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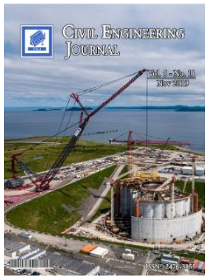

Investigated of Desalination of Saline Waters by Using Dunaliella Salina Algae and Its Effect on Water Ions

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Abstract

Nowadays, due to augmentation of population and expansion of cities and the limitation of fresh water sources in the world, existing water resources cannot meet the human needs. Desalination or reduction of salinity of water through biological methods involves the use of plant species, microorganisms, algae or a combination of them, which can be effective in reducing water salinity. The objective of this study was to investigate the desalination of saline waters by using Dunaliella salina algae. For this purpose, the experiments were carried out as factorial based on completely randomized design. The expected tests were conducted in a laboratory controlled condition for humidity, light and temperature (90 days). During this research, the Electrical Conductivity (EC) was measured daily. The results showed that salt absorption in Dunaliella salina algae was significantly different. High salt absorption was observed in Dunaliella salina at a concentration of 130mS.cm-1. Due to the constant humidity, light and temperature, salt removal from saline water was observed in laboratory conditions. The results of this study indicate that significant reduction of chlorine, sodium and bicarbonate levels were observed using Dunaliella salina algae. According to the results of this study, it seems that reducing salt absorption in algae is due to the use of salt in the metabolism and the growth and proliferation of algae. The absorption process in this study showed that the catch of Dunaliella salina has a good ability to remove salt and can be used as an appropriate suggestion for salt removal from saline water.

Keywords: Bio-desalination; Dunaliella Salina; Saline Water; Dry Weight.

1. Introduction

Today, because of using drinking water for uses such as agriculture and industry, the human race will suffer from water crisis in the near future [1-2] due to the world population growth and the limitation of global freshwater reserves, desalination from sea water has become more important [3-5]. The selection and development of desalination process is more efficient and less costly than existing methods, and also an essential requirement [6-8].

Arid countries are currently facing a water crisis, and given the population growth, industrial and agricultural development, this crisis will also increase as water requirements increase at various cost and intensify competition in various uses [6]. In arid and semi-arid countries, due to the conditions of the soil, the land, the climate of the region, and the lack of surface water and underground fresh water resources, the desalination of saline water (rivers and seas) and

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groundwater resources, is an important and inevitable option for water supply [9, 10].

Today, desalinization of saline water is carried out using various methods worldwide based on the quantity and quality of water required [11, 12]. The main methods of desalination include methods membrane for reverse osmosis (RO), multiple effect distillation process (MED), methods thermal evaporation, flash multi-stage (MSF), distillation vapor compression (VCD) and ion exchange (IX) each of these have their advantages and disadvantages that are applied in accordance with the regional conditions and needs [13-15].

The use of algae in salt removal from saline water, and the production of water for a variety of purposes, is a new and cost-effective concept. This is a new route that can be effective in solving the problem of desalinating at the lowest cost [16-18].

Algaes are simple plants species without stem and root and have chlorophyll matter. They range from small single-cell species to large and high-density species that have varied ability to grow in various aqueous and dry environments [19]. Most algaes species are able to grow properly in saline waters that absorb water solubility [20]. In this way, water salts, like other nutrients, are absorbed by the living creature (plant or animal) into biomass and ultimately, the absorption ratio of salts from water salinity will be reduced [7, 8]. In this case, some creatures are more likely to absorb water soluble than their nutritional needs, which is why they have more potential to reduce water salinity. Desalination or reduction of water salinity using biological methods involves the use of different species of macrofite (aquatic plants), microfite (algae) and micro-organisms or their combination, which can be effective in reducing the salinity of water [21].

Unlike organic plants, algae can complete their life cycle in a wide range of salinity, Micro-algae has been used for many years in treating wastewater of the third level to eliminate nitrogen and phosphorus compounds after lowering BOD and COD [22, 23]. Many microalgae such as Scenedesmus obliquus able to use the organic compounds under light conditions are mix-otrophic (In the biology of the growth process of organs using the combination of organic matter and minerals) and use an alternative secondary treatment to reduce the content of organic matter and nutrients designed to eliminate [24].

Some recent studies showed that the use of algaes in the desalinization of saline waters will have minimum cost. The results indicated positive effect of algae on desalinization of seawater, which is considered as new way for desalination of saline water by modern methods. Some results estimated that sewage with a quality of 35000 to 10900 $mg.L^{-1}$ salinity, 65 to 250 $mg.L^{-1}$ of oil and 56 to 212 BOD was treated with algae and results show that salinity to 2500 oil to 7 and BOD 15 $mg.L^{-1}$ were reduced [25].

Badawy et al. (2012) was designed to investigate the removal efficiency of various nutrients in saline water (TDS 40000 ppm) by using green Scenedesmus alga species through a continuous flow purification system in two reactors. They found that the total dissolved solids, sodium, chloride, and phosphate removal efficiency measured at the output of the second reactor is about 97%. In addition, removal of both nitrate and sulfate is about 93% [26].

Tien (2002) studied on several species of green algae and cyanophyce microscopy. The results showed that these species are capable of high salinity wastewater and exposure to high temperature and light intensity have the best production performance. Some species, such as the Scendesmus species, are capable of reducing the amount of salts in the highly saline wastewater (about triple) and increases the amount of salinity to the natural range, which is not a problem in the disposal of the sea. Species of Spirulina, Chlorella, Haematococcus and Dunaliella also produce high rates of biomass in high-salinity and high temperatures [48]**.** Some results show that the efficiency of removing various nutrients in saline water using green Scenedesmus algas species through a continuous flow purification system. The salt water was used from the Red Sea, the Ismaili province (TDS was 40000 ppm). Seaweed extract was added to each reactor for two consecutive reactors for 7days. Total soluble solids, sodium, chloride and phosphate the removal efficiency measured at the output of the second reactor was about 97% and the removal efficiency of each nitrate and sulfate was about 93% [11, 25, 26].

Microalgae Dunaliella salina is the most resistant species of eucaryate to salinity, seen in many saline environments such as lakes and saltwater lagoons. According to the results, the efficacy of the algae in high salinity can be effective in reducing the salinity of seawater, sewage, and recycle water from factories. In general, the results show that the use of green algae is beneficial for reducing water salinity. Fluctuations in physical and chemical factors such as salinity have significant effects on growth and biochemical composition of green microalgae [7, 19, 20].

Despite the great benefits of sweeteners, concerns have been raised about potential negative impacts on the environment. Due to the high evaporation of salt and added chemicals in the process of chlorination, pH adjustment, coagulation and flocculation, chlorination and addition of sediment materials can have negative effects on the environment. Algae not only have no negative environmental effects but can be used for other industries. Due to the high cost and saline problems in thermal and membrane methods, further studies have been done to reduce these problems. However, many studies have not been done in this study. In general, the results refer to the use of green algae to reduce water salinity. It is an easy and economical environmental solution for saline water in plants [25, 26].

Since more studies have been done on the purification and removal of heavy metals, the main objective of this project compared different species of micro algae is effectiveness in saline water desalination process.

2. Materials and Methods

2.1. Source of Water Used

High salinity water sample was collected from Sirjan Lake. The water sample was taken in sterilized bottles and kept for further studies. The source of water sample was transferred to the Central Lab that is located in Islamic Azad University, Tehran, Iran for analysis.

2.2. Microorganisms Used for Desalination

Dunaliella Salina

The algae is classified in Chlorophyceae category, Volvocales order and Chororophyta branch. Previously, this algae was placed in the family Polyblepharidaceae, but due to its similarity with Chlamydomonas algae, it was placed in Chlamydomonadaceae family [28-29]. Dunaliella Salina species are widely distributed in Iranian ecosystems due to their different physico-chemical characteristics [30]. The most resilient eukaryotic is the salinity found in many saline environments, including lakes and saline water lagoons. The salinity level for Dunaliella Salina is 3M (174 M), but the critical salinity points are 0.5 M (29 M) and 5 M (290 M).

Chlorella

Chlorella is a genus of single-celled green algae belonging to the division Chlorophyta. It is spherical in shape, about 2 to 10μm in diameter, and is without flagella. Chlorella contains the green photosynthetic pigments chlorophyll-a and -b in its chloroplast. Through photosynthesis, it multiplies rapidly, requiring carbon dioxide, water, sunlight and a small amount of minerals to reproduce.

Nano-Chloropysis

Nano-Chloropysis oculata is one of the single-cell marine algae belonging to the branch of Chlorophyta and the sub-branch of Eustigmatophyceae.

Scendesmus

The algae is from the Scenedesmaceae family and is one of the important single-cell algae belonging to the Chlorophyceae family. The species Scenedesmus obliquus, Kützing 1833 is an important commercial species for the removal of some toxic cations such as cadmium from water sources that are good for the industry [31].

2.3. Different Steps of the Research Method

The first stage: Different types of micro-algae and macrophyte species were found to be effective in desalination processes. In addition, the efficiency and effectiveness of each one were determined on the basis of library reviews.

The second stage: a laboratory scale pilot was designed to evaluate the effect of selected algae on reducing the salinity of the water and achieving the principles for the design and use of these algae for desalination.

The third stage: After transferring the water sample to the laboratory, algae were placed in the EC in proportion to their salinity (ranging salinity from 50 to 140 $dS.cm^{-1}$).

2.4. Algae Sample and Preservation Conditions

In this research, green algae including Dunaliella Salina, Chlorella, Nano-Chloropysis and Scendesmus were used. Samples were placed in the reactor under laboratory conditions. The PVC tank (5-liter) with input and output tanks at the same speed (speed adjusted by control valves) was considered as a temporary medium. For each treatment, three replicates (3 continuous systems) were considered. To each of the tanks, 3cc of algae was added in water with different salinity. The reactor was made of 10 liter transparent polyethylene bottles and the maximum supply of light necessary for optimal growth of algae in the 3500±350 lux daily reactor and at 25±2°C in the light protocol of 12 hours of light and 12 dark hours [32]. To generate water flow and rotation, and eventually transfer algae as well as provide carbon dioxide for photosynthesis and oxygen necessary for respiration, alternate water aerating was performed using air pump and Electrical Conductivity (EC) was measured for 7days by EC meter (Horiba-meter model Es-14 E). Algae in addition to physical factors including light, temperature and aeration need vital activity for medium and it includes micro and macro elements [33]. In this research, without using food elements, water salts, like other nutrients, were absorbed into the biomass by the living creature (plant or animal) and reduced to salinity by absorbing salts. In this regard, some creatures are more likely to absorb water salts than nutritional needs, and therefore have more potential to reduce water salinity. After the end of each period, the amount of chlorine, sodium and bicarbonate was also measured. At the end of each period, samples of water and algae were transferred to the laboratory and the amounts of sodium, chlorine, calcium, magnesium and bicarbonate were measured.

2.5. Statistical Analysis

This pattern had factorial based on completely randomized design with three application. Statistical analysis was performed by the SPSS statistical package. The SPSS procedure was used for analysis of variance to determine the statistical significance of treatment effects.

3. Results and Discussion

3.1. Comparison of Desalination in Different Algae

Data were analyzed following analysis of variance and means were compared based on Duncan at the 0.01 probability level (Table 1).

Table 1. Analysis of variance for desalination in different algae

Source of variance	df	Total square	Means square	f	Significance level
Treatment	10	7310.493	731.049	26.389	<0001
Error	202	5596.052	27.703	202	-
Total	212	12906.544	-	212	-

The highest desalination was observed in Dunaliella salina algae at different electrical conductivity (EC). In general, by decreasing the electrical conductivity (EC), the desalination rate in Dunaliella salina algae decreased significantly, so that these treatments had the lowest absorbance (Figure 1). Therefore, based on the experiments conducted, Dunaliella salina algae was selected for this research.

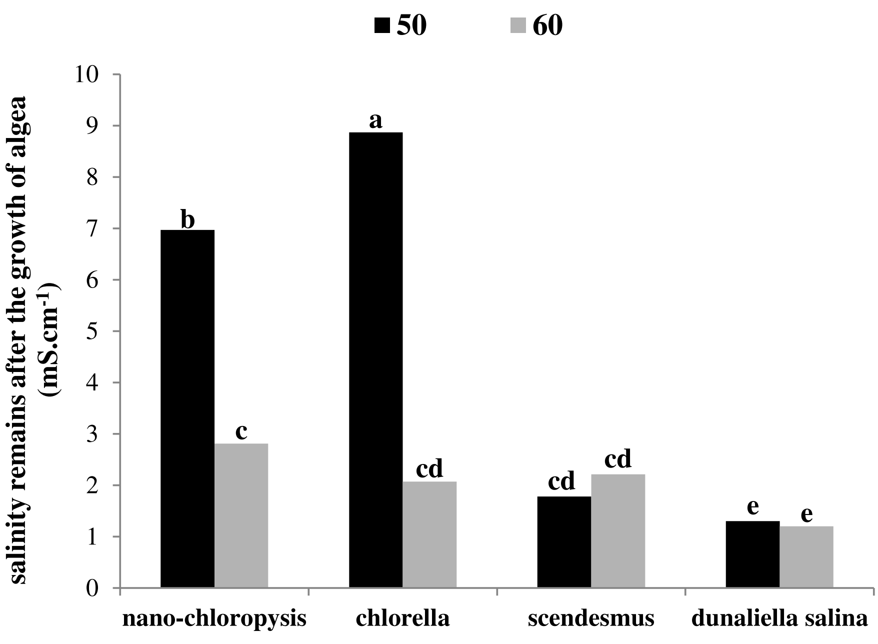

Figure 1. Comparison between different algae for desalination. Different small letters on the columns indicate treatment means significantly different at p<0.01

3.2. Comparison of Desalination in Dunaliella Salina Algae

The results of ANOVA test showed that salt absorption in different concentrations was significantly different (p <0.0001).

Table 2. Analysis of variance for desalination in Dunaliella salina algae

Source of variance	df	Total square	Means square	f	Significance level
Treatment	4	3432.527	858.132	3217.994	<0001
Error	10	2.667	0.267	-	-
Total	14	3435.193	-	-	-

The results show that in the Donalila salina algae in five different EC, including 50, 70, 100, 130 and 140 mS.cm^{-1}, with an increase in concentration from 70 to 130 mS.cm^{-1}, the absorption of salt in algae was significantly increased from 1.93 to 42.67 mS.cm^{-1} (about 22 times), but with an increase in electrical conductivity (EC) from 130 to 140 mS.cm^{-1}, the decrease in salt absorption was observed at 12.70 mS.cm^{-1}. At low concentrations of 50 and 70 mS.cm^{-1}, salt absorption in algae was not significant ($p \leq 0.05$).

According to the results, as shown in Figure 2, the highest absorption in Donalila salina algae was at a concentration of 130 mS.cm^{-1}. In Donalila salina algae, by providing environmental conditions including light, temperature and aeration, electrical conductivity (EC) reduction was observed to be 42.67 mS.cm^{-1} (7 days).In this research, the goal of reducing the salinity of water is economical and as a result, the micro and macro elements used in the algal growth medium did not add to the incubator. The results show that by increasing the electrical conductivity from 50 to 130 mS.cm^{-1}, the amount of desalination in algae increases about 7 times, significantly (1.33 to 22.5 mS.cm^{-1}), but increasing the electrical conductivity from 130 to 140 mS.cm^{-1} decreased the desalination in Dunaliella salina. The amount of desalination in algae was not significantly different at low electrical conductivity of 50 and 60mS.cm^{-1} ($p \leq 0.05$). Park et al. (2010) and Kesaano and Sims, (2014) investigated that the ability of these algae to tolerate extreme environmental conditions is unmatched in terms of light intensity, temperature, food availability and salinity levels among photosynthetic organisms [16, 18]. Since light intensity and salinity are two important and effective environmental factors for Dunaliella salina algae, so in a dense environment, Dunaliella salina can live in this environment. High salt concentrations lead to the production of reactive oxygen species through variation, which can be effective in absorbing salt from the environment. Therefore, microscopic algae are used as a clean and renewable resource in the biotechnology industry [34-41].

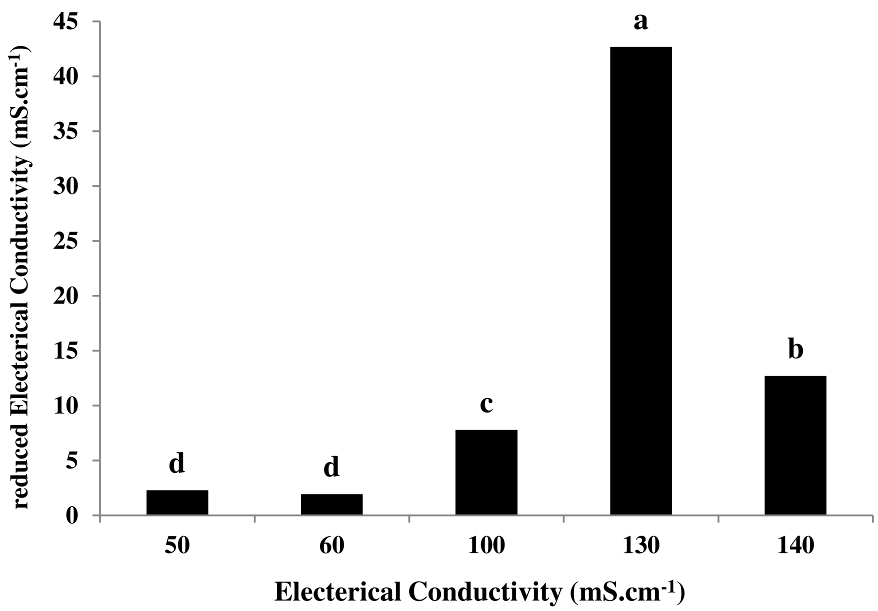

Figure 2. Comparison of electrical conductivity (EC) reduction in Donalila algae after 7 days growth. Different small letters on the columns indicate treatment means significantly different at $p < 0.01$

3.3. Comparison of Ion Absorption in Dunaliella Salina Algae (salinity of 130 mS.cm^{-1})

Data were analyzed following analysis of variance and means were compared based on Duncan at the 0.01 probability level (Table 4).

Table 4. Analysis of variance for ion absorption in different algae

Source of variance	df	Total square	Means square	f	Significance level
Treatment	2	87841.005	43920.503	1226.544	<0001
Error	6	214.850	-	-	-
Total	8	88055.855	-	-	-

3.4. Ion Absorption Rate (Salinity of 130 m S.cm^{-1})

Chloride absorption rate

The results show that chloride absorption in Dunaliella salina algae has a significant difference ($t=60.230$, $df=4$, $p<0.001$). The amount of chloride in saline water containing Dunaliella salina algae (1450.57 mg.l^{-1}) was significantly lower than saline water (2168.65 $mg.l^{-1}$). In other words, it can be said that Dunaliella salina reduced chlorine by 718$mg.l^{-1}$. The results of the research have shown that the presence of compounds in the algal surface, such as amines, phosphates, sulfites, sulfidryls, carboxyls, proteins, and saccharides, have been activated in many respects, such as ion exchange resins [21, 42]. These compounds, such as amino groups, when combined with proteins, have a positive charge and can absorb complex compounds, such as phenolic compounds, which absorb electrostatically charged negative electrically charged electrons, which have a negative electrical charge electrostatically absorbed. Therefore, chlorine is absorbed from the environment by algae and its amount decreases. In addition, algae based systems for bio-desalination have been in development for 3 decades, and despite being found to be potentially cheaper, more effective, and environmentally friendly than conventional treatment it has failed to become a standard process. As with algal biofuels the main problem is separating the cells from the liquid, which is an energy intensive process. According to the findings, algae in this group are likely to be present in an active anion group [43-50].

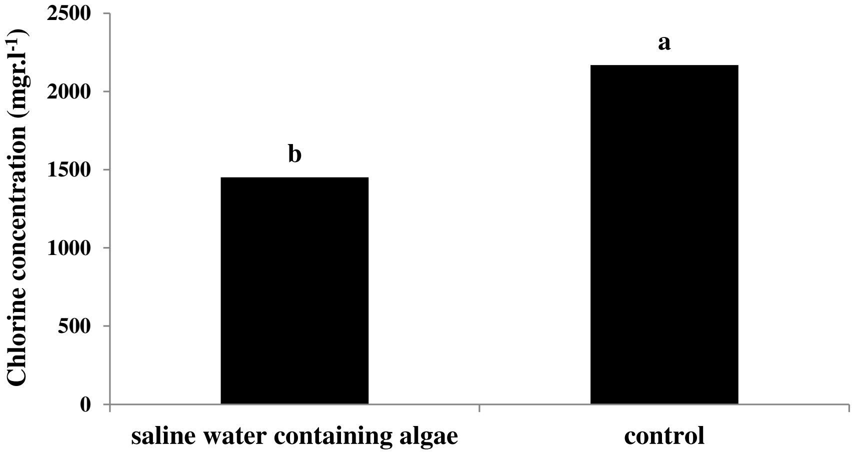

Figure 3. The results of the comparison of means chloride absorption. Different small letters on the columns indicate treatment means significantly different at $p < 0.01$

Sodium absorption rate

The amount of sodium in saline water (130 $mS.cm^{-1}$) and saline water containing Dunaliella salina alga had a significant difference ($t = 81.643$, $df= 4$ and $p \leq 0.001$). Sodium content in saline water containing Dunaliella salina algae (1480.28 $mg.l^{-1}$) was significantly lower than saline water (2100.20 $mg.l^{-1}$). In other words, Dunaliella salina algae reduced the sodium content by 620$mg.l^{-1}$. The results of Badawy et al. (2012) have been shown that increasing the absorption of sodium by algae is due to the fact that, over time, algae surface openings are opened and cations can enter the grooves [26]. As a result, absorption in algae is in two stages. So that absorption of the first stage is related to active algal groups that are exposed directly to cations. The absorption of the second stage is relatively low in long periods of time due to the cation bond to the surface groups. Tien, (2002) found that the magnitude of anions showed easy uptake of surface area and dry weight of algal cells. It was found to be the main factor influencing anion sorption and indicates favorable adsorption [48]. Biosorption using biomass derived from fresh water, algae, marine seaweeds and fungi have recently attracted growing interest of researchers. Many potential binding sites occur in algal cell walls and alginate matrices. Existing ligands, such as the carboxyl group or other functional groups in the cell wall of the algae, cause that cation to be absorbed. As a result, a negative charge is generated and the attraction between the negative charge and the cation increases the absorption on the cell surface and ultimately reduces the cation in the environment [51-53].

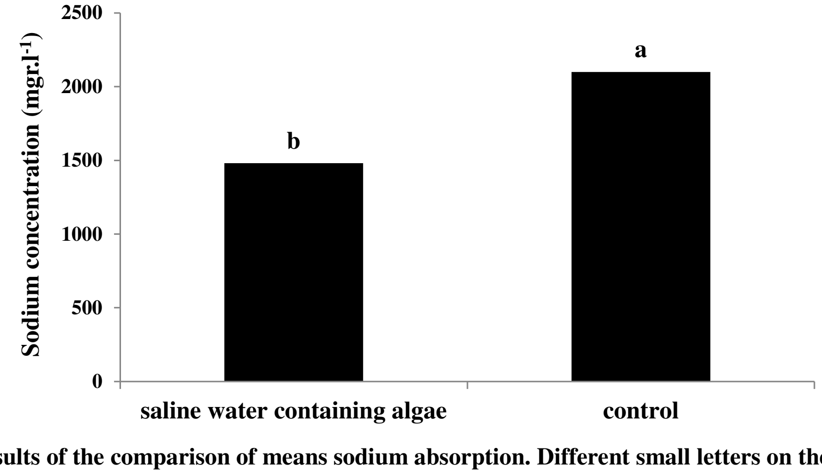

Figure 4. The results of the comparison of means sodium absorption. Different small letters on the columns indicate treatment means significantly different at $p < 0.01$

Bicarbonate absorption rate

There was a significant difference in the amount of bicarbonate in saline water and saline water containing Dunaliella salina algae (t = 6.124, df = 4 and p = 0.004). The amount of bicarbonate in saline water containing Dunaliella salina algae (1480.28 $mg.l^{-1}$) was significantly lower than saline water (2100.20 $mg.l^{-1}$). It can be said that Dunaliella salina algae reduces bicarbonate by 1.25 $mg.l^{-1}$. Carbon is an essential factor for the growth of microalgae. In general, the carbon source of microalgae under cultivation is atmospheric CO_2, which is naturally 300 ppm. However, it can be said that the metabolic efficiency and the combination of microalgae for use CO_2 and bicarbonate and carbonate as a carbon source can vary from species to species. Results indicated that one of the sources of algae consumption is carbon use. Therefore, bicarbonate is used to supply carbon and reduces its amount in the environment. White et al. (2013) showed that the presence of different concentrations of sodium bicarbonate on the growth of two species of marine microalgae is effective and it can be stated that it is considered as a food source for the growth and development of algae [54-56].

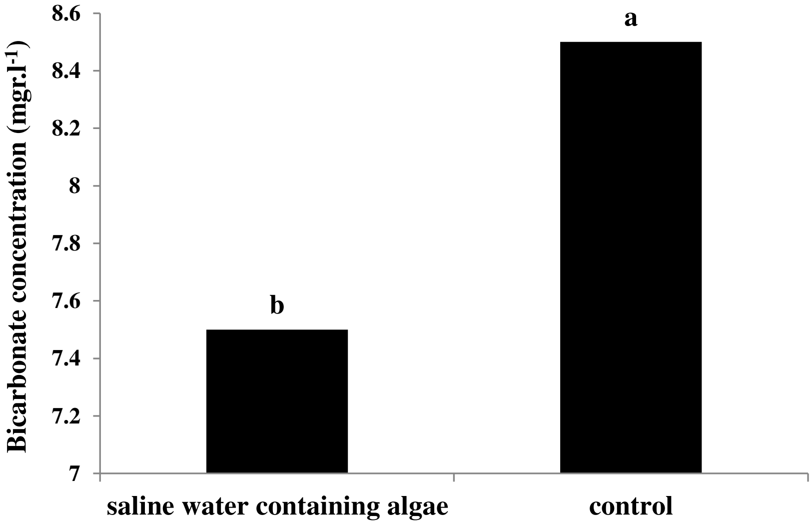

Figure 5. The results of the comparison of means bicarbonate absorption. Different small letters on the columns indicate treatment means significantly different at p < 0.01

3.5. Comparison of Dry Weight in Dunaliella Salina Algae

Data were analyzed following analysis of variance and means were compared based on Duncan at the 0.01 probability level (Table 5).

Table 5. Analysis of variance for dry weight in Dunaliella salina algae

Source of variance	df	Total square	Means square	f	Significance level
Treatment	4	0.994	0.249	330.03	<0.0001
Error	10	0.008	0.001	-	-
Total	14	1.002	-	-	-

The highest dry weight was observed in electrical conductivity of 130 $mS.cm^{-1}$, with dry weight of 1.08 gr. (Figure 6). Significant reduction in cations and anions, and on the other hand, the dry weight gain measured after the end of the test, indicates that these algae use elements of chlorine, sodium and bicarbonate to grow and reproduce as nutrients. Depending on the comparison of the amount of algae desalinating with the amount of dry matter, algae has the highest dry matter in salinity, which has the highest salt content. The main environmental factors and chemical composition affecting micro-algae growth are light, food, pH and temperature. Adjusting the above factors can greatly help them to grow better. Light and salinity are important factors that their oscillation influences chlorophyll, carotenoids and cell growth. The results of research El Sergany et al. (2014) show that the ability of algae to use salinity is higher with its availability, and algae plays a role in their growth and reproduction by reducing salinity as the absorption of salts and their use in metabolism [57].

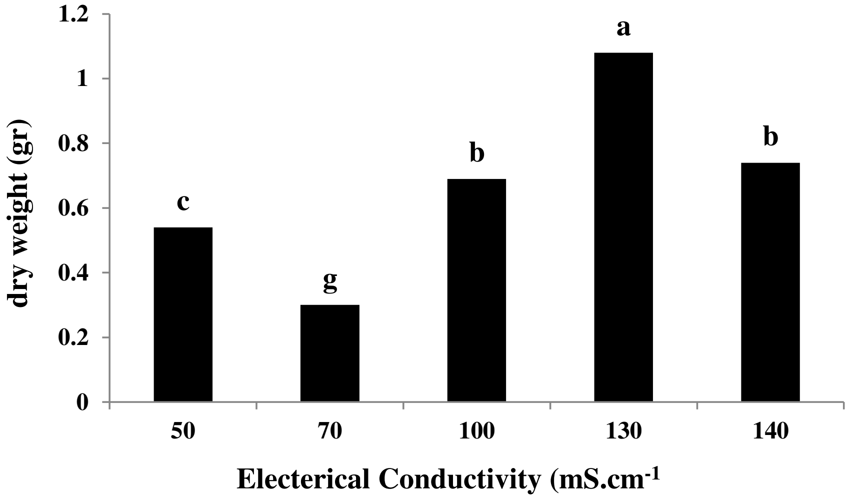

Figure 6. The results of the comparison of means dry weight. Different small letters on the columns indicate treatment means significantly different at p<0.01

4. Conclusion

In general, the results of this study indicate that the use of Dunaliella Salina will be effective in reducing salinity. In fact, this can be said to be an environmental to environmental problems caused by saline water. The growth of Dunaliella salina species in saline water has been very effective in absorbing salts and reducing salinity. Depending on the comparison of the amount of algae desalinating with the amount of dry matter, algae has the highest dry matter in salinity, which has the highest salt content. The results are expressed without supplementing the food needed for the growth of algae, and when added to the food, more salinity is reduced, which should be considered economically. Advantages of using this proposed system are low cost with easy construction, low performance and maintenance cost without any need for energy and in addition to reducing environmental pollution. The basis of algae usage in the removal of salt from saline water is a new concept for the production of fresh water, which is in fact a cheap and effective tool for eliminating salinity, which itself causes ecological safety in aquatic ecosystems. In addition, in this way, the water pollutants such as nitrogen compounds, heavy metals and phosphorus compounds are eliminated simultaneously. In general, it can be argued that the production of algae biomass plays a significant role in the production of biofuels, cosmetics, drugs and protein substances.

5. Conflicts of Interest

The authors declare no conflict of interest.

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