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# Utilization of Regional Natural Brines for the Indoor Cultivation of *Salicornia europaea*

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**Abstract:** Scaling agriculture to the globally rising population demands new approaches for future crop production such as multilayer and multitrophic indoor farming. Moreover, there is a current trend towards sustainable local solutions for aquaculture and saline agriculture. In this context, halophytes are becoming increasingly important for research and the food industry. As *Salicornia europaea* is a highly salt-tolerant obligate halophyte that can be used as a food crop, indoor cultivation with saline water is of particular interest. Therefore, finding a sustainable alternative to the use of seawater in non-coastal regions is crucial. Our goal was to determine whether natural brines, which are widely distributed and often available in inland areas, provide an alternative water source for the cultivation of saline organisms. This case study investigated the potential use of natural brines for the production of *S. europaea*. In the control group, which reflects the optimal growth conditions, fresh weight was increased, but there was no significant difference between the treatment groups comparing natural brines with artificial sea water. A similar pattern was observed for carotenoids and chlorophylls. Individual components showed significant differences. However, within treatments, there were mostly no changes. In summary, we showed that the influence of the different chloride concentrations was higher than the salt composition. Moreover, nutrient-enriched natural brine was demonstrated to be a suitable alternative for cultivation of *S. europaea* in terms of yield and nutritional quality. Thus, the present study provides the first evidence for the future potential of natural brine waters for the further development of aquaculture systems and saline agriculture in inland regions.

**Keywords:** carotenoids; glasswort; land-based aquaculture; seawater; phytochemicals; halophytes; salt composition; chlorophylls; artificial salt; saline agriculture

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## 1. Introduction

Water scarcity already affects 1.2 billion people worldwide, and this development will be exacerbated by the impacts of climate change in the future [1,2]. Faced with climate change-induced declines in drinking water resources and global population growth, alternative strategies for sustainable water use in agriculture are urgently needed to ensure food security and nutrition in the future [3]. Additionally, urbanization and limited agricultural land has led to a necessity for alternative cultivation systems, such as vertical farming, which offers efficient production sites and is therefore becoming extremely relevant in regard to future cultivation [4]. Importantly, with more than 95% of the world's

water resources being saline, aquatic food has been highlighted as one of the seven priorities to end hunger and protect the planet, and thus, saline agriculture is gaining increasing attention [5–7]. Consequently, the cultivation of salt-tolerant plants (halophytes) is an emerging field both in research and the food industry [8–11]. In this context, indoor cultivation with regional brine waters has three distinct advantages. First, indoor cultivation provides year-round fresh green leafy vegetables that are increasingly needed in today's and tomorrow's diets. Second, short supply chains result in fresh, sustainable products. Finally, the use of brine water in indoor aquatic systems is more cost-effective than the use of artificial seawater, which is also an important factor in terms of the economic potential of indoor aquatic cultivation systems [12].

Halophytes are widely distributed across several plant families. Next to the well-known salt-tolerant varieties of crops, such as quinoa, sugar beet, and barley, less attention has been paid to the herbs and vegetables that have been consumed in coastal regions for centuries [13–15]. Among these are members of the genera *Salicornia* which are found in coastal areas worldwide and are traditionally used as forage and fodder [16]. Given their natural distribution, members of *Salicornia* can be grown on saline soil and are used for its remediation [17–19]. In our research, we focus on *S. europaea* that is widespread on inland salt marshes and European coastal areas and is currently making its way into European supermarkets either as a fresh product or dried herb.

The nutritional value of *S. europaea* is mainly due to its richness in secondary metabolites, such as chlorophylls, carotenoids, saponins, flavonoids and flavanones, or lignans [20]. Epidemiological studies indicate that secondary plant metabolites or plant-based foods can lower the risk of various non-communicable diseases such as diabetes, cardiovascular diseases, eye-related disorders, and several types of cancer [21,22]. This also highlights halophytes as a potential source for new nutraceuticals [23]. Chlorophylls, for instance, can prevent DNA damage and thus have chemo-protective properties. Among the carotenoids, the main focus in green leafy vegetables is on β-carotene content because of its provitamin A activity as well as lutein and zeaxanthin because of their relevance in eye health, as for example they can help to prevent age-related macular degeneration [24,25]. In addition to their health-promoting properties, chlorophylls and carotenoids are essential for plant metabolism as they have a key role in photosynthesis. Therefore, chlorophylls and carotenoids are not only indicators of the nutritional value of food, but are also a part of the plant's adaptation system to changing environmental conditions.

As *S. europaea* is an annual plant and an obligate halophyte with salt tolerance up to 720 mM (4.2%), indoor cultivation with saline water is of great interest [8,26–28]. However, domestically, saline irrigation with seawater or water with added sodium chloride creates the problem of introducing salt into the regional cycle. To overcome these issues, we used regional brine water for cultivation. Natural brine springs are widely distributed and often available in inland areas. As natural brines can have different compositions, we used water from two natural brine locations in Germany.

Previous studies using *S. europaea* have already investigated how saline water affects growth, germination, and seed quality as well as demonstrated successful cultivation with seawater, sodium chloride solutions, brackish, or waste water, thereby highlighting *S. europaea* as a suitable model plant to test natural brines in this case study [8,9,28–32]. However, to date, none of these studies have addressed cultivation with natural brines. Therefore, to test the suitability of natural brine for saline indoor cultivation, (1) we investigated whether *S. europaea* can be cultivated with natural brines, (2) we characterized the basic composition of the salt-enriched nutrient solutions, and (3) we determined the yield and concentration of selected secondary metabolites to assess nutritional quality.

## 2. Materials and Methods

### *2.1. Chemicals*

Methanol and acetonitrile (Chemsolut for LC/MS; Th. Geyer), tetrahydrofuran (HiPerSolv Chromanorm for LC-MS), and dichloromethane (PESTINORM for GC-capillary analysis) were purchased from VWR International GmbH (Darmstadt, Germany). Isopropanol was obtained from Merck KGaA (Darmstadt, Germany). Ammonium acetate (≥97%), formic acid (Rotipuran, ≥98%), tert-butyl methyl ether, sodium chloride (plant treatment, >99.8%), and sodium hydrogen carbonate (≥99.5%) were obtained from Carl Roth GmbH (Karlsruhe, Germany). The carotenoid standards were purchased from CaroNature GmbH (Munsingen, Switzerland). Chlorophyll standards (chlorophyll *a* and *b*), sodium carbonate (≥99%), potassium hydrogen phosphate (≥99.0%), sodium bromide (≥99%), sodium chloride (IC, ≥99%), sodium nitrate (≥99%), sodium sulfate (≥99%), sulfuric acid (≥95%), and oxalic acid (≥98%) were purchased from Sigma Aldrich Chemie GmbH (Taufkirchen, Germany).

### *2.2. Natural Brines*

Two different natural brines were used in the present study: brine water from location 1 (BW1: Bad Saarow, Brandenburg (N 52° 17.47 E 14° 3.62); 2.37% salt (Na 8.65 g $l^{-1}$, Ca 0.52 g $l^{-1}$, Mg 0.25 g $l^{-1}$, Cl 14.57 g $l^{-1}$, $SO_4^{2-}$ 0.29 g $l^{-1}$, $HCO_3$ 0.24 g $l^{-1}$)) and brine water from location 2 (BW2: Heiligenstadt, Thuringia (51° 22.61 E 10° 8.63); 27.5% salt (Br 96.8 mg $l^{-1}$, Na 100.8 g $l^{-1}$, K 1.34 g $l^{-11}$, Ca 1.53 g $l^{-1}$, F 4.4 mg $l^{-1}$, Cl 163.6 g $l^{-1}$, Mg 1.6 g $l^{-1}$, S 7.0 mg $l^{-1}$)). Both locations, approximately 360 km apart, are situated in the inner land of Germany and provide natural geothermal brines used in therapeutic spas.

### *2.3. Plant Material and Cultivation Conditions*

The seeds of *Salicornia europaea* were purchased from Rühlemann's Kräuter and-Duftpflanzen (Horstedt, Germany). The plants were germinated and grown on Grodan® delta (4 × 4 × 4.5 cm; rock wool) growth media in a climate chamber with the following settings: light intensity, 200 µmol $m^{-2}$ $s^{-1}$; temperature, day 20 °C, night 16 °C; $CO_2$, 400 ppm; photoperiod, 12/12 h (day/night); humidity, 75%. Four weeks after germination, the plants were transferred to pots containing 1/3 soil (substrate type T, pH 5.9, N 180 mg $l^{-1}$, $PO_4^{2-}$ 180 mg $l^{-1}$, K 260 mg $l^{-1}$, Mg 130 mg $l^{-1}$) and 2/3 fine quartz sand with the grain size of 0.5–1 mm (Euroquarz GmbH [Laußnitz, Germany]) with six plants per pot on average. The plants were irrigated with a nutrient solution ($NH_4NO_3$ 0.6 mmol $l^{-1}$, $Ca(NO_3)_2$ 1.04 g $l^{-1}$, $KNO_3$ 0.81 g $l^{-1}$, iron chelate 8 $e^{-6}$%, $KH_2PO_4$ 0.31 g $l^{-1}$, $MgSO_4$ 0.54 g $l^{-1}$, $MnSO_4$ 2.5 mg $l^{-1}$, $Na_2[B_4O_5(OH)_4]\cdot 8H_2O$ 3.6 mg $l^{-1}$, $CuSO_4$ 0.2 mg $l^{-1}$, $Na_2MoO_4$ 0.1 mg $l^{-1}$, $ZnSO_4$ 0.4 mg $l^{-1}$) and enriched with salts of different origin and concentrations for the treatments.

### *2.4. Experimental Design*

To investigate the feasibility of brine water for halophyte cultivation, five different saline water treatments were applied: a control, a sodium chloride solution (2.4% NaCl (24 g $l^{-1}$), an artificial sea water salt Tropic Marine™ (2.4% TM (24 g $l^{-1}$)), which is commonly used in aquaculture and is more comparable for several experimental approaches, and the two natural brines BW1 (2.37% salt; brine water location 1 (Bad Saarow)) and BW2 (2.4% salt; brine water location 2 (Heiligenstadt)) (Figure 1) [33]. The control condition (1% NaCl (10 g $l^{-1}$), control group) was applied, as it proved to be the optimal growth condition for *S. europaea* in a preliminary experiment (unpublished data). The salt concentration of 2.4% of the treatment group was chosen regarding to the natural salt concentration of BW1 (Bad Saarow) and is also in the salt tolerance range of up to 4.2% of *S. europaea* [28].

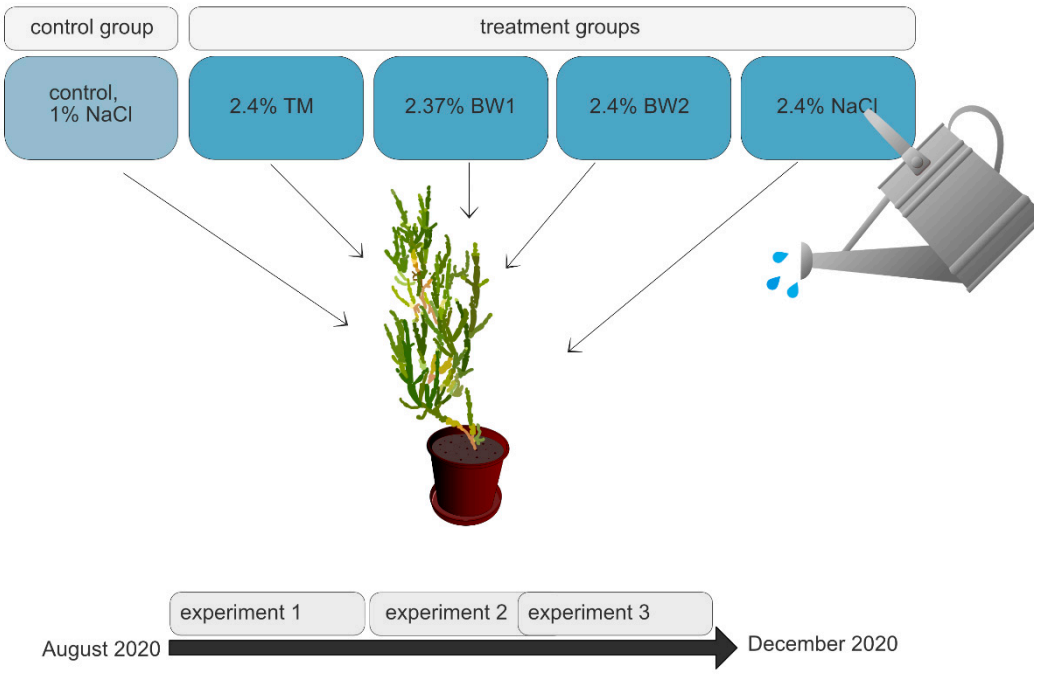


**Figure 1.** Experimental design to determine feasibility of natural brines for cultivation of *S. europaea*. TM, artificial sea water Tropic Marine; BW1, brine water location 1 (Bad Saarow); BW2, brine water location 2 (Heiligenstadt); NaCl, sodium chloride solution.

### *2.5. Experimental Procedure*

The treatment started six to seven weeks after germination under cultivation conditions and plants were harvested after three weeks of treatment. Per experiment five biological replicates were in each treatment group and the experiment was repeated three times (experiments 1, 2, and 3). Experiments 1, 2, and 3 (exp. 1, 2, and 3) represent three independent repetitions. Experiment 1 was performed in August to October 2020 and experiments 2 and 3 in September to November 2020 (Figure 1). While harvesting, fresh weight of the aboveground part of the plant was determined and then plants were frozen immediately in liquid nitrogen and freeze dried for one week. For further analysis, samples were homogenized by grinding (for 3–5 × 50 s with 3–5 metal beads (Ø 9 mm) at 25 Hz) using a Retsch mill (Retsch MM 400; Retsch GmbH, Haan, Germany).

### *2.6. Purchased Salicornia Product*

To compare nutritional quality with a purchased product grown under field conditions in seawater, a fresh *Salicornia* was purchased from an online store in 2019. Based on its specifications, the plant was harvested from the Northeast Atlantic FAO 27 IVc where it was grown naturally. The sample was freeze dried for one week and prepared for further analysis as described above.

### *2.7. Determination of Anion Concentrations in the Saline Solutions*

Chloride, nitrate, phosphate and sulfate content in the salt solutions were determined by ion exchange chromatography (IC). For this, 120 µL nutrient solution was diluted up to 12 mL ultrapure water, including 400 µL sodium bromide (0.6 mg $mL^{-1}$) as an internal standard. The samples were shaken for better homogenization. The measurements were performed with an ion chromatograph 930 Compact IC Flex (Metrohm AG, Herisau, Schweiz) equipped with a conductivity detector with suppression system. The injection volume was 20 µL at a flow rate of 0.7 mL $min^{-1}$ with an eluent consisting of $Na_2CO_3$ (3.2 mmol $l^{-1}$) and $NaHCO_3$ (1 mmol $l^{-1}$) and a Metrosep A Supp5 column (Metrohm AG,

Herisau, Schweiz; 250 mm, 4 mm). The final concentration of anions was calculated with an external calibration from standards of chloride, phosphate, nitrate, and sulfate using MagIC Net 3.2 software.

### *2.8. Determination of Chlorophylls and Carotenoids in the Plants*

Carotenoids and chlorophylls were determined as previously described in Frede et al. [34] with slight modifications. First, 10 mg homogenized, lyophilized plant material was extracted three times with 0.5 mL methanol/tetrahydrofuran (1:1, v/v). The samples were evaporated under nitrogen stream until dryness, dissolved in 50 µL dichloromethane and 200 µL isopropanol, and then filtered through PTFE-filter tubes (0.2 µm, Thermo Fischer Scientific Inc., Wilmington, USA) before transferring to HPLC vials. The analysis was performed with an Agilent Technologies 1290 Infinity UHPLC coupled with a ToF (Agilent Technologies Sales and Services, GmbH & Co. KG, Waldbronn, Germany). The analytes were separated on a C30 Carotenoid column (YMC Co. Ltd., Kyoto, Japan; 100 × 2.1 mm, 3 µm) at 20 °C. Identification was performed based on HR-MS data and UV/VIS spectra. Quantification was achieved by external calibration with carotenoid standards of all-*trans*-isomers from β-carotene, lutein, zeaxanthin, and (9*Z*)-neoxanthin as well as chlorophyll *a* and *b* standards at the wavelength of 450 nm. The quantification results of individual chlorophylls and carotenoids are cumulated as the total chlorophyll and total carotenoids.

### *2.9. Statistical Analysis*

Statistical analysis was performed with SigmaPlot 14.0. Statistical differences were tested by a one-way ANOVA followed by a post hoc test using the Holm–Sidak method ($p \leq 0.05$) when normal distribution and equal variance were present. When either normal distribution or equal variance was absent, a Kruskal–Wallis one-way ANOVA on ranks was performed, followed by the Tukey test. Normal distribution was tested with the Shapiro–Wilks normality test and equal variance with the Brown–Forsythe equal variance test. Data are presented as means ± standard deviation (SD) of the three independent experiments (experiments 1, 2, and 3).

## 3. Results

### *3.1. Composition of the Different Saline Solutions*

To determine the differences between the different saline solutions, we analyzed the anion concentrations. The values were very similar in all three experiments and no significant difference was found in anion concentrations at the beginning and the end of each experiment (Table S1 in Supplementary Materials). Exemplary data from experiments 2 and 3 are shown in Figure 2 and data from experiment 1 as well as the end of experiments 2 and 3 can be found in supplemental Table S1. The control group showed the lowest amount of chloride with 6.40 ± 0.02 g $l^{-1}$. In the treatment groups, the chloride content ranged from highest 15.04 ± 0.04 g $l^{-1}$ (2.4% NaCl) and lowest 12.14 ± 0.06 g $l^{-1}$(TM), whereas measured differences are due to different compositions of the salt and/or brine water composition. The nitrate content is almost equivalent and ranges between 1.02 ± 0.00 g $l^{-1}$ (2.4% NaCl) and 0.94 ± 0.00 g $l^{-1}$ (2.4% TM). The phosphate content is similar in all solutions (ranged from 0.15 ± 0.01 to 0.20 ± 0.00 g $l^{-1}$), and slightly decreased in BW1 brine water. TM contained the highest amount of sulfate salts (1.69 ± 0.00 g $l^{-1}$) within the treatment groups, while 2.4% NaCl contained the lowest amount (0.21 ± 0.00 g $l^{-1}$). The two brine water solutions fall in between, with BW1 having a higher chloride content (14.44 ± 0.03 g $l^{-1}$) and lower sulfate content (0.46 ± 0.00 g $l^{-1}$) than BW2 ($Cl^-$, 13.02 ± 0.04 g $l^{-1}$; $SO_4^{2-}$, 0.62 ± 0.00 g $l^{-1}$). These findings indicate that the nitrate and phosphate contents originate mainly from the nutrient solution.

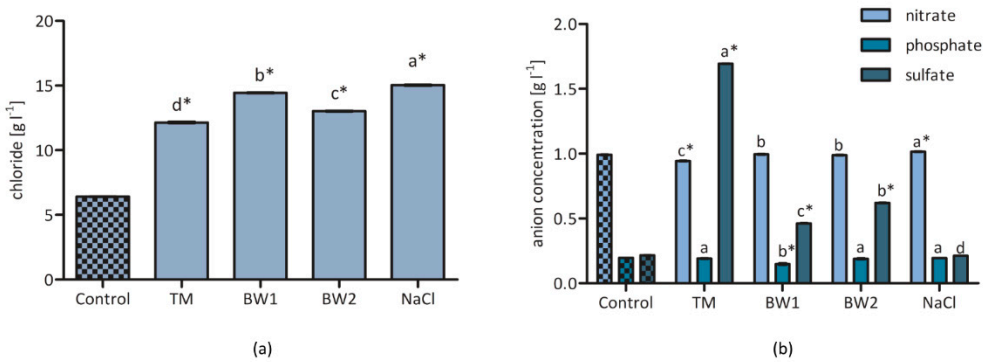


**Figure 2.** Anion composition of saline solutions. Concentration of (**a**) chloride and (**b**) nitrate, phosphate, and sulfate in salt solutions of the control and treatment groups at the beginning of experiments 2 and 3. Means ± SD of one independent experiment. Asterisks indicate significant differences between individual treatments and the control within one component ($p \leq 0.05$). Small letters indicate significant difference between the treatments within one component ($p \leq 0.05$). TM, artificial sea water Tropic Marine; BW1, brine water location 1 (Bad Saarow); BW2, brine water location 2 (Heiligenstadt); NaCl, sodium chloride solution.

### *3.2. Impact of Treatment on Yield and Phytochemicals*

#### 3.2.1. Impact of Treatment on Yield

The fresh weight was strongly affected by the experimental period. Fresh weight of the control was highest in experiment 1 (4.68 g) and almost half as high in experiments 2 (2.56 g) and 3 (2.27 g) (Figure 3). One reason could be the seasonal difference since experiment 1 was sown in August and experiment 2 in late September.

There were no significant differences within treatments. Related to the control, fresh weight was significantly reduced in all treatments, most dramatically with 2.4% NaCl at 0.42-fold (Figure 3). However, all nutrient solutions were suitable for the cultivation of *S. europaea*.

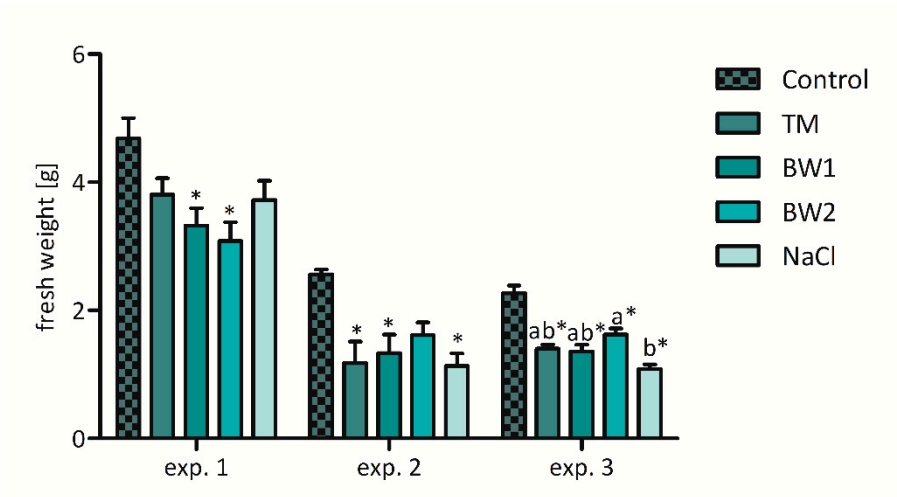


**Figure 3.** Fresh weight of shoots of *Salicornia europaea* of 10-week-old plants. Means ± SD of three independent experiments (exp.). Asterisks indicate significant differences between individual treatments and the control within an experiment ($p \leq 0.05$). Small letters indicate significant difference between the treatment groups within one experiment ($p \leq 0.05$). TM, Tropic Marine BW1, brine water location 1 (Bad Saarow); BW2, brine water location 2 (Heiligenstadt); NaCl, sodium chloride solution.

### 3.2.2. Impact of Treatment on the Phytochemical Content

The total chlorophyll content ranged from 1853.27 ng mg$^{-1}$ to 3477.02 ng mg$^{-1}$ (Table 1). There were no significant differences within the treatment group, but the chlorophyll content was significantly reduced (0.1–0.3-fold) in BW1 and BW2 compared to control (optimal growth conditions). The chlorophyll a/b ratio was unaffected (Table 1). The total carotenoid content ranged from the lowest value 356.89 ng mg$^{-1}$ (BW1, exp. 3) to the highest value 551.23 ng mg$^{-1}$ (control, exp. 2) (Table 2). There were no significant differences between the treatments. Compared to the control, only BW2 showed a difference. However, for all treatments, there was a trend toward a slight 0.1–2-fold reduction of total carotenoids. The experiment 3 showed no differences for either the total chlorophyll or the total carotenoid content (Tables 1 and 2) and also had the lowest values of all three experiments. Individual carotenoids showed significant differences within experiments 1 and/or experiment 2, but no particular pattern. Zeaxanthin, however, showed the lowest amounts in the control group.

**Table 1.** Content of total chlorophylls, chlorophyll *a*, chlorophyll *b* and chlorophyll a/b ratio in shoots of *Salicornia europaea* of 10-week-old plants. Means ± SD of one independent experiment (exp.). Asterisks indicate significant differences between individual treatments and the control within an experiment ($p \leq 0.05$). TM, Tropic Marine; BW1, brine water location 1 (Bad Saarow); BW2, brine water location 2 (Heiligenstadt); NaCl, sodium chloride solution; 1, 2, 3, three different experiments; ns, not significant.

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Total Chlorophyll (ng mg$^{-1}$ DW)** | | | | | | | | | | | | |
| | exp. 1 | | | | exp. 2 | | | | exp. 3 | | | |
| Control | 2843.01 | ± | 156.23 | | 3477.02 | ± | 116.65 | | 2592.50 | ± | 100.07 | ns |
| TM | 2430.70 | ± | 161.34 | | 2876.78 | ± | 348.73 | | 2310.38 | ± | 278.75 | ns |
| BW1 | 2365.12 | ± | 322.58 | * | 2706.29 | ± | 113.64 | * | 1853.27 | ± | 188.28 | ns |
| BW2 | 2251.70 | ± | 210.14 | * | 2722.68 | ± | 154.77 | * | 2248.02 | ± | 428.07 | ns |
| NaCl | 2499.83 | ± | 313.34 | | 2813.55 | ± | 97.74 | | 2106.85 | ± | 616.33 | ns |
| **Chlorophyll *a* (ng mg$^{-1}$ DW)** | | | | | | | | | | | | |
| | exp. 1 | | | | exp. 2 | | | | exp. 3 | | | |
| Control | 2229.06 | ± | 131.31 | | 2755.64 | ± | 85.36 | | 1978.65 | ± | 124.72 | ns |
| TM | 1905.73 | ± | 145.10 | | 2299.47 | ± | 274.90 | * | 1836.28 | ± | 221.58 | ns |
| BW1 | 1853.07 | ± | 267.48 | | 2149.04 | ± | 106.27 | * | 1404.60 | ± | 178.87 | ns |
| BW2 | 1748.37 | ± | 169.81 | * | 2158.34 | ± | 114.23 | * | 1725.75 | ± | 384.16 | ns |
| NaCl | 1966.04 | ± | 258.95 | | 2225.54 | ± | 69.43 | * | 1648.19 | ± | 493.74 | ns |
| **Chlorophyll *b* (ng mg$^{-1}$ DW)** | | | | | | | | | | | | |
| | exp. 1 | | | | exp. 2 | | | | exp. 3 | | | |
| Control | 613.95 | ± | 35.82 | | 721.38 | ± | 31.52 | | 613.85 | ± | 49.17 | |
| TM | 524.96 | ± | 21.17 | * | 577.32 | ± | 76.08 | | 474.10 | ± | 59.31 | * |
| BW1 | 512.04 | ± | 56.68 | * | 557.25 | ± | 16.38 | * | 448.67 | ± | 20.07 | * |
| BW2 | 503.33 | ± | 41.23 | * | 564.34 | ± | 41.09 | * | 522.27 | ± | 48.43 | |
| NaCl | 533.79 | ± | 56.44 | | 588.00 | ± | 28.89 | | 458.66 | ± | 124.38 | * |
| **Chlorophyll a/b Ratio** | | | | | | | | | | | | |
| | exp. 1 | | | | exp. 2 | | | | exp. 3 | | | |
| Control | 3.63 | ± | 0.18 | ns | 3.82 | ± | 0.05 | ns | 3.25 | ± | 0.41 | ns |
| TM | 3.63 | ± | 0.20 | ns | 3.99 | ± | 0.15 | ns | 3.88 | ± | 0.15 | ns |
| BW1 | 3.61 | ± | 0.18 | ns | 3.86 | ± | 0.18 | ns | 3.13 | ± | 0.37 | ns |
| BW2 | 3.47 | ± | 0.09 | ns | 3.83 | ± | 0.09 | ns | 3.28 | ± | 0.50 | ns |
| NaCl | 3.68 | ± | 0.17 | ns | 3.79 | ± | 0.07 | ns | 3.58 | ± | 0.23 | ns |

**Table 2.** Content of total carotenoids, lutein, β-carotene, and zeaxanthin in shoots of *Salicornia europaea* of 10-week-old plants. Means ± SD of one independent experiment (exp.). Letters indicate significant differences within treatments in one experiment and one component ($p \leq 0.05$). Asterisks indicate significant differences between individual treatments and the control within an experiment ($p \leq 0.05$). Small letters indicate significant difference between the treatment groups within one experiment ($p \leq 0.05$).TM, Tropic Marine; BW1, brine water location 1 (Bad Saarow); BW2, brine water location 2 (Heiligenstadt); NaCl, sodium chloride solution; 1, 2, 3, three different experiment; ns, not significant.

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Total Carotenoids (ng mg$^{-1}$ DW)** | | | | | | | | | | | | |
| | **exp. 1** | | | | exp. 2 | | | | exp. 3 | | | |
| Control | 480.7 | ± | 26.39 | | 551.23 | ± | 17.64 | | 439.7 | ± | 17.38 | ns |
| TM | 425.6 | ± | 25.21 | | 488.78 | ± | 67 | | 403.66 | ± | 47.53 | ns |
| BW1 | 418.17 | ± | 44.57 | | 458.16 | ± | 21.41 | | 356.89 | ± | 11.03 | ns |
| BW2 | 398.87 | ± | 31.96 | * | 459.23 | ± | 25.97 | * | 409.66 | ± | 45.02 | ns |
| NaCl | 441.16 | ± | 34.31 | | 468.53 | ± | 20.91 | | 373.19 | ± | 99.08 | ns |
| **Lutein** | | | | | | | | | | | | |
| | exp. 1 | | | | exp. 2 | | | | exp. 3 | | | |
| Control | 179.23 | ± | 14.58 | | 193.68 | ± | 12.66 | | 170.25 | ± | 17.16 | |
| TM | 174.76 | ± | 6.628 | a | 197.62 | ± | 15.88 | a | 169.18 | ± | 16.85 | |
| BW1 | 167.25 | ± | 19.17 | a | 173.47 | ± | 9.341 | ab | 144.52 | ± | 12.83 | |
| BW2 | 140.49 | ± | 9.456 | b* | 161.62 | ± | 11.59 | b* | 157.35 | ± | 8.80 | |
| NaCl | 156.97 | ± | 7.494 | ab | 157.5 | ± | 5.648 | b | 130.87 | ± | 32.07 | * |
| **β-Carotene** | | | | | | | | | | | | |
| | exp. 1 | | | | exp. 2 | | | | exp. 3 | | | |
| Control | 136.31 | ± | 8.777 | | 158.5 | ± | 6.431 | | 144.66 | ± | 15.72 | |
| TM | 123.77 | ± | 6.796 | | 145.89 | ± | 14.67 | a | 125.31 | ± | 12.11 | |
| BW1 | 120.02 | ± | 11.08 | | 128.34 | ± | 5.715 | * | 110.76 | ± | 5.68 | |
| BW2 | 105.36 | ± | 8.281 | * | 126.86 | ± | 7.95 | *b | 124.74 | ± | 12.08 | |
| NaCl | 120.4 | ± | 12.88 | | 131.3 | ± | 7.865 | *ab | 105.1 | ± | 30.47 | * |
| **Zeaxanthin** | | | | | | | | | | | | |
| | exp. 1 | | | | exp. 2 | | | | exp. 3 | | | |
| Control | 13.532 | ± | 3.347 | | 8.0821 | ± | 1.1 | ns | 9.116 | ± | 4.29 | ns |
| TM | 12.147 | ± | 2.435 | | 21.509 | ± | 6.484 | ns | 15.683 | ± | 5.28 | ns |
| BW1 | 15.524 | ± | 3.835 | | 13.795 | ± | 4.066 | ns | 19.581 | ± | 0.73 | ns |
| BW2 | 13.854 | ± | 1.839 | | 12.484 | ± | 3.361 | ns | 16.076 | ± | 2.27 | ns |
| NaCl | 21.027 | ± | 2.194 | * | 22.356 | ± | 15.45 | ns | 19.746 | ± | 5.24 | ns |

## 4. Discussion

Given the global wide distribution of natural brines and their known complex chemical composition, often coupled to local geothermal structures, there is a high potential for their further application to respond to the crop production needs and requirements of regional agricultural [35,36]. In Germany, natural brine springs are widespread and of economic importance for the chlor-alkali electrolysis industry as well as for therapeutic applications in the spa [37,38]. So far, natural brine has not been used for agricultural purposes. Here, the choice of cultivated crop will be crucial, and we predict that the cultivation of halophytes could significantly contribute to resource efficient farming of halophytes or other aquatic organisms as demonstrated for *S. europaea.*

Previous studies have shown that salt concentration and composition can influence the growth of halophytes [9,28–31]. The differences in growth between treatment and control groups are potentially the result of the lower chloride content in the control solution enabling higher biomass production. Even *S. europaea* is an obligate halophyte, yet salt tolerance has a limit at which the plant cannot cope with increasing salinity and responds

by inhibiting growth and altering metabolism. Chloride, along with sodium, is the main stressor in salt stress and affects plant growth and photosynthesis. Several studies showed growth inhibition with increasing salinity concentration in halophytes, not only for *Salicornia* species, but also for *Chenopodium quinoa*, *Sarcocornia fructosia*, and *Cochlearia officinalis* [14,29,39–41]. He, Silliman and Cui [9] showed a reduction in the growth of the aboveground part of *S. europaea* at 4 to 10% soil salinity. Moreover, Orlovsky, Japakova, Zhang and Volis [30] investigated the effect of different salt compositions on germination and growth of *S. europaea.* Their study revealed that a mixed chloride-sulfate salt has a positive effect on growth compared to pure chloride salt. Interestingly, no significant differences in growth related to anion composition were observed in the present study, thereby suggesting that the use of natural brine for cultivation of *S. europaea* is both feasible and a suitable alternative for seawater use in the mainland.

Variations in the contents of chlorophylls and carotenoids are of interest as changes in these pigments provide a good indication of oxidative stress. The chlorophyll a/b ratio is an important parameter that, when altered under stress conditions, provides an indication of altered photosystem activity. As the chlorophyll a/b ratio was not changed in the present study, this indicates that the plants had an unaffected photosynthetic rate. Plants that cannot cope with salinity stress show reduced chlorophyll content and photosynthetic activity. In green bean (*Phaseolus vulgaris* L.) plants for example, ElSayed, et al. [42] showed a decrease in photosynthetic quantum yield and total chlorophyll content already from a salt concentration of 200 mM (1.2%) sodium chloride. Along with the chlorophylls, carotenoids are important pigments in the photosystems and plants can adapt chlorophyll and carotenoid content in their photosystems under suboptimal photosynthetic conditions, which can occur during salt stress [43]. The absence of changes in the total carotenoid content between treatments also indicates that the plants are not suffering from suboptimal photosynthetic conditions. Thus, the altered pigment levels between treatment and control groups are likely due to reduced growth.

Food quality can be influenced by salinity in both glycophytic crops (e.g., *Lactuca sativa,* Eggplant, *Cucumis sativus,* and *Solanum lycopersicum*) as well as in halophytes (e.g., *Crithmum maritimum* and *Salicornia persica*) [29,44–47]. Chlorophylls and Carotenoids are nutritionally valuable compounds that are associated with health-promoting effects and are particularly abundant in green leafy vegetables. Moreover, carotenoids and chlorophylls have an antioxidant capacity and can prevent DNA damage and lipid peroxidation, and thus, have anti-carcinogenic effect [22,48,49]. In addition, the carotenoids lutein and zeaxanthin have a positive effect on eye health and β-carotene has provitamin-A activity [24,25]. The comparison of the values of total chlorophylls and carotenoids (Tables 1 and 2) of the treatment group with a purchased product of *S. europaea* from the North Sea coast (3–3.5% salt) (Table S2 in Supplementary Materials) showed that the values were in the same range. Furthermore, compared to other vegetables, such as spinach and kale, which are known to be a good sources for carotenoids and chlorophylls, *S. europaea* has a comparable content of carotenoids and chlorophylls [50,51]. Thus, on the basis that no changes in the fresh weight and the selected metabolites were found, we assume that natural brine can be utilized for the production of nutrient-rich vegetables.

In summary, this case study demonstrates the potential of brine water for indoor aquaculture systems. To realize its full potential, further research is needed on the cultivation of halophytes in regional indoor brine water systems and the adaptation of the system to other aquatic organisms such as algae or shrimp, as well as mechanistic approaches to study the influence of brine water on halophytes. Here, initial approaches to determine the optimal growth conditions for halophytes are needed. In detail, lowering the chloride content could be beneficial for yield and as well as the nutrient profile. In this respect, further natural brines should be examined to better assess their regional potential. Finally, among others, (bio-)technological developments, such as wireless senor technologies, au-

tomatized irrigation systems, renewable energy, and culture compartments, as well as research unleashing the biodiversity of halophytes as alternative vegetables and mechanistic approaches are required to implement saline food systems [52–54].

## 5. Conclusions

Due to the freshwater scarcity, new approaches in agriculture are needed to ensure food security in the future. Urban agriculture contributes to the availability of affordable, healthy, fresh food and can improve food security even with less arable land. Additionally, this study highlights that natural brine is a possible alternative to the use of seawater or artificial seawater for the cultivation of the halophyte *Salicornia europaea* and is therefore a promising approach for urban indoor farming without being dependent on shrinkable potable water resources. This is an important step towards more sustainable saline food systems offside coastal regions. Furthermore, the example of *S. europaea* demonstrates that halophytes have a much overlooked potential as a nutritious and health-promoting food source. As such, they can improve the biodiversity of plant-based diets and hence the uptake of plant secondary metabolites and contribute to a healthier diet. Thus, further research aimed at optimizing growth conditions for different halophyte species would better allow the potential of natural brines for sustainable local solutions to be exploited for aquaculture and saline agriculture.

**Supplementary Materials:** The following are available online at www.mdpi.com/article/10.3390/su132112105/s1, Table S1: Anion concentrations in nutrient solutions at the beginning and end of the experiments, Table S2: Chlorophyll and carotenoid content of purchased *S. europaea*.

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