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Research Article

Salinity reduction by *Sarcocornia neei* in hydroponics: implications in marine aquaculture wastewater remediation

Silvia Gómez[1], **Carlos Felipe Hurtado**[1], **Jaime Orellana**[2] & **José Andrés Gallardo**[1]
[1]Escuela de Ciencias del Mar, Pontificia Universidad Católica de Valparaíso, Valparaíso, Chile
[2]Erwin Sander Elektroapparatebau GmbH, Uetze-Eltze, Germany
Corresponding author: Silvia Gómez (silvia.gomez@pucv.cl)

ABSTRACT. Salinization has become one of the main environmental problems affecting the worldwide ecological balance. The effluent discharge of saline industrial effluents, such as marine aquaculture, and its rapid increase in global production in the last decades has become an important part of this problem. The halophyte *Sarcocornia neei* (Lag.) has proven its ability to remove and assimilate nutrients from saline wastewater, making it a promising candidate for application in phytoremediation systems. In this study, its ability to reduce water salinity was evaluated. For this purpose, the plants were reared for 53 days in artificial effluents at four different salinities: A) 0, B) 10, C) 20, and D) 30 g L^{-1}. A significant salt reduction was obtained in all treatments with NaCl addition. The maximum decrease was achieved in treatment C, reducing 6.91 ± 1.52 g L^{-1}, approximately 35% of the total added at the beginning of the trials. Plants tolerated all salt content ranges; no mortality or salt stress symptoms were observed in any treatment. The results obtained are a first approach suggesting that *S. neei* could be used for bio-desalination of saline wastewater. Further studies are needed to evaluate its application in pilot systems integrated into saline industrial effluents.

Keywords: *Sarcocornia neei*; halophyte plants; salt removal; marine aquaculture; aquaculture wastewater; bioremediation; hydroponics

INTRODUCTION

Environmental salinization is becoming more prevalent worldwide, causing damage to soil structure, reducing fertility, and negatively impacting agro-industrial activities and drinking water availability. Some industrial effluents are a significant sodium source, increasing salinization. In addition, nutrients contained in these effluents increase the risk of eutrophication in the environments where they are discharged (Jesus et al. 2017). Marine aquaculture, which has grown rapidly in the last few decades, has enlarged the discharge of saline effluents and become a significant part of this problem. Other contributors include slaughterhouses, dairy farms, and reverse osmosis reject water (Ventura & Sagi 2013, Orlofsky et al. 2020). The use of halophyte plants for bioremediation of saline effluents has been studied in recent years to provide a partial solution to this problem. *Salicornia* and *Sarcocornia* are among the most promising genera of halophytes for application in the phytoremediation of saline waters and soils. For this reason, several aspects of their cultivation have recently been described, evaluating their biomass production and nutrient removal rate from different types of domestic and industrial wastewater (Shelef et al. 2012, Buhmann et al. 2015, Custódio et al. 2017, Orlofsky et al. 2020, Ranjbar et al. 2022).

Even with the potential of using halophytes as biotechnological tools, their use to reduce wastewater salinity has been less studied than other subjects, such as their tolerance to salinity or its removal capacity of other nutrients (Jesus et al. 2017, Caparrós et al. 2022).

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Although halophytes have proven efficient in removing salt from soils, the water bio-desalination concept is novel, and research is still beginning (Sahle-Demessie et al. 2019, Garcia et al. 2020).

Halophyte plants can grow and complete their life cycle in habitats with high salinity. They tolerate at least 200 mM NaCl (11.7 g L^{-1}) and normally obtain higher growth rates when salt is present in the environment (Custódio et al. 2017). These plants, representing around 1% of the planet's flora, can survive in hypersaline environments, such as salt marshes, coastal regions, wetlands, or estuaries, and can be grown even with full-strength seawater irrigation (Shelef et al. 2012, Ventura & Sagi 2013, Angeletti & Cervellini, 2015). In addition to salinity tolerance, their ability to remove nutrients from water and soils has been demonstrated in several investigations (Brown et al. 1999, Webb et al. 2012, Waller et al. 2015). Halophyte cultivation is also important for human and animal consumption due to its nutritional value, in terms of antioxidant vitamins and minerals, as an alternative source of omega-3 polyunsaturated fatty acids (Ventura et al. 2011a).

The hydro halophyte *Sarcocornia neei* (Lag.), recently merged with *Salicornia neei*, is a perennial plant belonging to the Amaranthaceae family. It is primarily found along the Pacific coast of South America and is considered a vital component of estuarine and coastal ecosystems (Alonso et al. 2017, Piirainen et al. 2017, Diaz et al. 2020).

Recent studies have demonstrated the ability of *S. neei* to remove and assimilate inorganic and organic nitrogen, carbon, and phosphorus compounds from hypersaline aquaculture wastewater and its potential for bioabsorption of heavy metals and other soil contaminants, making it a promissory candidate for application in phytoremediation systems (Diaz et al. 2020, Sepúlveda et al. 2020, Beyer et al. 2021). The objective of this study was to evaluate the salinity reduction performance of *S. neei* in hydroponics, assessing its potential use as a bio-desalination tool in saline wastewater treatment, also evaluating the biomass obtained by the plants at different salinities to optimize its desalination performance.

MATERIALS AND METHODS

Sarcocornia neei seedlings (initial wet weight: 2.93 ± 0.58 g) were collected in the Salinas de Pullally wetland (32º24′50.6"S, 71º24′33.3"W), Valparaíso Region, Chile. The extraction method from the natural environment was carried out as described by Beyer et al. (2021). Upon arrival at the laboratory, a cleaning process with distilled water was conducted in order to eliminate the residual adhering soil. Five plants were chosen for initial dry weight determination (60ºC to constant weight). The plants were acclimatized in freshwater hydroponics for 35 days to strengthen the rooting (photoperiod 12:12 h, 20ºC) (Fig. 1) (Pinheiro et al. 2020).

The experiments to evaluate salinity reduction consisted of assessing four different salinities in the cultivation of *S. neei* in a hydroponic system, A) 0, B) 10, C) 20, and D) 30 g L^{-1}. Each treatment had three replicates (with plants) and three independent units functioning as controls (without plants) to determine salt removal rates by *S. neei* and to evaluate possible water losses by evaporation.

An artificial light system was installed to ensure photosynthesis (H-MET 250 W Greenlight, Brazil), with a constant photoperiod (12:12 h) to prevent plant flowering (light intensity: 1150 µmol m^{-2} s^{-1}; daily light integral: 49.68 mol m^{-2} d^{-1}) (Ventura et al. 2011b). The experimental setup consisted of 24 independent units, one seedling per unit, and six units per treatment. They were implemented in an incubation chamber (BioRef, Chile), where the temperature was maintained at 19.35 ± 0.67ºC (Webb et al. 2012). All the units were lightly and constantly aerated to provide oxygen for the plant roots (HT-500 air pump, Sunsun, China). All units were sealed with a flexible transparent film (Parafilm) to prevent evaporation (APHA 2017), and a plastic tube was implemented in each unit to allow gas exchange (Quintã et al. 2013).

The artificial saline effluents were prepared by adding NaCl (Dilaco, Chile) (Brown et al. 1999). At the beginning of the experiments, distilled water was added to the units (569.20 ± 27.69 mL; n = 24), maintaining the plants under these conditions for 48 h. The addition of NaCl to ensure the correct adaptation of the plants was achieved by increasing 5 g L^{-1} every second day in the experimental units until the corresponding salinities were reached in each treatment (Waller et al. 2015).

A 24 h period was established before the start of parameter measurement, which was performed every 3-7 days during the 53 days of experiments. Temperature and conductivity were measured with a portable multiparameter probe (Hach HQ40d). The initial (day 1) and final (day 53) wet weight of the plants were determined with a precision scale. Dry weight was determined after dehydration (60ºC to constant weight). To perform an adequate standardization of electrical conductivity (EC) (S m^{-1}) and salinity values (g L^{-1}) and to detect possible evaporation phenomena in the

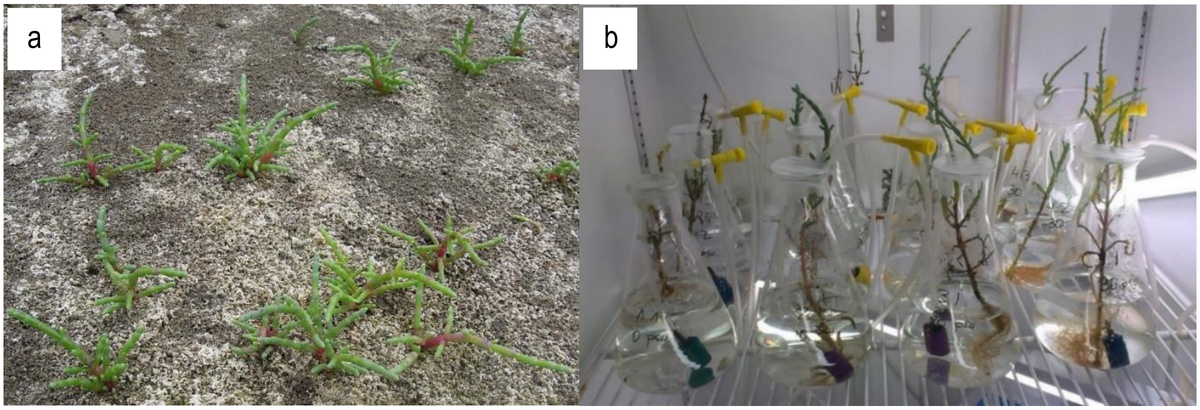

Figure 1. a) Plants at the extraction site, b) *Sarcocornia neei* seedlings during acclimatization.

hydroponic system, a standardization phase (10 days) was carried out prior to the implementation of the plants.

Salinity data were obtained through water conductivity measurements according to the Electrical Conductivity Method (APHA 2017, Sahle-Demessie et al. 2019). The technique relates the conductivity of a KCl solution at different temperatures (Eq. 1) to the conductivity of the samples to be determined (Eq. 2).

$$C\ (KCl\ solution\ at\ t)\ (\mu S\ cm^{-1}) = -0.0267243\ t^3 + 4.663647\ t^2 + 861.3027640\ t + 29035.1640851 \quad (1)$$

$$Rt = C\ (sample\ at\ t)/C\ (KCl\ solution\ at\ t) \quad (2)$$

By obtaining ΔS (Eq. 3), the salinity values were given by the relationship between the factors obtained and a series of numerical indices (a_0 = 0.0080; a_1 = -0.1692; a_2 = 25.3851; a_3 = 14.0941; a_4 = -7.0261; a_5 = 2.7081; b_0 = 0.0005; b_1 = -0.0056; b_2 = -0.0066; b_3 = -0.0375; b_4 = 0.0636; b_5 = -0.0144) determined by the standardization of KCl conductivity at different temperatures (Eq. 4).

$$\Delta S = [t - 15/1 + 0.0162\ (t - 15)]\ (b_0 + b_1 R_t^{1/2} + b_2 R_t + b_3 R_t^{3/2} + b_4 R_t^2 + b_5\ R_t^{5/2}) \quad (3)$$

$$S = a_0 + a_1 R_t^{1/2} + a_2 R_t + a_3 R_t^{3/2} + a_4 R_t^2 + a_5 R_t^{5/2} + \Delta S \quad (4)$$

Data analyses were conducted using GraphPad-Prism version 8.4.3. Data were tested for normality and homogeneity of variance using the Shapiro-Wilk test. The differences between initial and final salinity concentration, electrical conductivity, and plant biomass were determined by paired samples t-test ($P < 0.05$). The Tukey test ($P < 0.05$) analyzed the significant differences. The differences in salinity removal and electrical conductivity between treatments were determined by one-way repeated measure ANOVA analysis.

RESULTS

Sarcocornia neei obtained significant salt removal in all treatments with NaCl addition. Treatments B, C, and D resulted in significant reductions compared with treatment A, with no statistical differences between them (Fig. 2). The highest salinity reduction was obtained in treatment C (20 g L^{-1}), eliminating 6.91 ± 1.52 g L^{-1} in 53 days, corresponding about 35% of the total NaCl added at the beginning (a reduction of 0.15 g L^{-1} per g of dry weight per day). In treatments B (10 g L^{-1}) and D (30 g L^{-1}), removals of 4.33 ± 0.39 and 3.32 ± 0.47 g L^{-1} were obtained, also implying significant salinity reduction, corresponding to 0.11 and 0.09 g L^{-1} per g of plant in dry weight per day.

The electrical conductivity (EC) values obtained during the experiment are shown (Fig. 3). EC averages were respectively 0.04 ± 0.01, 1.66 ± 0.32, 3.75 ± 0.47, and 5.11 ± 0.21 S m^{-1} for each treatment. All treatments with NaCl addition showed significant differences between the initial and final EC obtained. In terms of the reduction achieved, there were differences between treatments B and C, compared to A and D. The higher removal was obtained in C (0.96 ± 0.22 S m^{-1}), representing a 22.67 ± 4.39% reduction of the initial value. In terms of percentage, treatment B was more efficient (39.58 ± 5.07%), corresponding to a reduction of 0.82 ± 0.09 S m^{-1}. Treatments A and D reduced EC by 0.0024 ± 0.006 and 0.19 ± 0.075 S m^{-1}, respectively.

An increase in plant biomass was obtained in all treatments. Significant differences were observed in the weight gain obtained by the plants between treatment A compared to B and C (Fig. 4). In the last two treatments, the relative increment in wet weight was 37.87 ± 8.79% (treatment B) (corresponding to an increase in wet and dry weight of 1.01 ± 0.28 and 0.20 ± 0.06 g, respectively) and 31.09 ± 3.57% (treatment C) (1.02 ± 0.14 g wet weight and 0.20 ± 0.03 g dry weight) after 53 days.

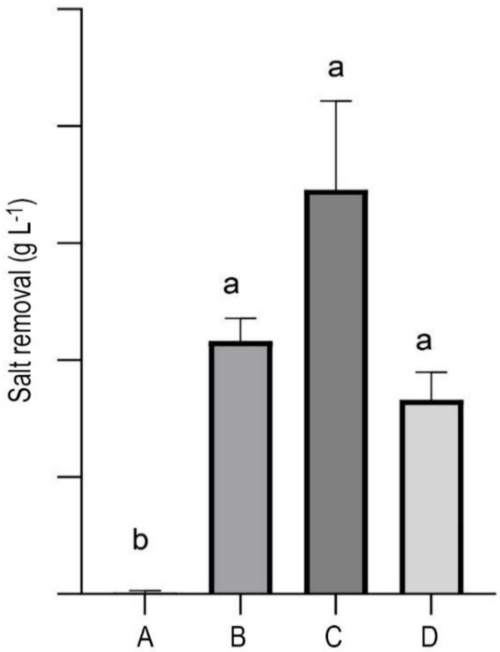

Figure 2. Salt removal achieved by *Sarcocornia neei* (g L^{-1} NaCl, 53 days). Treatments: A (0 g L^{-1}): 0.02 ± 0.038 g L^{-1}; B (10 g L^{-1}): 4.33 ± 0.39 g L^{-1}; C (20 g L^{-1}): 6.91 ± 1.52 g L^{-1}; D (30 g L^{-1}): 3.32 ± 0.47 g L^{-1}. Significant differences are expressed with different letters ($P < 0.05$).

DISCUSSION

S. neei tolerated the wide range of salinities; no plant mortality or salt stress symptoms were observed in any treatment. No salt precipitation or evaporation phenomena were observed, reflected in the non-existence of significant differences in the volume of water inside the units and the maintenance of salinity values in the controls throughout the process, averaging respectively 0.04 ± 0.03, 9.86 ± 0.12, 20.68 ± 0.67 and 31.50 ± 0.65 g L^{-1} at the end of the experiences.

In the present study, artificial effluents were simulated to evaluate the ability and efficiency of *S. neei* to reduce salinity in water. To the best of our knowledge, this is the first study evaluating the water desalination potential of *S. neei* and its possible application in bioremediation systems associated with saline effluents. To compare the achieved results with other halophyte species, *Spartina maritima* (Curtis) Fernald in a hydroponic system with an initial water salinity of 9.6 g L^{-1} reported removals of 10.4 ± 4.0% total salts after 23 days (Jesus et al. 2017). In the same study, *Juncus maritimus* (Lam.) also demonstrated salt removal capacity, although significantly lower (1.0 ± 2.5%). *Bassia indica* (Wight) A.J. Scott also achieved significant salinity removal when implemented in hydroponics, which was reflected in EC reductions of 22.2% in a mild saline solution (0.5 S m^{-1}) and 12.9% in a hypersaline solution (1.6 S m^{-1}), reducing salinity by 20 to 60% in 48 days (Shelef et al. 2012). In another trial with *Portulaca oleracea* (L.), however, biomass gain and removal of other nutrients were obtained, but this species did not present the expected sodium and

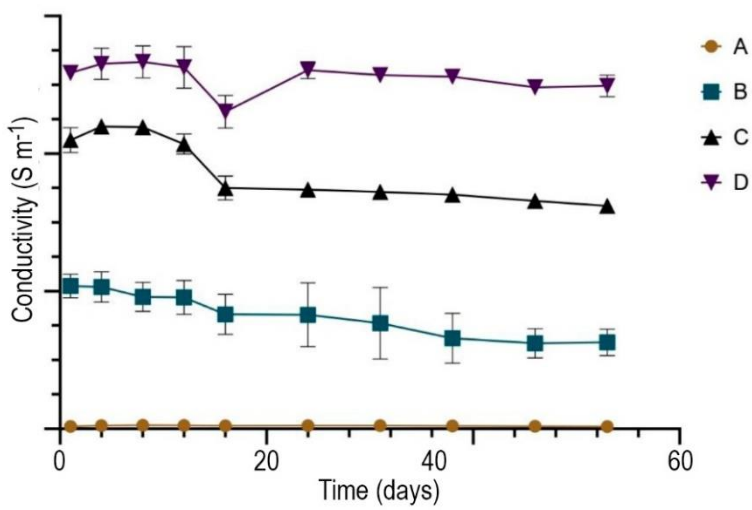

Figure 3. Electrical conductivity (S m^{-1}) measured in each treatment. The reduction achieved at the end of the experiments. Treatments: A (0 g L^{-1}): 0.0024 ± 0.006 S m^{-1}; B (10 g L^{-1}): 0.82 ± 0.09 S m^{-1}; C (20 g L^{-1}): 0.96 ± 0.22 S m^{-1}; D (30 g L^{-1}): 0.19 ± 0.075 S m^{-1}.

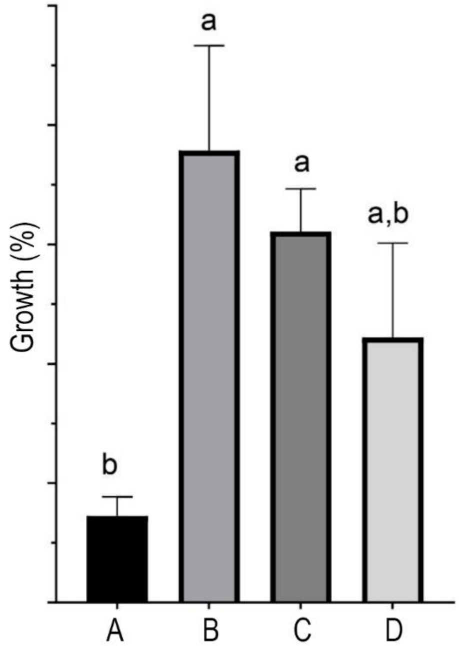

Figure 4. The relative increase in wet weight (%) achieved by *S. neei* (53 days). Treatments: A (0 g L^{-1}): 7.26 ± 1.59%; B (10 g L^{-1}): 37.87 ± 8.79%; C (20 g L^{-1}): 31.09 ± 3.57%; D (30 g L^{-1}): 22.20 ± 7.90%. Significant differences were found between treatments A *vs.* B and C ($P < 0.05$).

chloride removal capacity after five weeks at different salinities (De Lacerda et al. 2015). In the present study, after 53 days, the maximum NaCl removal by *S. neei* was around 35% (~7 g L^{-1}), with a maximum EC reduction of 0.96 S m^{-1} (22.67%) in treatment C (20 g L^{-1}). Reductions in EC values were most significantly observed around day 20 of experiments, consistent with other halophytes where salinity reduction was observed from 23 days of hydraulic retention in hydroponic systems (Jesus et al. 2017).

The lower plant biomass obtained in treatment A (distilled water) could be advantageous for implementing *S. neei* in integrated systems associated with marine aquaculture effluents. Other halophytes also reported higher growth rates when irrigated with saline water instead of fresh or distilled water (Hasanuzzaman et al. 2014, Custódio et al. 2017). *Salicornia* and *Sarcocornia* spp. when cultivated in hydroponics also reported lower biomass production when grown in freshwater or at low salinity concentrations (1 g L^{-1}) (Ventura et al. 2011a, Quintã et al. 2013).

On the other hand, the reduced weight gain achieved by *S. neei* in treatment D could be because halophytes reduce their growth rate at high salinities to store photosynthetic energy and reduce protein synthesis, as free amino acids help adjust osmotic balance (Parida et al. 2016). These results are consistent also with studies conducted with *Sarcocornia* spp., where biomass production was inhibited when irrigated with seawater (Ventura et al. 2011a). Therefore, *S. neei* could be classified as an extreme halophytic plant (optimum growths at 12-24 g L^{-1} NaCl), with salinities higher than 30 g L^{-1} allowing survival but inhibiting its growth (Ventura & Sagi 2013). Studies carried out with *Salicornia* spp. in the natural environment determined its optimum salinity at 14.23 g L^{-1}, but also being able to survive at higher and lower salinity ranges (Kaijser et al. 2019).

Salt accumulation in plant tissues was not analyzed in this preliminary study. However, the results of reduced water salinity and increased plant biomass indicate that this could be a feasible explanation, as with other halophyte species (Shelef et al. 2012, Orlofsky et al. 2020). Also, during these trials, no salt precipitation occurred in the culture water, nor was salt accumulation observed externally in aerial plant tissues (Jesus et al. 2017).

This study shows early results using *S. neei*, a halophyte plant native to the coasts of Chile and other Latin American countries, to reduce NaCl in hydroponic systems. The data show that *S. neei* can obtain significant reduction rates without signs of stress or mortality at different salinities, suggesting its novel use in water bio-desalination. According to the data obtained, 10 and 20 g L^{-1} are the most suitable salinities to achieve better performance of *S. neei* reducing NaCl in hydroponics.

S. neei has emerged as a promising tool for treating industrial saline wastewater in land-based marine aquaculture facilities. Previous studies have demonstrated its potential in reducing the environmental impacts of saline effluent discharge resulting from fish culture. Beyer et al. (2021) reported that *S. neei* could effectively remove nitrate (NO_3-N), total ammonia nitrogen, phosphate, and total inorganic nitrogen from a *Seriola lalandi* (Valenciennes, 1833) aquaculture saline effluent. These nutrients are important components of eutrophication processes in aquatic environments. The study also reported *S. neei* growth rates of 2.80 ± 0.30 g m^{-2} d^{-1} and biomass productivity rates of 0.17 ± 0.02 kg m^{-2} after 61 days of trials. Furthermore, the adaptability of this species to high salt concentrations makes it an ideal candidate for use as a biofilter for marine aquaculture effluents. Diaz et al. (2020) reported significant nutrient removal rates when simulating artificial aquaculture effluents at very high salinities, ranging from 40.0 to 48.8 g L^{-1}.

The results of the present study suggest that using *S. neei* could allow partial desalination of saline effluents before their discharge into the environment. Further research is required to assess the plant's salinity reduction capacity in larger-scale systems integrated into aquaculture facilities. It would also be interesting to evaluate sodium accumulation in plant tissues, to optimize the bio-desalination process.

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