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Research Article

Scenedesmus obliquus and *Chlorella vulgaris* in biodesalination of deep aquifer well water in Sistan region, Iran

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Abstract

The use of microalgae is a new and cost-effective biological method for the desalination of saline and brackish waters. For this purpose, after sampling and preparing BG-11 medium with deep aquifer well water, some physicochemical parameters of water were measured and the possibility of reducing the salinity of deep aquifer well water by *Scenedesmus obliquus* and *Chlorella vulgaris* algae with cell densities of 1.25 and 2.5×10^6 cells/mL was investigated. The results showed that the highest salinity, total dissolved solids value (TDS), Na^+, Hardness, Nitrate, Phosphate removal and the highest biological desalination rate were observed in *S. obliquus* algae with a density of 2.5×10^6, respectively with 9.6%, 27.14%, 7.34%, 25%, 67.01 %, 97.32% reduction, and 28.5±2.50 % for biological desalination rate which showed a significant difference compared to *C. vulgaris* algae. The highest Chloride removal was observed in *C. vulgaris* with a density of 2.5×10^6 cells/mL from 2840 to 828.33±108.45 mg/L (70.83% reduction). The results of dry biomass and algal cell numbers indicated that the highest values were assigned to low and high densities of *C. vulgaris* algae, with values of 1.03±0.08 and 0.99±0.04 g/L, and 46.35±4.45 and 43.53±4.86 ($\times10^6$cells/mL), respectively. According to the results, both algae have the ability to reduce deep aquifer well water salinity. Therefore, considering the impact of higher algae densities on further desalination, it is recommended that higher densities be tested.

Introduction

Due to the use of drinking water for purposes such as agriculture and industry, humanity will face a water crisis in the near future. Climate change, population growth, overexploitation of freshwater resources, and their inability to regenerate lead to the problem of freshwater shortage worldwide. Hence, it is essential to use all available water resources (Obotey Ezugbe and Rathila, 2020). Inland saline groundwater is an important resource in many arid and semi-arid regions and has met water needs for various purposes in many countries. In the United States, more than 95% of desalination plants use inland saline water resources (Mickley, 2006). All existing desalination methods such as multi-stage distillation, reverse osmosis membrane, ion exchange, steam-condensation distillation, membrane distillation, electrodialysis, and multi-stage flash evaporation require high electricity consumption, thus increasing the production cost by 3–7 times than other sources (Pistocchi *et al*., 2020; Dhakal *et al*., 2022). In addition to energy costs, there are serious issues related to the above desalination systems, including greenhouse gas emissions, massive discharge of thermal and ultra-saline effluent into the environment and the harmful effects of wastewater on ecosystem life (Einav *et al*., 2003; Ashwaniy and Perumalsamy, 2017). Desalination using renewable energy sources such as solar, geothermal and, wind energy is much more environmentally friendly, but it is more expensive compared to conventional desalination methods (Shatat and Riffat, 2014). Biological desalination is a new desalination method based on surface biosorption (non-metabolic mechanism) or bioaccumulation (metabolism-related mechanism), in which various salt-tolerant organisms absorb salts from salt water. In this method, water salts can be absorbed by living organisms (plants or animals) like other nutrients and converted into biomass, and as a result, the salinity of the water is also reduced. Compared to conventional desalination methods, biological desalination systems, while saving energy, have simpler technology and engineering complexity, and also have fewer environmental impacts (Barahoei *et al*., 2021; Nadersha and Aly Hassan, 2022). Microalgae can desalinate water through osmosis, ion exchange, or biosorption (Chisti, 2007). In addition, there are extensive applications for cultivated algal biomass in high-salinity environments after harvest, including the production of lipids for use in fuel and animal feed production (Sahle-Demessie *et al*., 2019). The unicellular green algae of *C. vulgaris* has been widely used in a wide range of salt concentrations and has a fast growth rate and high nutrient uptake capacity (Laliberte *et al*., 1997; Gaur *et al*., 2021). Despite the fact that *C. vulgaris* is often found in freshwater, it has been shown to tolerate salinities up to 20 g/L NaCl (Sahle-Demessie *et al*., 2019). *S. obliquus* is another freshwater microalgae that has shown high tolerance to salinity stress (Mohammed and Shafea, 1992; Demetriou *et al*., 2007) and is able to adapt to changing environments with high vitality and high reproductive rates (Lewis, 1995; Martinez *et al*., 2000). The use of

unconventional water resources especially deep groundwater resources has been the focus of attention in the Sistan region and has been proposed as a solution to provide water in the face of a water shortage crisis resulting from persistent droughts. Deep aquifer well No. 3 is located in Nimroz city (Iran). Due to the salinity of the water, purification is done using reverse osmosis technology. Purification by the above method requires a high amount of energy. In addition to energy costs, there is a fundamental and important problem of environmental impacts caused by extremely salty desalination effluent. Extensive research is being conducted to reduce these problems and replace environmentally friendly methods with conventional desalination in different parts of the world. Algae-assisted biological desalination is a method with low cost and equipment and is environmentally friendly. In addition, there are several other uses of microalgae in salt water other than its use for desalination. For example, they can be used for the purpose of detoxifying minerals and heavy metals (Sanchez *et al*., 2015). Therefore, considering the problem of desalination effluent, the present study was conducted to investigate the effectiveness of microalgae *C. vulgaris* and *S. obliquus* in reducing the salinity of water in deep aquifer well No. 3 in the Sistan region.

Materials and methods

Selection of algae

In this study, two algal species were selected based on previous studies due to their growth rate, tolerance to salinity range, and water salinity reduction, which included 1- *C. vulgaris* (Louye *et al*., 2019; Sahle-Demessie *et al*., 2019; Figler *et al*., 2019; Ghobashy *et al*., 2022) and 2- *S. obliquus* (Gan *et al*., 2016; Sahle-Demessie *et al*., 2019; Figler *et al*., 2019; Wei *et al*., 2020).

Sampling of the water from deep aquifer well

Deep aquifer well (No. 3) is located in Nimroz city (61°25′E longitude and 31°7′N latitude), with a depth of 1790 m located in the north of Sistan and Baluchistan province. Sampling of deep aquifer well water was carried out directly from the well outlet pipe using clean 20-liter containers in the winter of 2024 and transferred to the Aquatic Breeding and Farming Laboratory of the Hamoun International Wetland Institute located in Research Institute of Zabol (Iran).

Algae culture medium preparation

BG-11 culture medium was prepared with distilled water (control) and deep aquifer well water (experimental treatments) based on the compositions listed in Table 1.

Table 1: Ingredients of BG-11 culture medium used for algae cultivation

Macro and micro-elements (g)	
$NaNO_3$	75
$K_2HPO4.3H_2O$	2
Na_2CO_3	1
$MgSO_4.7H_2O$	3.75
$CaCl_2.2H_2O$	1.80
EDTA-Na_2	0.05
$Fe(NH3)_2$Citrate	0.30
Citric acid	0.30
H_3BO_3	2.86
$MnCl_2.4H_2O$	1.81
$ZnSO_4.7H_2O$	0.22
$Na_2MOO_4.2H_2O$	0.39
$CuSO_4.5H_2O$	0.08
$CO(NO_3)_2.6H_2O$	0.05

BG-11 medium contains essential elements for algae growth, i.e. microelements used at a rate of 1 mL per liter and macro elements used at a rate of 10 mL per liter of culture medium. The nutrient media was autoclaved at 121°C for 15 minutes to avoid contamination before the algae inoculum (Ahamefule *et al*., 2020).

Preparation of algae species and cultivation conditions

The *S. obliquus* strain was obtained from Ravis Sabz Gene Company (Iran) and the *C. vulgaris* species (Abdf 21144) was obtained from the National Algae Bank. The strains were cultured in 250 mL Erlenmeyer flasks at a ratio of 1:10 in BG-11 medium. The flasks were incubated at 26 ± 1°C temperature under fluorescent lamps of 3500 lux intensity and 12:12 h light/dark cycle for 21 days. The flasks were shaken three times a day to prevent them from attaching to the walls. An air pump was used to circulate the water and create flow and also to move the algae. The grown algae were subsequently used for inoculation in the desalination experiment.

Biodesalination experiment

For the desalination test, 4 treatments were used including 1- *S. obliquus* algae with a cell density of 1.25×10^6 cells/mL, 2- *C. vulgaris* algae with a cell density of 1.25×10^6 cells/mL, 3- *S. obliquus* algae with a cell density of 2.5×10^6 cells/mL, and 4- *C. vulgaris* algae with a cell density of 2.5×10^6 cells/mL. After initial cultivation of algae in BG-11 medium prepared with distilled water, the density of grown algae was counted with a Neubauer hemocytometer slide and a portion of the high-density grown algae stock was inoculated into flasks containing BG-11 medium prepared with deep well water to give an initial cell concentration of 1.25×10^6 and 2.5×10^6 cells/mL (Ahamefule *et al*., 2020). Erlenmeyer flasks (500 mL) were used for the desalination experiments. A control treatment including algae cultivation in BG-11 medium prepared with distilled water was considered to control the growth of the treatments. For optimal algae growth, the items mentioned in the previous section were provided. This experiment was performed in triplicate for each treatment for 21 days (El-Sergani *et al*., 2014; Minas *et al*., 2015; El-Nadi *et al*., 2019).

Algal growth measurement

Algae counting and determination of microalgae cell number were performed using a Neubauer hemocytometer under a light microscope equipped with a camera on days 0, 6, 10, and 21 after algae cultivation in deep aquifer well water:

Cells average number = number of cells (per mL of the sample) $\times10^4$ × dilution factor

Their optical densities were also measured at a wavelength of 680 nm using a spectrophotometer. SGR (μmax) (1/day) was calculated using the following equation:

$\mu max = LnN_1 - lnN_0/t_1\text{-}t_0$

Where, N_0 and N_1 represent the cell concentration at times t_0 and t_1 of the logarithmic growth phase.

Dry algal biomass was obtained using the method proposed by Lavens and Sargeloos (1996). According to this method, 21 days after algae cultivation, the culture medium containing algae was centrifuged at 4000 rpm for 8 min. After the sedimentation of algae and separation of the supernatant, the residue in the Petri dish was emptied and kept at 60°C for 1 day and then weighed on a digital scale with an accuracy of 0.0001 g.

$DW = W_2 – W_1$

Where, W_1 is the weight of an empty Petri dish and W_2 is its weight containing microalgae after drying.

Measurement of physicochemical parameters of water

The Salinity, TDS, and EC were measured using a portable handheld meter. Total hardness (calcium and magnesium), nitrate, phosphate, magnesium and iron were measured in terms of mg/L using a Palin Test photometer (Palintest™ 8000, Tyne & Wear, U.K.). Sodium and potassium were measured using a flame-photometer (Elico Technologies CL 361, Hyderabad, India). Chloride was assayed using direct titration with $AgNO_3$ solution in the presence of potassium chromate reagent. The physicochemical parameters mentioned above were measured again after preparing BG-11 culture medium with deep aquifer well water on the first day of the experiments and 21 days after algae cultivation to calculate the percentage of changes in each of the physicochemical parameters compared to the first day.

Evaluation of desalination rate and bioaccumulation capacity of Cl

In this study, TDS was used to evaluate the desalination rate of deep aquifer well saline water. The initial TDS of deep aquifer well water before algae addition was recorded as TDS_i and at the end of the desalination experiment, the algal solution was centrifuged at 4000 rpm for 8 min, the supernatant was filtered through a 0.45 µm filter and analyzed for final TDS (TDS_f). The algae residue was dried at 60°C and the weight difference was used to measure the net mass of dry algae (m). The removal efficiency (desalination rate) and the chloride anion bioaccumulation capacity were determined by measuring the initial (Cl_i) and the final chloride anion concentration (Cl_f) using direct titration against $AgNO_3$. The desalination rate will be calculated with the following equations (Gan *et al*., 2016; Wei *et al*., 2020 ;Ghobashy *et al*., 2022):

Desalination rate (%) = $(1\text{-}TDS_f / TDS_i) \times 100$

Bioaccumulation capacity of Cl = $(Cl_i - Cl_f) / m$

Statistical analysis

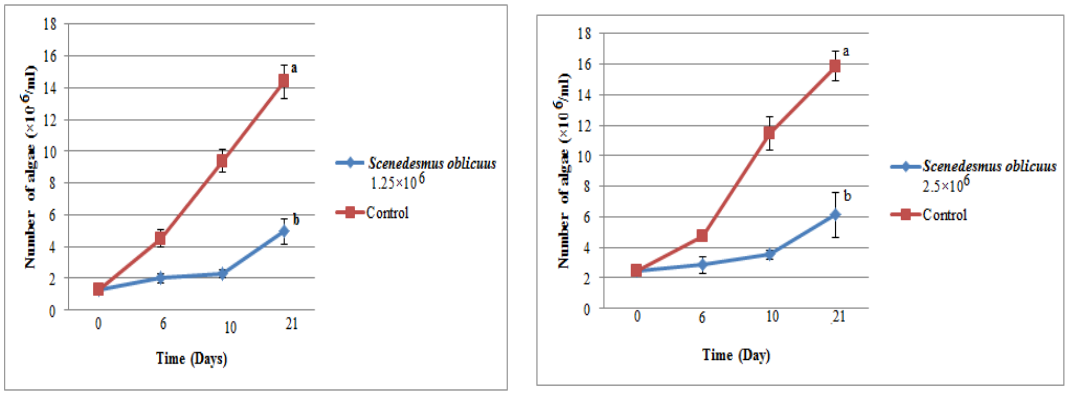
Initially, the normality of the data was checked using the Kolmogorov-Smirnov test. After ensuring the normality of the data, the analysis was performed using the One Way ANOVA method. The Tukey's test was used to compare the means and $p<0.05$ was considered as the statistical inference threshold. SPSS software was used to perform all the above statistical analyses and Excel software was used to draw graphs. The experimental numerical

data were expressed in mean ± standard deviation.

Results

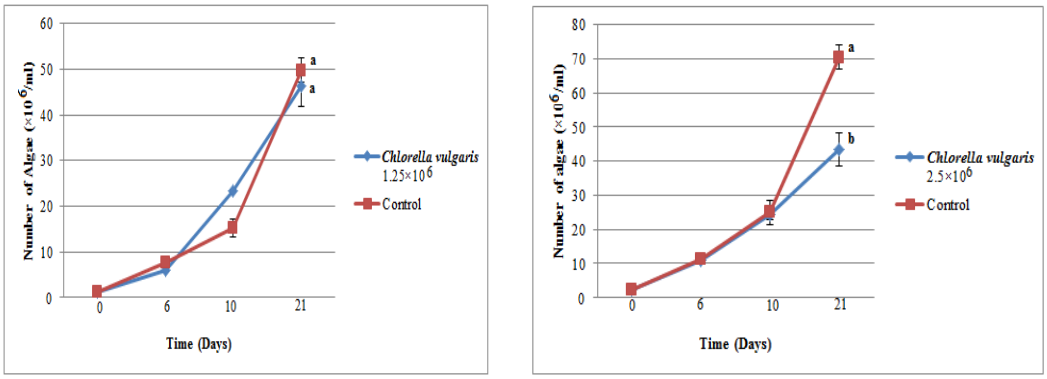
Algae growth in deep well water salinity

Figure 1 A, B shows the cell number of *S. obliquus* algae on days 0, 6, 10, and 21 after cultivation in deep aquifer well water salinity (12.5 ppt) compared to the control treatment (BG-11 medium prepared with distilled water) for each algal density. Although the results indicated an increasing trend during days 10 to 21 of cultivation, a significant decrease (p<0.05) in cell number was very evident on all days of the study compared to the control treatment of each density, and the greatest differences were observed on day 21 of the test in 1.25×10^6 treatment of this algae with a number of 4.97+0.79 ($\times10^6$) compared to the control treatment (14.37+1.03) and 2.5×10^6 treatment with a number of 6.14 +1.08 ($\times10^6$) compared to the control (15.8+0.95). However, *C. vulgaris* algae showed better growth and a number increase in the two studied densities in deep aquifer well water, and the 1.25×10^6 treatment of this algae did not show a significant difference with the control treatment on the days studied, However, the 2.5×10^6 treatment of this algae showed a significant decrease (p<0.05) on the final day of the experiment (Fig. 2).



A B

Figure 1: Growth of control and cultures of *Scenedesmus obliquus* treated with BG-11 medium prepared with deep aquifer well water. A: 1.25×10^6 and B: 2.5×10^6



A B

Figure 2: Growth of control and cultures of *Chlorella vulgaris* treated with BG-11 medium prepared with deep aquifer well water. A: 1.25×10^6 and B: 2.5×10^6

The results of the algae biomass after the end of the experimental period and drying in the oven indicated that the highest value was assigned to the low and high densities of *C. vulgaris* with values of 1.03±0.08 and 0.99±0.04 g/L, respectively, followed by the high density of *S. obliquus* with 0.88±0.12 g/L, which showed no significant difference with *C. vulgaris* (Fig. 3).

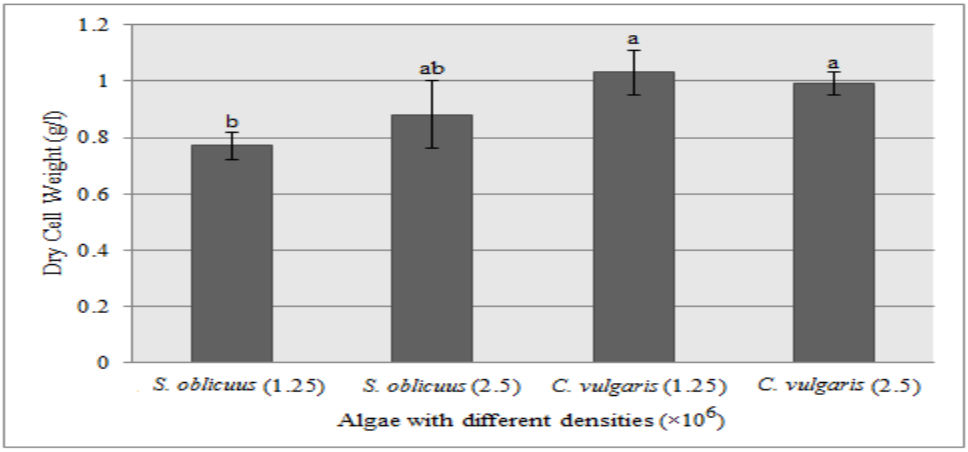


Figure 3: Comparison of dry biomass of algae over a 21-day period at different densities

After the completion of the 21-day culture period, a comparison of the number of algal cells per ml showed that the low and high densities of *C. vulgaris* algae were the highest with values of 46.35±4.45 and 43.53±4.46 (×10^6 cells /mL), respectively, followed by the high and low densities of *S. obliquus* algae, 6.14±1.48 and 4.97±0.79 (×10^6 cells /mL), which showed a significant difference with *C. vulgaris* (p<0.05) (Fig. 4).

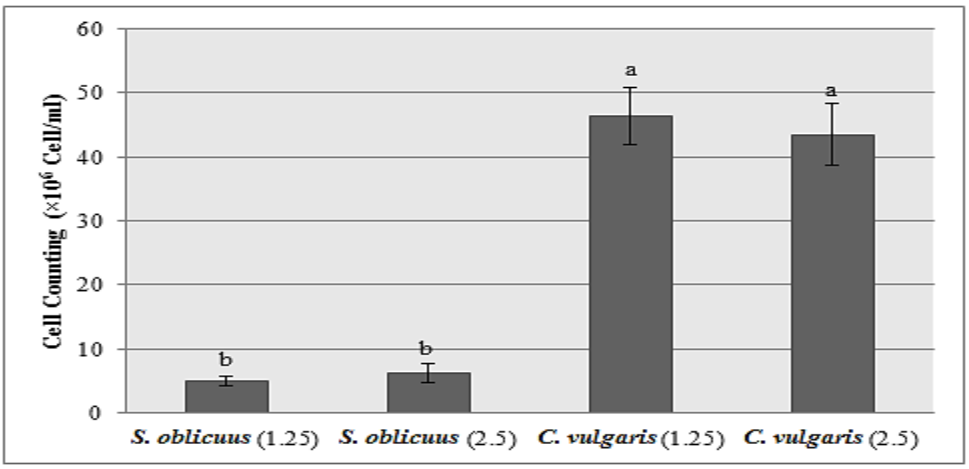


Figure 4: Comparison of algae counts per ml over a 21-day period at different densities

The specific growth rate and Optical density values at the end of the experimental period also showed similar results, and the specific growth rate in *C. vulgaris* with different densities showed a significant difference (p<0.05) with *S. obliquus* (Figs. 5 and 6).

The results of the physicochemical parameters analysis of the BG-11 culture medium prepared with deep aquifer well water (before algae treatment) for use in the desalination test with the two mentioned algae and after algae treatment (end of the 21-day experiment period) are given in Table 2. As shown in the table, the salinity of the water used was 12.5 g/L at the beginning of the experiment.

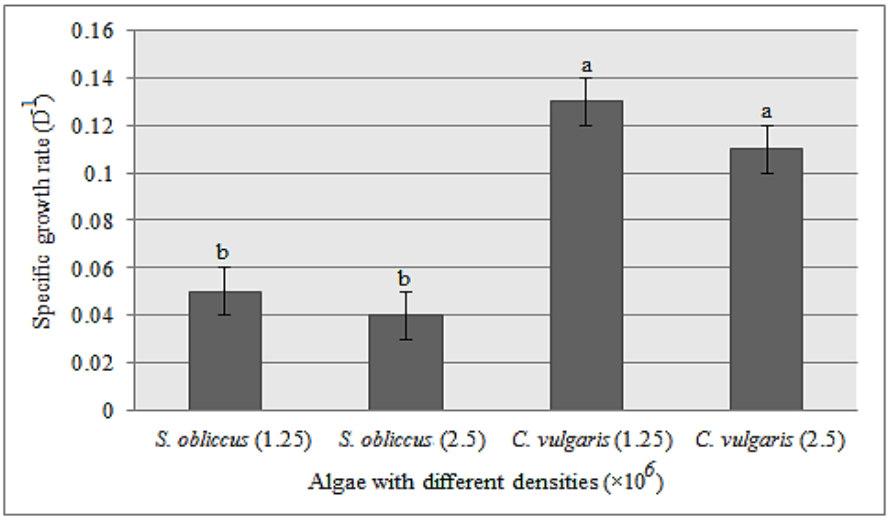


Figure 5: Comparison of specific growth rates of algae over a 21-day period at different densities

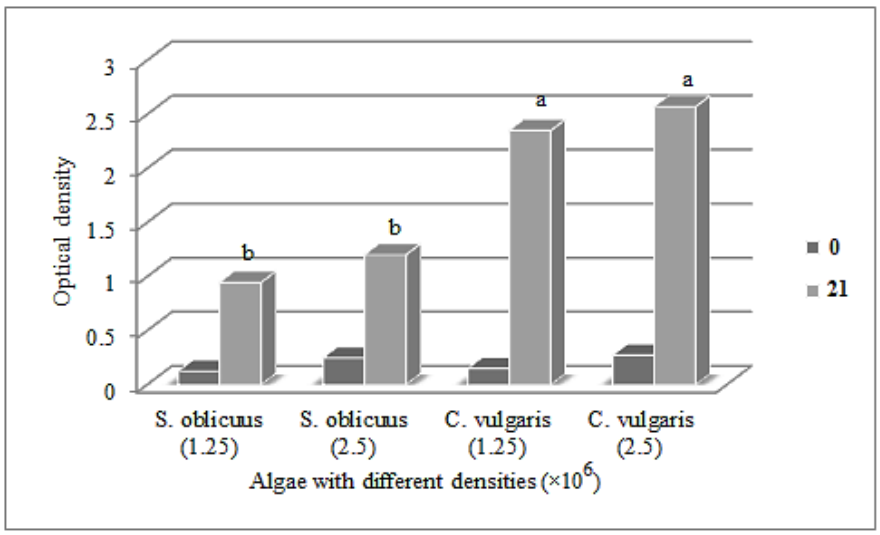


Figure 6: Comparison of Optical density of algae at 0 and over a 21-day period at different densities

Physicochemical parameters of water

A comparison of salinity changes in different treatments on days 1, 6, 10, 17, and 21 after algae growth is given in Figure 7. On all the days studied, the salinity uptake rate in the *S. obliquus* with a density of 2.5×10^6 was higher than the other treatments and in the 1.25×10^6 treatment this algae was lower than the other treatments. At the end of the experimental period, the highest salinity absorption was observed in the high-

density treatment of *S. obliquus*, which decreased from 12.5 to 11.30±0.10 ppt, also showing a 9.6% decrease (Figure 8) and a significant difference ($p<0.05$) compared to the lower density of this algae. The TDS value of the water used at the beginning of the period and before the algae treatment was 17.2 g/L. After the algae treatment, the TDS value reached 12.53±0.46 and 13±0.20 g/L in the treatments of 2.5×10^6 and 1.25×10^6 of *S. obliquus*, respectively (Table 2) and showed a significant difference ($p<0.05$) with *C. vulgaris* treatments. Also, the value of TDS removal in these treatments was 27.14 and 24.42%, respectively, which showed a significant increase compared to *C. vulgaris* (Fig. 9).

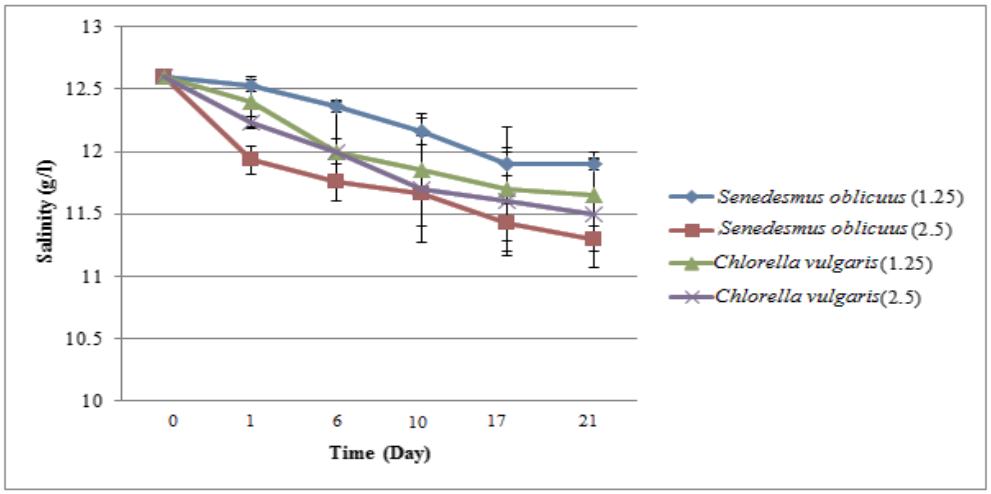


Figure 7: Comparison of salinity changes after the growth of *Scenedesmus obliquus* and *Chlorella vulgaris* with different densities on different days of the deep aquifer well water desalination experiment

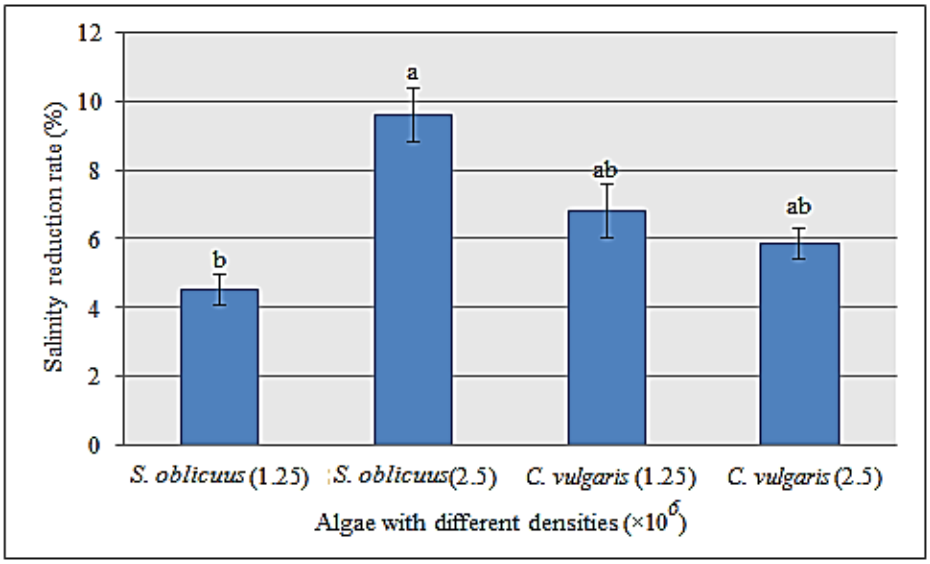


Figure 8: Comparison of the percentage of salinity reduction after the growth of *Scenedesmus obliquus* and *Chlorella vulgaris* over a 21-day period at different densities

Comparison of EC changes at the end of the period showed the greatest decrease in this parameter from 28000 µmohs/cm at the beginning of the cultivation period to

24000 ± 1000 (14.29% decrease) at a low-density of *C. vulgaris*, and then to 25333 ± 577.33 (9.53% decrease) at high density of *S. obliquus*, however, no significant difference was observed (Table 2; Fig 10). The highest Na+ absorption rate was observed at high density of *S. obliquus* algae, which decreased from 958.3 at the beginning of the period to 888 ± 5 mg/L at the end of the period, showing a decrease of 7.34% (Fig 11) and a significant difference compared to other treatments (p<0.05).

Table 2: Analysis of physicochemical parameters of deep aquifer well water containing BG-11 culture medium at the beginning and over a 21-day period at different densities

First of the test period Before algae treatment BG-11 culture medium prepared with deep well water		**over a 21-day period After algae treatment**			
		***Scenedesmus obliquus* (cell/ml)**		***Chlorella vulgaris* (cell/ml)**	
Parameters	**Results**	**1.25×10^6**	**2.5×10^6**	**1.25×10^6**	**2.5×10^6**
Salinity (g/L)	**12.5**	11.93 ± 0.05 ^{a}	11.30 ± 0.10 ^{b}	11.65 ± 0.35 ^{ab}	11.76 ± 0.05 ^{ab}
TDS (g/L)	**17.2**	13 ± 0.20 ^{b}	12.53 ± 0.46 ^{b}	14.6 ± 0.20 ^{a}	15.06 ± 0.23 ^{a}
EC(µ mohs/cm)	**28000**	27000 ± 0 ^{a}	25333 ± 577.35 ^{b}	24000± 1000 ^{b}	25500 ± 500 ^{ab}
Sodium (mg/L)	**958.3**	917.60 ± 5.04 ^{b}	888 ± 5 ^{c}	936.72 ± 9. 57 ^{a}	936.33±5.20 ^{a}
Chloride (as Cl^{1-}) (mg/L)	**2840**	2059 ± 71 ^{a}	1313.3 ± 35.50 ^{b}	1136± 284 ^{bc}	828.33±108.45 ^{c}
Potassium (mg/L)	**16.4**	14 ± 0.20 ^{a}	13.30 ± 0.17 ^{b}	12.90 ± 0.30 ^{b}	13.36 ± 0.15 ^{b}
Magnesium (mg/L)	**4600**	± 57.73 ^{b} 1933.3	2066.7 ± 57. 73^a	2100± 0.0 ^{a}	2166.7± 57. 73^a
Iron (mg/L)	**0.37**	0.00 ± 0.00 ^{b}	0.0033 ± 0.005 ^{b}	0.02 ± 0.005 ^{a}	0.03 ± 0.01 ^{a}
Total Hardness (mg/L)	**2000**	± 57.73 ^{b} 1516.7	1500 ± 0.0 ^{b}	1825 ± 75 ^{a}	1666.7±104.08 ^{ab}
Nitrate (mg NO3/L)	**1060.4**	678.7 ± 73.70 ^{b}	349.80 ± 37.40 ^{c}	937.20± 83.60 ^{a}	1009.1± 6.72 ^{a}
Phosphate (mg P/L)	**16.5**	0.62 ± 0.02 ^{ab}	0.50 ± 0.05 ^{b}	0.69 ± 0.07 ^{a}	0.66 ± 0.06 ^{a}

Note: Values are means±SD of three replications. Means in the same raw with different superscripts are significantly different by Tukey's Test (p<0.05).

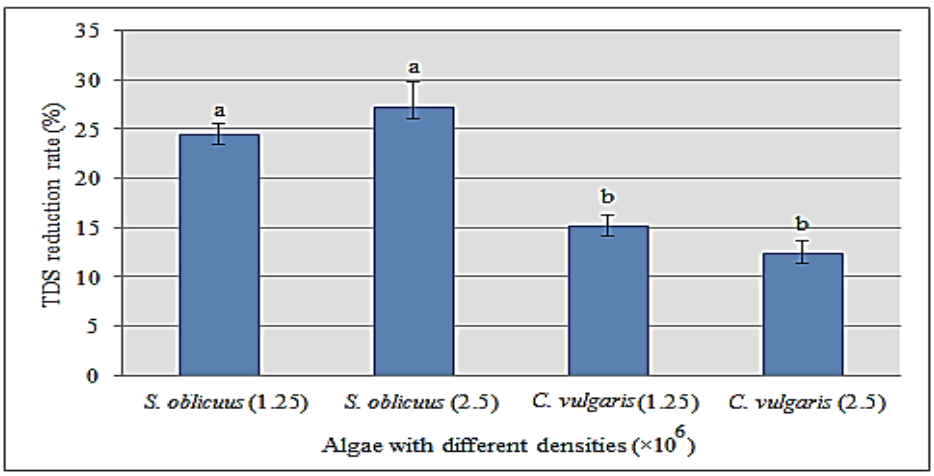


Figure 9: Comparison of the percentage of TDS reduction after the growth of *Scenedesmus obliquus* and *Chlorella vulgaris* over a 21-day period at different densities

Figure 12 indicated a 70.83±3.81% reduction in the chloride content of deep aquifer well water by high densities of *C. vulgaris* algae, its value decreased from 2840 to 828.33 ± 108.45 mg/L (Table 2), which showed a significant difference with the densities of *S. obliquus* algae, also the lowest percentage of removal was observed in low-density of *S. obliquus* algae (27.5±2.5). The potassium level at the beginning of the period was 16.4 mg/L, at the end of the algae growth period and after algae harvesting, it decreased to 12.90±0.30 mg/L (21.35% decrease) in the low-density of *C. vulgaris* followed by 13.30±0.17 mg/L (18.91% decrease) in the high density of *S. obliquus* and 13.36±0.15 (18.5% decrease) in the high density of *C. vulgaris* to. However, no significant difference was observed between these treatments (Table 2; Fig13).

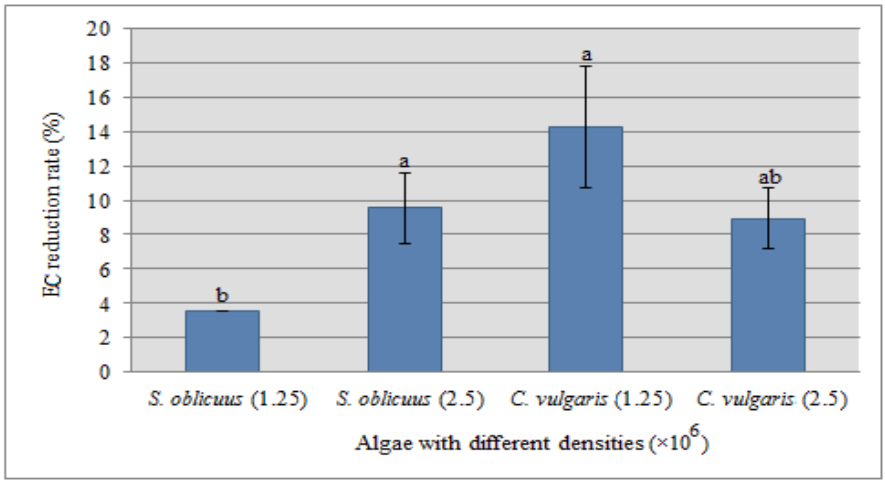


Figure 10: Comparison of the percentage of EC reduction after the growth of *Scenedesmus obliquus* and *Chlorella vulgaris* over a 21-day period at different densities

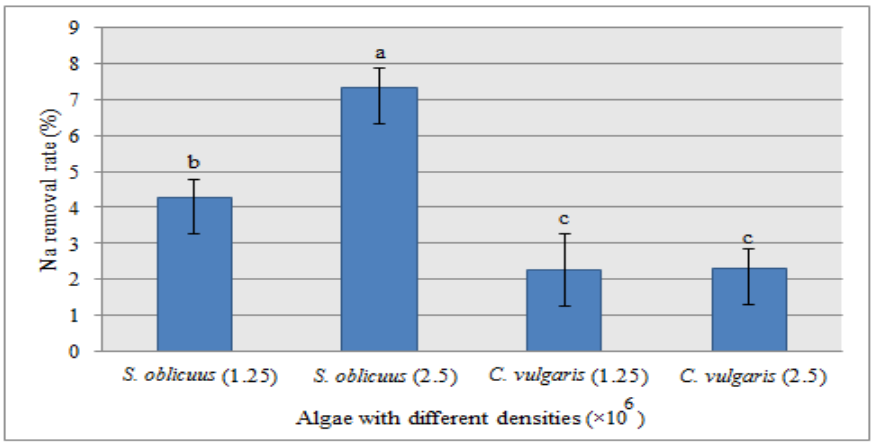


Figure 11: Comparison of the percentage of Na^+ reduction after the growth of *Scenedesmus obliquus* and *Chlorella vulgaris* over a 21-day period at different densities

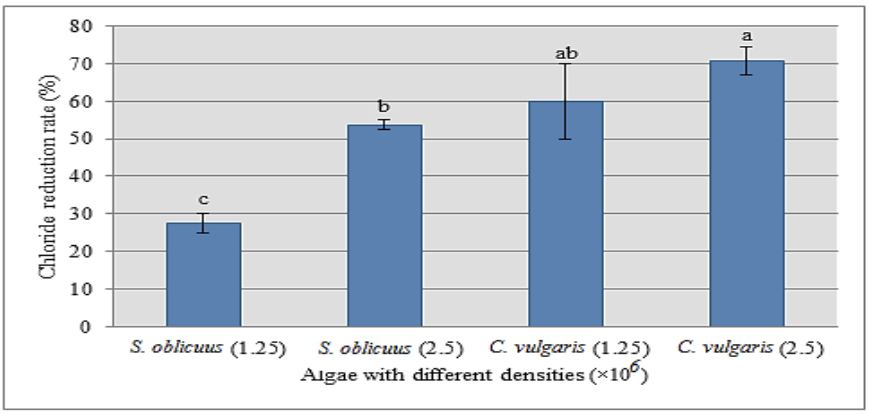


Figure 12: Comparison of the percentage of Chloride reduction after the growth of *Scenedesmus obliquus* and *Chlorella vulgaris* over a 21-day period at different densities

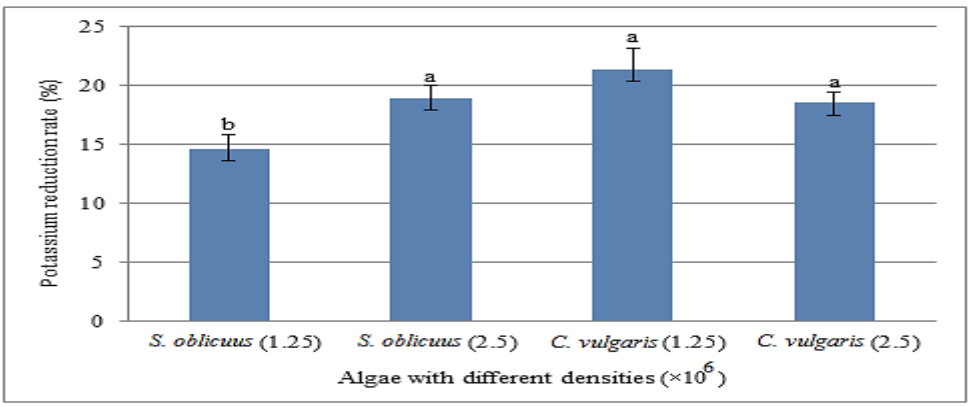


Figure 13: Comparison of the percentage of Potassium reduction after the growth of *Scenedesmus obliquus* and *Chlorella vulgaris* over a 21-day period at different densities

The highest magnesium uptake was observed in the low density treatment of *S. obliquus* algae, which was able to reduce the magnesium level from 4600 at the beginning of the period to 1933.3±57.73 mg/L (57.97% reduction) at the end of the period and showing a significant difference (p<0.05) with other treatments (Table 2; Fig.14).

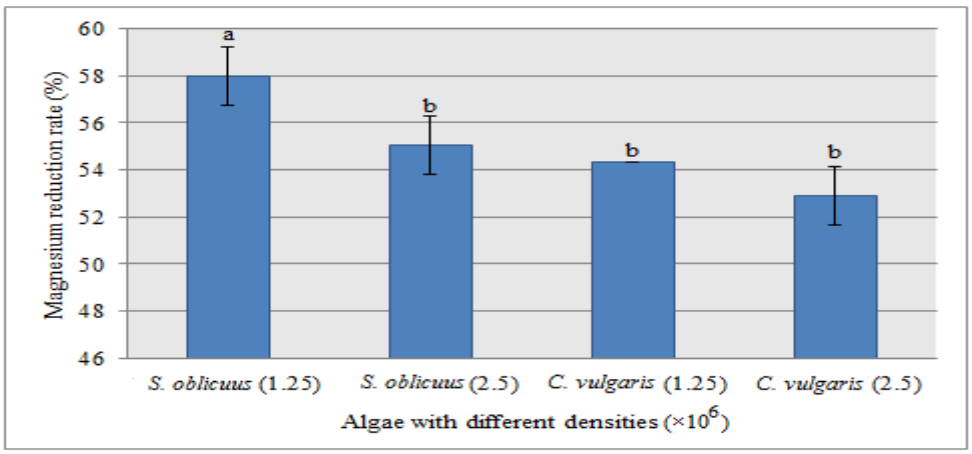


Figure 14: Comparison of the percentage of Magnesium reduction after the growth of *Scenedesmus obliquus* and *Chlorella vulgaris* over a 21-day period at different densities

The *S. obliquus* algae absorbed more iron at both densities compared to *C. vulgaris* algae densities, and the iron content decreased from 0.37 mg/L at the beginning of the period to 0 (100% reduction) and 0.0033±0.005 (90% reduction) at high and low densities of this algae compared to densities of *C. vulgaris* algae, and the differences were statistically significant (Fig. 15; $p<0.05$). The highest total hardness removal rate was observed in the high and low treatments of *S. obliquus* algae, which were able to reduce the total hardness from 2000 at the beginning of the period to 1500 mg/L (25% reduction) and 1516.7±57.73 (24.16% reduction), respectively, and showed a significant difference ($p<0.05$) with other treatments (Table 2; Fig. 16).

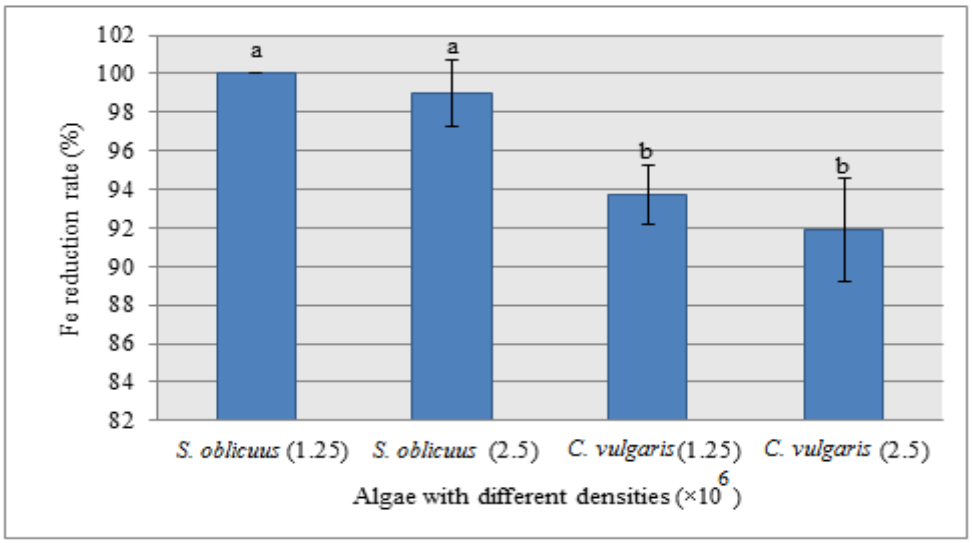


Figure 15: Comparison of the percentage of Fe reduction after the growth of *Scenedesmus obliquus* and *Chlorella vulgaris* over a 21-day period at different densities

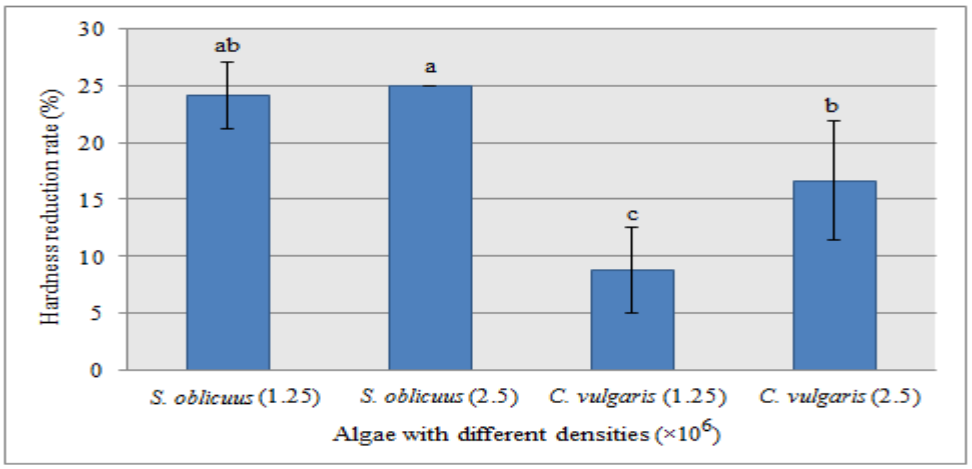


Figure 16: Comparison of the percentage of Total hardness reduction after the growth of *Scenedesmus obliquus* and *Chlorella vulgaris* over a 21-day period at different densities

As shown in Table 2 and Figure 17, the comparison of nitrate changes in the two algae indicated a significant difference ($p<0.05$) between high density of *S. obliquus* algae with other treatments in the uptake rate by reducing from 1060.4 mg/L

to 349.80±37.40 mg/L (67.01% reduction). In our study, the phosphate level decreased from 16.5 mg/L at the beginning of the period to 0.50±0.05 (97.32% reduction) after treatment with high density of *S. obliquus* algae, which showed a significant difference (p<0.05) compared to both densities of *C. vulgaris* algae at the end of the period, in the rate of phosphate absorption and reduction (Table 2; Fig.18).

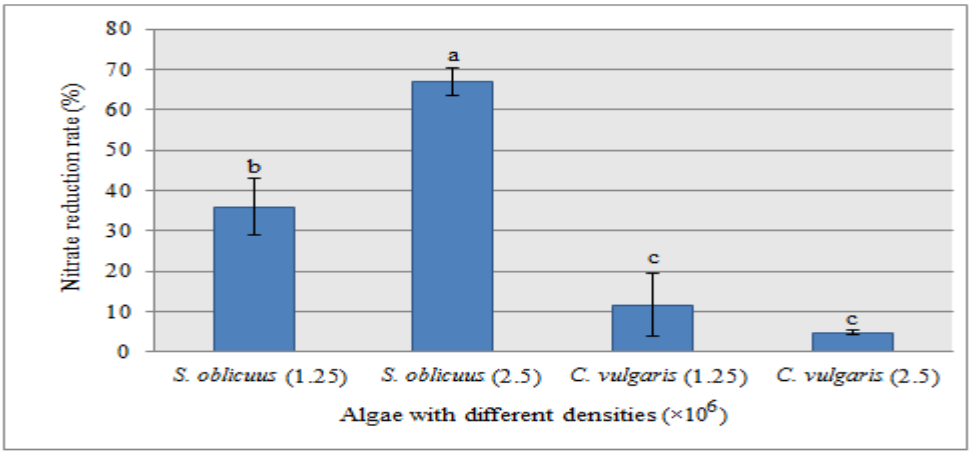


Figure 17: Comparison of the percentage of Nitrate reduction after the growth of *Scenedesmus obliquus* and *Chlorella vulgaris* over a 21-day period at different densities.

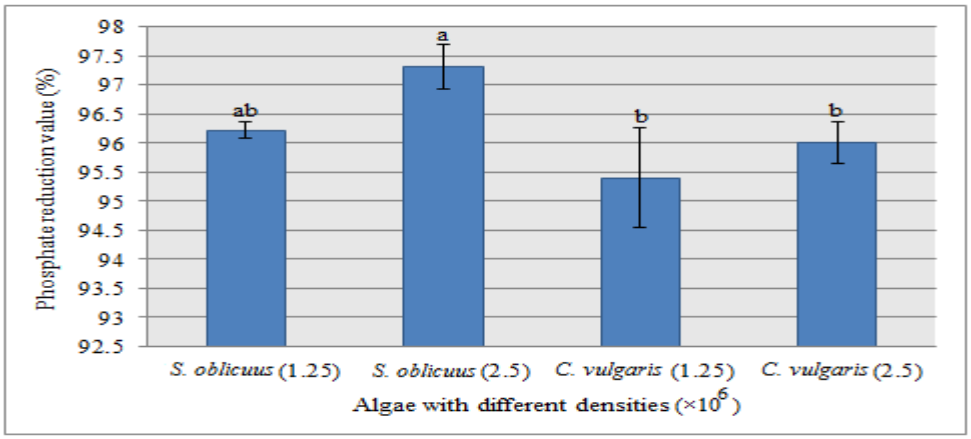


Figure 18: Comparison of the percentage of Phosphate reduction after the growth of *Scenedesmus obliquus* and *Chlorella vulgaris* over a 21-day period at different densities

Biological desalination rate and chloride bioaccumulation capacity

The results of the biological desalination rate with algae indicated a significant increase (p<0.05) in desalination rate in the high densities of algae *S. obliquus* with 28.5±2.50% compared to densities of algae *C. vulgaris* (Fig. 19).

The highest chloride bioaccumulation capacity at the end of the 21-day experiment period was observed in the high density of *C. vulgaris* algae (1997.2±117.74 mg/g dry weight of algae) which showed a significant difference (p<0.05) with the other treatments (Fig. 20).

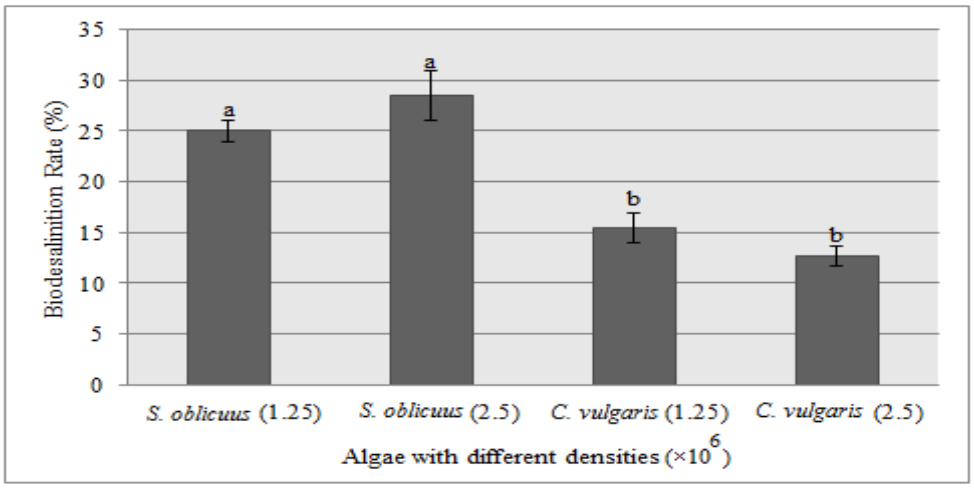


Figure 19: Comparison of the percentage of biological desalination rate after the growth of *Scenedesmus obliquus* and *Chlorella vulgaris* over a 21-day period at different densities

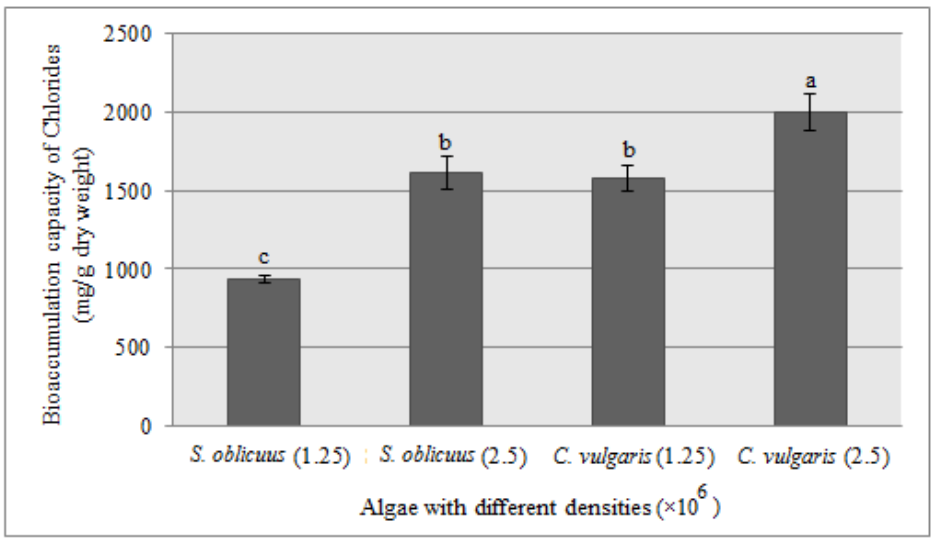


Figure 20: Comparison of the Bioaccumulation capacity of Chlorides after the growth of *Scenedesmus obliquus* and *Chlorella vulgaris* over a 21-day period at different densities

Discussion

Our study investigated the changes in physicochemical parameters of deep aquifer well water (No. 3) in the Sistan region using *S. obliquus* and *C. vulgaris* microalgae for the biological desalination for 21 days. Our results showed a decrease in biomass, algal cell number and specific growth rate in *S. obliquus* compared to *C. vulgaris*, after cultivation in deep aquifer well water, which is similar to the Maru study *et al*. (2020) on *S. obliquus* that the highest cell density (8.4×10^6) and biomass (450 mg/L) were observed at a lower concentration (2.5 g/L salt) in the salinities from 0 to 15 g/L NaCl, and with increasing salinity, algal growth parameters decreased. Also, in the study of Barahoei *et al*. (2021), the growth and number of *C. vulgaris* cells decreased with increasing NaCl salt concentration above 4000 ppm, which suggested that disruption of the K^+/Na^+ balance in algal cells due to the presence of Na^+ and Cl^- ions could be the

reason for the growth reduction. Increasing the concentration of Na^+ and Cl^- ions leads to an increase in osmotic pressure furthermore, when salinity values exceed the tolerance level of algal cells, the osmotic pressure exceeds the tolerance threshold of the Na^+ and Cl^- pumps, resulting in cell damage, disruption of the cell division process, and a decrease in growth rate (Barahoei *et al*., 2021). Microalgae respond to salinity and other stress conditions (e.g. dehydration, cold, excessive osmotic pressure) through various adaptive mechanisms such as morphological changes and growth patterns, as well as physiological and biochemical processes (Hiremath and Mathad, 2010). Stress adaptation is also accompanied by metabolic adjustments that lead to the synthesis and accumulation of several organic solutes and also osmolytes such as glycine betaine, polyols, amino acids, sugars, and proline (Hasegawa *et al*., 2000; Hoque *et al*., 2007). These osmotic adjustments in response to high salinity reduce oxidative damage caused by free radical production and protect intracellular structures (Hare *et al.,* 1998; Hiremath and Mathad, 2010). The slower growth of *S. obliquus* algae may be due to high osmotic pressure on algal cells and the process of cellular dehydration (Zafar *et al*., 2022).

Bioaccumulation is a metabolically dependent mechanism in which salts accumulate in algal cells. However, biosorption is a non-metabolic mechanism and involves the physical binding or adhesion of ions and molecules to the algal cell surface (Moghazy *et al*., 2022). In our study, given the reduced growth in *S. obliquus* and the observation of greater desalination (negative relationship) compared to *C. vulgaris*, it is likely due to the higher surface area to volume ratio of *S. obliquus* than *C. vulgaris*, so the biosorption mechanism occurred more than bioaccumulation which is consistent with the results of Wei *et al*. (2020) that showed both biosorption and bioaccumulation play a role in salt removal and salinity reduction in *S. obliquus*. They also reported that two thirds of the NaCl removal occurred within the first 30 min of contact time, which was mainly attributed to the biosorption process. A consortium of five UK universities set out to study the potential of using cyanobacteria to remove Na^+ and Cl^- from seawater, suggesting that algal strains with fast growth and larger cell surface area and internal aggregative volume would lead to better biosorption and bioaccumulation of salts (Amezaga *et al*., 2014) In the present study, maximum algal growth was observed in *C. vulgaris* compared to *S. obliquus* densities, which is consistent with the results of Luangpipat and Chisti (2017), who cultured *C. vulgaris* algal using BG-11 medium in freshwater, brackish water and seawater. They found the amount of dry algal biomass after the end of *C. vulgaris* cultivation experiments in freshwater, brackish water, and seawater to be 2.5, 3.5, and 2.9 g/L, respectively. Various studies have described the ability of freshwater *C. vulgaris* to survive and grow at salinities comparable to or even higher than seawater (Church *et al.,* 2017). Our study indicated a greater reduction in salinity by higher density of *S. obliquus* algae

compared to lower density This is in accordance with the results of the study by El-Sargani *et al*. (2014) and Balasubramaniyan *et al*. (2024) who reported that the TDS removal efficiency is proportional to the algae dosage amount. Also, in Figler's study (2019), the *S. obliquus* species showed a desalination rate of up to 26% compared to other algae. In our study, the type of algae in salinity harvesting showed no significant difference between *C. vulgaris* algae at both densities and the high density of *S. obliquus* algae. Previously, the results of a study investigating the efficiency of halophyte algae in reducing salinity with salinity concentrations from 2 to 20 g/L reported a salt harvesting efficiency of 36% at 25°C for *Scenedusmus* sp. and *C. vulgaris* algae (Sahle-Demessie *et al*., 2019). Our results showed that the high density of S. *obliquus* was more efficient than *C. vulgaris* in removing sodium, and iron ions, which is similar to the results of Demetriou *et al*. (2007) and Mohammed and Shafea (1992) who showed *S. obliquus* could absorb sodium, calcium and magnesium ions. Our results were also consistent with the results of El-Sayed and Abdel-Maguid (2010) in terms of sodium uptake by *Scenedesmus* sp. algae grown in treatments with different concentrations of 0, 25, 50, 75 and 100% Red Sea water (with a salinity of 45 g/L). In addition, other studies have also shown that *S. obliquus* removes sodium ions in alkaline brine with a removal efficiency of 3.15 g/L NaCl (Yaho *et al*., 2013). The reason for the reduction of sodium from water occurs in two ways: 1. Cellular structures can cause bioaccumulation by absorbing ionic pollutants into them. This is an aerobic metabolic process that is carried out with the consumption of energy in a living organism (Vijayaraghavan and Yun, 2008; Velasquez and Dussan, 2009). Various reports have indicated the removal of metal ions from water by the process of bioaccumulation of microalgae (Rangsayatorn *et al*., 2002; Gupta and Rastogi 2009). In addition to the accumulation of Na^+ ions in the cell structure of algae, which occurs over long periods of time, the cell walls of different algae have different functional groups. For example, green algae usually have a high percentage of proteins on their cell walls, providing amino acid functional groups (imidazole, hydroxyl, amine, sulfhydryl, amide, and carboxyl). Functional groups play a pivotal role in various salt removal mechanisms (Ivanova *et al*., 2012). Wei *et al*. (2020) used FTIR to analyze the surface properties of the microalga *S. obliquus*. They identified various functional groups, including -OH or -NH, -COOH and aldehyde with negative charges, which lead to effective biosorption of cations in water through electrostatic attraction. So, due to the binding of cations to functional groups on the surface of the algae cell wall, we see a reduction of salt in the algae medium (Gan *et al*., 2016).

Our results showed that the high density of *S. obliquus* was more efficient than *C. vulgaris* in removing phosphate and nitrate ions. Our results were also consistent with the results of El-Sayed and Abdel-Maguid (2010) in terms of nitrogen, phosphorus uptake by *Scenedesmus* sp. algae grown in Red Sea water (with a salinity of 45 g/L).

Algae consume nitrate and phosphate to synthesize osmotic regulators, which could explain the reduction of nitrate and phosphate in the studied water (Moudrikova *et al*., 2017). Zafar *et al*. (2021) reported that salinity had no effect on the reduction of nitrate and phosphate ions by the *Phormidium keutzingianum* algae also, higher salinities could not significantly affect the uptake of nitrate and phosphate, but higher N:P ratios could affect the uptake of these nutrients by the algae.

Our results indicated that *C. vulgaris* algae removed more chloride anion than *S. obliquus* algae. Figler *et al.* (2019) showed that *C. vulgaris* was able to remove significant amounts of chloride (up to 29%) compared to other algae species from the genera *Scenedesmus*, *Chlorococcum*, *Desmodesmus*, and *Monoraphidium*. Also, in our study, the highest chloride anion bioaccumulation capacity at the end of the experimental period was related to *C. vulgaris* algae compared to *S. obliquus* algae, which was in accordance with the results of Ghobashy *et al.* (2022), which reported the highest amount of chloride bioaccumulation in *C. vulgaris* algae compared to *Spirulina maxima* and *Scenedismus arcuatusa* algae at all seawater dilutions (25, 50, 75, and 100% dilution).

Our results showed higher potassium uptake by *C. vulgaris*, which was consistent with the results of a study by Zand *et al*. (2018) with 54% potassium removal efficiency by *C. vulgaris* from primary petroleum wastewater. The mechanisms of cation uptake by *C. vulgaris* include surface deposition, physical and biological adsorption, active transport, and passive diffusion (Gupta *et al*., 2008).

Wei *et al*. (2020) found that higher algae doses resulted in better salt removal. However, the salt removal efficiency was not directly proportional to the algae dose. They suggested that this was due in part to insufficient mixing between the algae and the salt solution and the equilibrium between the salts on the algae's surface and in the salt solution. Without sufficient mixing, the algae cells could easily coagulate in the large algae aggregates, affecting the salt removal efficiency. This could explain why low *C. vulgaris* densities were more effective in removing some of the contaminants than high densities.

Conclusion

Our results can be used for the pretreatment of deep aquifer well water (No. 3) to reduce treatment costs. According to the results obtained in our study, both algae have the ability to reduce deep aquifer well water salinity. A comparison of the two algae showed that despite the decrease in growth and biomass, *S. obliquus* algae was able to reduce salinity, TDS, sodium, and nutrients such as nitrate and phosphate more than *C. vulgaris* algae, and showed a higher desalination rate. It seems that the surface absorption process, due to the larger cell size and greater cell surface area of *S. obliquus* compared to *C. vulgaris*, was more involved in bioaccumulation. The *C. vulgaris* algae was also able to grow better in deep aquifer well water. It is recommended to: 1- Desalination

experiments with higher algae densities, 2- Measurement of ion content in algal biomass to confirm the observed reduction in Na^+, K^+ and Cl^- due to biological adsorption, 3- Analysis of potential pH changes caused by microalgal activity that may affect ion solubility and sedimentation.

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Conflicts of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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