 0.05 a	3.42 ± 0.05 b	3.40 ± 0.05 b
Sulphur (S)	5.29 ± 0.10 a	5.11 ± 0.10 a	4.58± 0.10 b	4.62 ± 0.10 b
Iron (Fe)	93,515 ± 3954.79 c	122,757 ± 3954.79 b	132,720 ± 3954.79 ab	136,384 ± 3954.79 a
Boron (B)	15.32 ± 0.15 b	14.56 ± 0.15 c	14.81 ± 0.15 c	16.51 ± 0.15 a
Manganese (Mn)	19.39 ± 0.50 c	19.60 ± 0.50 c	24.30 ± 0.50 b	29.74 ± 0.50 a
Sodium (Na)	1.84 ± 0.04 ab	1.07 ± 0.04 c	1.73 ± 0.04 b	1.91 ± 0.04 a
Zinc (Zn)	21.70 ± 0.72 c	22.38 ± 0.72 c	35.08 ± 0.72 b	70.18 ± 0.72 a
Copper (Cu)	24.89 ± 0.12 d	30.74 ± 0.12 c	40.84 ± 0.12 b	60.41 ± 0.12 a
Molybdenum (Mo)	1.23 ± 0.21 c	1.86 ± 0.21b c	2.36 ± 0.21 ab	2.73 ± 0.21 a

*Data are expressed as mean ± standard deviation of three repetitions. Significant differences ($p \leq 0.05$) among treatments are indicated with different letters. §Composition of macrominerals (g kg^{-1}): P, K, Ca, Mg, and S. §§Composition of microminerals (mg kg^{-1}): Fe, B, Mn, Na, Zn, Cu, and Mo. T1: control; T2: 2.0 g L^{-1} NaCl; T3: 2.5 g L^{-1} NaCl; and T4: 3.0 g L^{-1} NaCl.

and T1 showed the lowest levels of this microelement, and were statistically similar to each other. The lowest value of Na was observed in T2, while the other treatments were similar to each other. Zn showed higher concentrations as the salinity level increased in the medium, although T2 was statistically similar to T1 (control). For Cu, statistically different means were observed for each of the treatments tested, with the highest concentration of this element observed in T4. The same trend was observed in Mo, with the highest concentration recorded in T4.

The total content of minerals in *A. maxima* could be attributed to the biosorption capacity that the cyanobacterium presents towards metal ions, and to the pH of the culture medium; since it intervenes in the solubility of the metal, as well as in the degree of dissociation of the functional groups contained in the cell wall of cyanobacteria (Michalak *et al*., 2019).

The biomass of *A. fusiformis* produced in a profitable nutrient medium (commercial fertilizer NPK10-20-20, sodium bicarbonate $NaHCO_3$ and NaCl at a concentration of 18 and 1 g L^{-1} respectively) showed higher Na and P content; while cultures in Zarrouk medium presented higher concentrations of Ca, Mn, Mg, Fe, and Zn, with the nutrient medium and tap water being the main factors related to the mineral composition (Michael *et al*., 2019). The levels of Ca and P found in *A. platensis* are the results of the differentiated cell metabolism of cyanobacteria (Alvarenga *et al*., 2011).

In this study, mineral concentrations of the samples of *A. maxima* cultivated with different NaCl concentrations were observed to be higher than in other reports, which shows the great potential of this microorganism to grow in the tropical conditions of the region of Cordoba, Veracruz, Mexico, offering an important source of nutrients that complements the mineral requirements in the human diet.

Concentration of photosynthetic compounds

Under salinity conditions such as those established in this study, it is possible to observe damage to the photosynthesis process, both a structural and functional level. The analyses showed that the chlorophyll-*a* content was affected under saline stress conditions; T1 and T2 were statistically higher than T3 and T4, with significant differences between them ($p \leq 0.001$). The levels of phycocyanin (PC) showed a negative relationship with the NaCl concentration in the nutrient medium; obtaining a maximum value without significant differences in T1 and T4 with values of 43.46 ± 0.14 and 43.23 ± 0.14 mg g^{-1}, respectively. However, T2 and T3 had lower PC values with significant differences between them and with the rest of the treatments (Figure 3B).

In the powdered biomass of *A. platensis*, the amount of chlorophyll-*a* was affected when applying 35.06 g L^{-1} NaCl. Meanwhile, PC levels increased to 23.37 g L^{-1} NaCl, and at 35.06 g L^{-1} no damage was reported in PC production under laboratory conditions (Liu *et al*., 2016). In this study, the results obtained for chlorophyll-*a* and PC of powdered *A. maxima* were not influenced by the concentrations of NaCl added to the nutrient medium (Figure 3). The concentration of photosynthetic pigments depends to a large

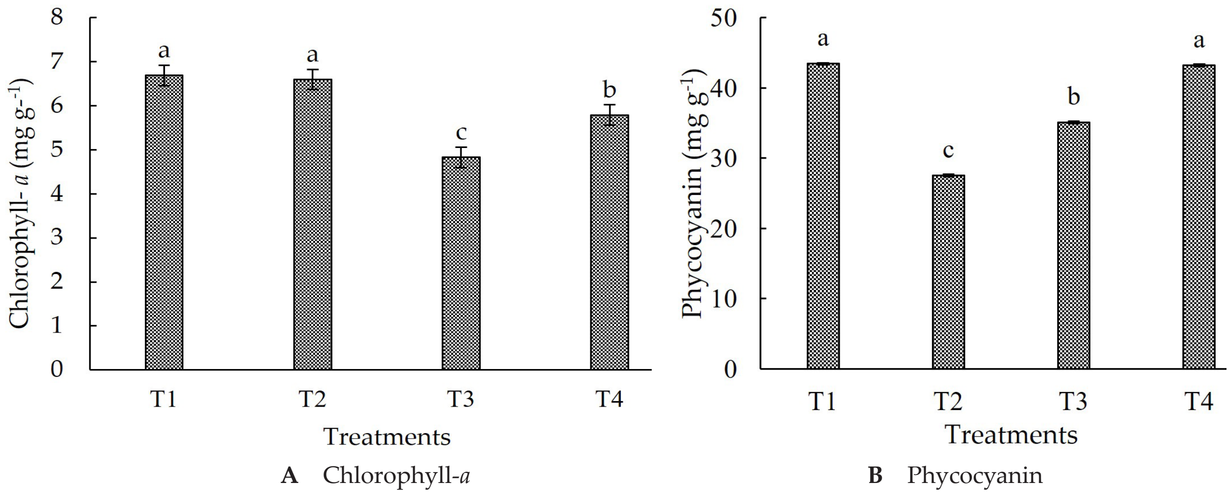

Figure 3. Pigment content in the powdered biomass of *Arthrospira maxima* cultivated in different NaCl concentrations under tropical conditions in the region of Cordoba, Veracruz, Mexico. A: chlorophyll-*a*; B: phycocyanin (FC). T1: control; T2: 2.0 g L^{-1} NaCl; T3: 2.5 g L^{-1} NaCl; and T4: 3.0 g L^{-1} NaCl.

extent on other factors such as available nutrients, irradiance, and light-harvesting capacity (Ajayan *et al*., 2012).

Infrared Spectroscopy (FTIR)

The FTIR spectra of T1, T2, T3, and T4 showed similarity among the absorption bands of functional groups associated with the presence of proteins, carbohydrates, lipids, and nucleic acids (Figure 4). Outstanding in the protein region were amide I at 1647

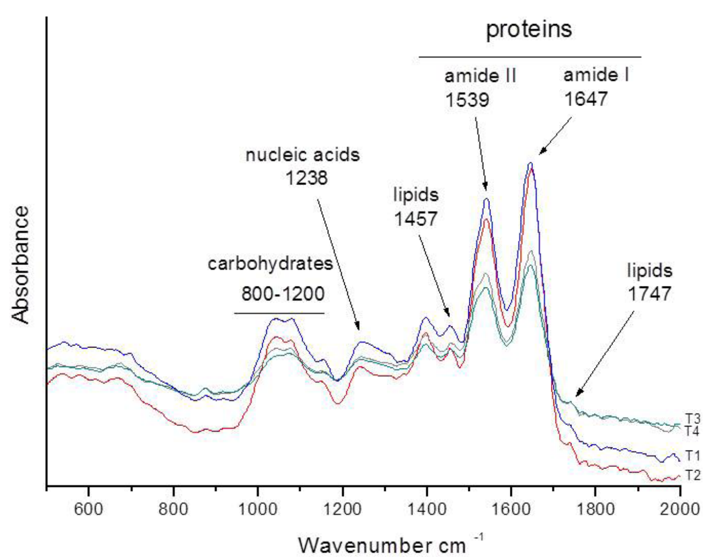

Figure 4. FTIR spectra in the powdered biomass of *Arthrospira maxima* cultivated in different NaCl concentrations under tropical conditions in the region of Cordoba, Veracruz, Mexico. T1: control; T2: 2.0 g L^{-1} NaCl; T3: 2.5 g L^{-1} NaCl; and T4: 3.0 g L^{-1} NaCl in the region of 500 to 2000 cm^{-1}.

cm^{-1} associated with the C=O bond and amide II at 1539 cm^{-1} NH bond. Lower intensity bands were observed in the region of 1200 - 800 cm^{-1} corresponding to the C-O-C and C-O bonds associated with the presence of carbohydrates. The presence of lipids was confirmed by bands at 1457 and 1747 cm^{-1} associated with CH_3 and C=O bonds. These results are similar to those reported by Lupatini-Menegotto *et al*. (2019) for *A. platensis*. On the other hand, the FTIR analysis indicates that the treatments were influenced by the salinity of the medium, highlighting this effect in the protein region where a decrease in absorption is seen in the amide I and II bands concerning to TI, related with a decrease in protein concentration observed in the proximal analysis (Table 1).

Antioxidant activity

The values of antioxidant activity in the powder obtained from *A. maxima* exposed to different NaCl concentrations show a positive relationship between antioxidant activity and NaCl levels; that is, at a higher concentration of NaCl, a higher antioxidant activity was recorded (Figure 5). At T4, the antioxidant activity was statistically higher (35.61 ± 2.21 %) than the rest of the treatments. The minimum activity (15.98 ± 2.21 %) was obtained at T1 (Figure 5). Coincidentally, the *A. platensis* strains exerted high antioxidant activity at 23.37 and 35.06 g L^{-1} NaCl (Liu *et al*., 2016). On the other hand, when the NaCl concentration is higher than 35.06 g L^{-1}, the antioxidant activity decreases. A higher antioxidant activity has been observed in *A. platensis* exposed to saline stress in a pH range from 7.5 – 11.0, compared to those cultivated under normal conditions and with a pH closer to neutrality (Ismaiel *et al*., 2016).

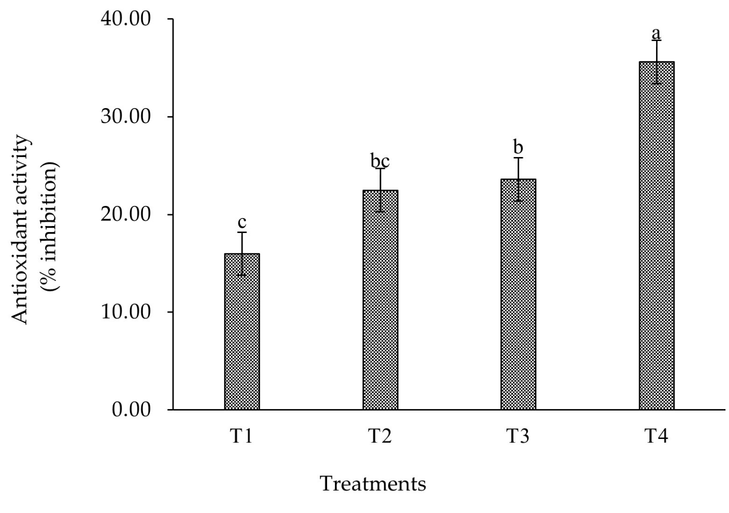

Figure 5. Antioxidant activity of the powdered biomass of *Arthrospira maxima* cultivated in different NaCl concentrations under tropical conditions in the region of Cordoba, Veracruz, Mexico. T1: control; T2: 2.0 g L^{-1} NaCl; T3: 2.5 g L^{-1} NaCl; and T4: 3.0 g L^{-1} NaCl.

The results obtained in this study are similar to those reported previously. The antioxidant activity of *A. maxima* is not only associated with the presence of phycocyanin and allophycocyanin; but with richness in insulin-like proteins, fatty acids (sulpholipids), and sulphated polysaccharides (Wan *et al*., 2016). It should be noticed that the powdered biomass of *A. maxima* evaluated in this study presents an excellent source of minerals, which are associated with the antioxidant and chelating activity provided by this cyanobacterium (Martínez-Palma *et al*., 2015).

CONCLUSIONS

Under the experimental conditions of this study, the addition of NaCl to the nutrient medium of *Arthrospira maxima* did not significantly affect cell growth, nor did it modify the morphology of the filaments or the content of photosynthetic compounds. However, the high concentration of salts decreases the concentrations of proteins and minerals. Therefore, the physical and chemical characterization showed that the powdered biomass of *A. maxima* cultivated under different NaCl concentrations in the tropical climate conditions of the region of Cordoba, Veracruz, Mexico has nutritional properties with the potential to be used as a food source for human consumption or as a functional ingredient in the development of innovative biotechnological products.

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