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Growing *Salicornia europaea* L. with Saline Hydroponic or Aquaculture Wastewater

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Abstract: Among halophyte plants, *Salicornia* species (also known as glasswort or sea asparagus) are increasingly grown in open fields and greenhouses for edible or non-edible purposes. Their salinity tolerance makes it possible to irrigate *Salicornia* plants with saline waters and even seawater, which cannot be used by other crop species. In this work, *S. europaea* (L.) was cultivated in pots under the typical climatic conditions of the fall season in the Mediterranean region and irrigated with non-saline standard nutrient solution (SNS) or saline wastewater discharged from a greenhouse semi-closed hydroponic (substrate) culture of tomato or a saltwater recirculating aquaculture system (RAS) with Gilthead sea bream (*Spaurus aurata* L., which was used as such or after dilution (50:50) with SNS. Plant growth was not significantly affected by the composition of irrigation water, while higher antioxidant capacity (measured using the DPPH assay) and concentration of photosynthetic pigments, phenols, flavonoids, and ascorbic acid were found in the shoots of SNS plants than in those of plants irrigated with wastewater. The level of lipid peroxidation and H_2O_2 production significantly increased in the SNS plants, which also showed higher activity of superoxide dismutase and lower activity of catalase. These results suggest that *S. europaea* can be cultivated using wastewater with moderate to high salinity discharged from greenhouse hydroponic crops or RASs, and that salt is not strictly required for the growth of this species. Using non-saline nutrient solution can result in moderate oxidative stress that improves the shoot quality of *S. europaea*.

Keywords: halophytes; glasswort; greenhouse effluents; plant antioxidant system; recirculating aquaculture system

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1. Introduction

There is an ever-growing interest in the cultivation of halophytic plants due to the increasing soil salinization and scarcity of freshwater, which are both exacerbated by climatic changes and anthropic activities [1]. Their tolerance to high salt concentrations makes halophytic species promising crops for the exploitation of saline water and even seawater, which cannot be used by other crop species [2]. Many aquaculture and greenhouse production systems generate large amounts of wastewater, with moderate to high concentrations of NaCl and polluting agents, such as nitrogen and phosphorus [3,4]. In the context of the circular economy, halophytic species could be grown hydroponically using saline wastewater from greenhouse crops or recirculating aquaculture systems (RASs) to reduce the environmental impact and running costs of these production systems (e.g., Puccinelli et al. [5,6]).

Among halophytic plants, several species of the genus *Salicornia* (in the Amaranthaceae and popularly known as glasswort, marsh samphire, or sea asparagus) are promising new

food or non-food crops in both open fields and greenhouses [7–9]. One of the most studied *Salicornia* species is *S. europaea* L. (Syn.: *S. perennis* Willd. subsp. *perennans*; *S. patula* Duval-Jouve), which is widely distributed in the Mediterranean coastal areas [9,10]. As an edible crop, *Salicornia* shoots are consumed, either raw or boiled, due to their pleasant salty taste and nutritional value [2]; they are rich in dietary fibers, minerals, unsaturated fatty acids, important amino acids, vitamins, and several antioxidant compounds. Moreover, the presence of bioactive secondary metabolites makes *Salicornia* extracts effective against oxidative stress and some pathologies, such as diabetes, asthma, hepatitis, cancer, and gastroenteritis [11].

The goal of this work was to assess the response of *S. europaea* to irrigation with saline wastewater discharged from a greenhouse semi-closed hydroponic (substrate) culture of tomato or a saltwater RAS with Gilthead sea bream (*Spaurus aurata* L.). In previous works with two facultative halophytes, Buck's horn (*Plantago coronopus* L.) [5] and sea beet (*Beta vulgaris* L. spp. maritima) [6], grown in water culture, the crop yield was markedly reduced by using greenhouse or RAS effluents with very similar salinity and ion composition to those used in the present work. *Salicornia europaea* is an obligate halophyte, and its growth is stimulated rather than depressed by irrigation with saline water. Thus, we hypothesized that greenhouse and RAS effluents would not affect the growth of *S. europaea*, which, on the contrary, would be decreased by irrigation with a non-saline nutrient solution.

Plant growth, shoot concentration of some minerals and antioxidant compounds, and the activity of some antioxidant enzymes were measured in plants grown in pots in a greenhouse under the typical climatic conditions of the fall season in the Mediterranean area.

2. Materials and Methods

2.1. Plant Material and Growing Conditions

Four-month-old potted plants of *S. europaea* were sourced from a commercial nursery; they were propagated by seeds and individually cultured in a 1 L (14 mm diameter) plastic pot in a substrate consisting of peat and green compost. At the University of Pisa (Pisa, Italy), the plants were grown for six weeks from mid-July to September 2021 in a greenhouse, with a crop density of approximately 38 pot m^{-2}. All the plants were drip-irrigated every 1–3 days with a fresh nutrient solution. *Salicornia europaea* is a short-day plant, and day length was established as an 18 h day length (06:00 a.m. to 12:00 p.m.) by using dim light provided by LED strips (24 V, 30 W m^{-1}) to prevent flowering [12].

The average daily solar radiation and air temperature inside the greenhouse were 9.53 MJ m^{-2} d^{-1} and 25.9 °C, respectively. The nutrient solution used to irrigate the plants was differentiated at the beginning of September 2021; during the experiment, which lasted 63 days, the irrigation dose was weekly adjusted to maintain a drain fraction between 25% and 35%.

2.2. Experimental Design and Nutrient Solutions

The experimental design consisted of four irrigation treatments, each replicated three times; each block hosted 48 plants (12 plants per treatment). The plants were irrigated with standard nutrient solution (SNS) or the effluent from a semi-closed substrate culture of tomato (HE) or from a RAS with Gilthead Sea bream, which was used as such (AE100) or diluted (50:50) with tap water (AE50).

The SNS was prepared by dissolving a relevant quantity of inorganic salts in tap water, which contained 1.50 mM Na. The HE was sourced from an independent experiment on the effects of salinity on tomatoes grown in the substrate and consisted of the nutrient solution discharged after one month of recirculation when tomato plants were 85 days old (from planting date). Tomatoes were grown in stone wool, with a crop density of 3.2 plants m^{-2}. The AE was discharged from an RAS that consisted of six cylindrical tanks (volume was 0.425 m^3); a nitrifying biofilter; a blower for water aeration a chiller for water temperature control (set point temperature was 23 °C); UV lamps for water disinfection. The AE was collected when fish were at the on-growing stage, and the fish density was approximately

25 kg m^{-3}. In the RAS, the water contained 25 g L^{-1} of the synthetic sea salt Instant Ocean in tap water [13].

Both HE and AE were collected two days before starting the experiment, filtered to eliminate solid debris, and then kept in light-tight irrigation tanks after pH adjustment to 5.5 with sulphuric acid. The SNS was also prepared before the experiment and stored in a light-tight tank.

The electrical conductivity (EC) and the concentration of Na and nutritive elements in tap water and nutrient solutions are reported in Table 1. The concentrations of mineral elements in the SNS were within the ranges recommended for hydroponic nutrient solutions [14] and were equal to those of the control solution used in similar experiments conducted previously [5,6,13].

Table 1. Mineral composition, electrical conductivity (EC), and pH of the standard nutrient solution (SNS) and hydroponic or aquaculture effluents used to irrigate *Salicornia europaea* plants grown in pots in a greenhouse. The effluents were collected from a semi-closed substrate culture of tomato (HE) or a recirculating aquaculture system (RAS) with Gilthead Sea bream, which was used as such (AE100) or diluted (50:50) with tap water (AE50). The aquaculture effluent also contained 0.10, 14.70, and 18.01 mg L^{-1} of organic nitrogen, total and dissolved organic carbon, respectively, and negligible levels of N-NH_4 and N-NO_2.

	Tapwater	**Nutrient Solutions**			
		SNS	**HE**	**AE50**	**AE100**
N-NO_3 (mM)	-	10.00	10.48	2.30	5.64
P-PO_4 (mM)	-	1.50	0.21	1.08	0.66
K (mM)	0.14	9.00	16.23	4.18	8.20
Ca (mM)	3.32	4.50	7.01	5.95	7.40
Mg (mM)	0.99	2.50	5.83	20.55	40.10
Na (mM)	1.50	1.50	59.75	204.50	408.40
B (μM)	5.56	40.00	20.10	153.51	301.01
Cu (μM)	0.31	3.00	4.96	0.55	0.79
Fe (μM)	1.43	40.00	30.54	3.49	5.45
Mn (μM)	0.55	10.00	0.73	0.70	0.95
Zn (μM)	8.56	10.00	3.25	7.85	7.14
EC (dS m^{-1})	1.01	2.71	10.14	18.45	37.18

In both experiments, the pH and EC of nutrient solutions were repeatedly checked, and the pH was adjusted to 5.5–6.0 with sulphuric acid when needed; the EC did not vary substantively during the experiment. On two occasions, three and six weeks after the beginning of the experiment, the water drained out from the bottom of the pot was collected in each treatment to measure pH and EC, which were within 15% and 32% of the values of the nutrient solutions.

2.3. Plant Growth and Shoot Mineral Concentration

At the end of the experiment, four plants were sampled from each replicate for the determination of shoot fresh (FW) and dry weight (DW). The DW was determined after drying fresh samples in a ventilated oven at 70 °C until reaching constant weight.

Dried samples were digested with a mixture (5:2) of nitric acid (65%) and perchloric acid (35%) at 240 °C for 1 h, and mineral elements were determined as follows: Ca, Mg, K, and Na by atomic absorption spectrophotometry; P by UV/VIS spectrometry (Olsen's method). The content of organic nitrogen was determined using the Kjeldahl method. Nitrate (NO_3) concentration was measured spectrophotometrically in dried samples that were extracted with distilled water (100 mg DW in 20 mL) at room temperature for 2 h, using the salicylic-sulfuric acid method. The mineral concentration was expressed on a DW basis.

2.4. Shoot Concentration of Secondary Metabolites and Antioxidant Capacity

Four shoots of individual plants were also sampled from each replicate at the end of the experiment. They were rapidly frozen in liquid nitrogen and stored at −80 °C until analysis.

Total chlorophylls and carotenoids were determined after extraction of 1 g of shoot samples in 10 mL of 100% (*v*/*v*) methanol. All samples were left at 4 °C overnight in the dark before reading the absorbance at 662 nm, 645 nm, and 470 nm. The pigment concentration was determined according to Lichtenthaler [15].

Shoot samples (0.25 g FW) were also extracted in 2 mL of 70% (*v*/*v*) methanol at 4 °C for 30 min of incubation; afterwards, the extracts were centrifuged for 20 min. The supernatant was used for the spectrophotometric determination of the concentration of total phenols and flavonoids as well as the total antioxidant capacity, as previously reported [13,16]. Total phenolic concentration was expressed as milligrams of gallic acid equivalents (GAE) per gram of FW, the total flavonoid content as milligrams of (+)- catechin equivalents (CE) per gram FW, and the total antioxidant capacity as µmol Trolox equivalent per gram FW (TEAC).

The concentration of ascorbate (AsA), dehydroascorbate (DAsA), and total ascorbate (AsA, DAsA, TAsA;) was measured spectrophotometrically (at 515 nm) in shoot samples extracted with 6% trichloroacetic acid according to Kampfenkel et al. [17].

2.5. Hydrogen Peroxide Production, Lipid Peroxidation, and Activities of Antioxidant Enzymes

Three fresh shoot samples for each irrigation treatment were used for the determination of H_2O_2, lipid peroxidation, and the activity of superoxide dismutase (SOD, EC 1.15.1.1) and catalase (CAT, EC 1.11.1.6).

Endogenous H_2O_2 levels were determined following Brennan and Frenkel [18]. The concentration of peroxide in acetone extracts was determined by comparing absorbance against a standard curve representing the titanium–H_2O_2 complex over a range from 0 to 20 µmol mL^{-1}. All H_2O_2 measurements were normalized to tissue FW.

Lipid peroxidation was determined by the thiobarbituric acid reactive substances (TBARS) assay [19]. TBARS content was expressed as nanomoles of MDA equivalents (MDAe) per gram of FW.

Enzymes were extracted from fresh shoots (200 mg FW) with 2 mL of 50 mM sodium phosphate (Na/P) buffer (pH7.0) containing 1.0 mM EDTA, 1.0 mM PMSF, and 0.2% insoluble PVPP (*w*/*v*), according to Pistelli et al. [20]. After centrifugation at 12,000× *g* at 4 °C for 20 min, the supernatant was used to measure the concentration of soluble proteins and the enzymatic activities.

The soluble protein content was determined according to Bradford [21] using bovine serum albumin as standard.

Enzymatic assays were carried out at 25 °C. Catalase activity (CAT, EC 1.11.1.6) was determined by monitoring the decline in absorbance at 240 nm for 1 min due to the decomposition of H_2O_2 in a reaction mixture containing 8.8 mM H_2O_2 (Aebi, 1974). The activity of CAT was expressed in international units (U) equal to the amount of CAT necessary to decompose 1.0 µM of H_2O_2 per minute. Superoxide dismutase (SOD, EC 1.15.1.1) activity was assayed by measuring its ability to inhibit at 560 nm the photoreduction of nitroblue tetrazolium (NBT), as reported by Beyer and Fridovich [22]. The activity of SOD is given in U equals the amount of enzyme required to cause 50% inhibition of the NBT. The specific activity of both SOD and CAT is reported as units per mg protein.

2.6. Statistical Analysis

Statistical analysis was performed using JMP Statistical Software (JMP Pro 17.0.0; SAS Institute, Cary, NC, USA, Software). Data were tested for the normality of distribution using Shapiro Wilk's test and for homogeneity of variances using Levene's test, and they were then subjected to 1-way ANOVA followed by Tukey's Honestly Significant Difference

(HSD) or the Kruskal–Wallis test according to the variance homogeneity, with a cut-off significance of $p < 0.05$ for mean separation.

A principal component analysis (PCA) was also carried out after the standardization of the data to investigate whether there were clusters between leaf quality attributes and to identify clusters across different irrigation treatments.

3. Results

Plant FW and DW did not significantly differ across the four irrigation treatments (Figure 1). No important differences were found in shoot dry matter content and succulence, which averaged 100.0 g kg^{-1} FW and 9.14 g H_2O kg^{-1} DW, respectively.

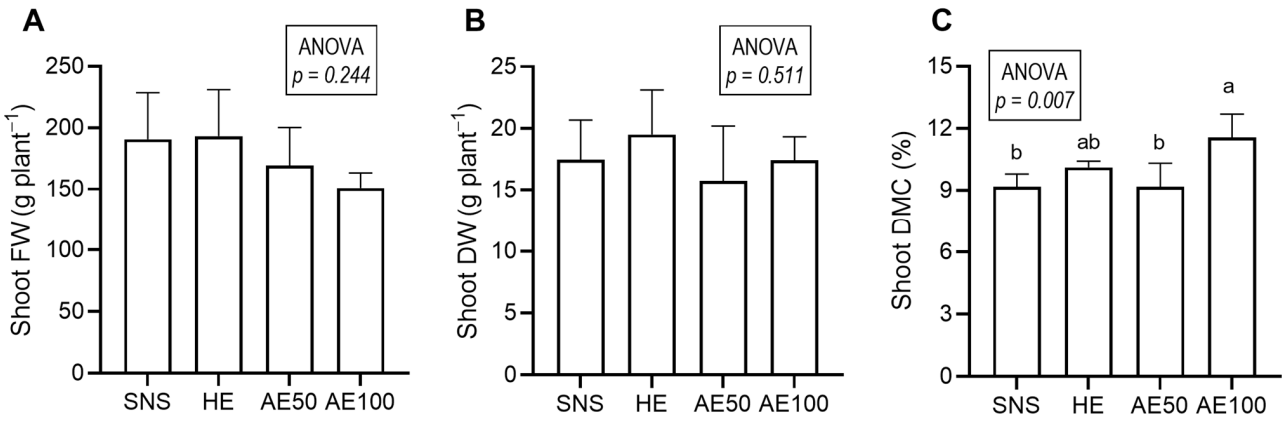

Figure 1. Shoot fresh (**A**) and dry weight (**B**), and dry matter content (**C**, DMC; DW/FW%) of *Salicornia europaea* plants grown in pots in a greenhouse and irrigated with standard nutrient solution (SNS) or the effluent from a semi-closed substrate culture of tomato (HE) or a recirculating aquaculture system with Gilthead Sea bream, which was used as such (AE100) or diluted (50:50) with tap water (AE50). Mean values (n = 3; ±SE) flanked by different letters were statistically different for $p \leq 0.05$ (Tuckey's test).

In contrast, the irrigation treatment did not affect the shoot concentration of all the macroelements, except Mg (Table 2). The concentration of organic N was greater in SNS and HE plants than in those irrigated with AE50 or AE100 (Table 2).

Table 2. Shoot concentration (on a dry weight basis) of macronutrients in *Salicornia europaea* plants grown in pots in a greenhouse and irrigated with standard nutrient solution (SNS) or the effluent from a semi-closed substrate culture of tomato (HE) or a recirculating aquaculture system with Gilthead Sea bream, which was used as such (AE100) or diluted (50:50) with tap water (AE50). Mean values (n = 3; ±SE) flanked by different letters were statistically different for $p \leq 0.05$ (Tuckey's test or Kruskal–Wallis test *).

	Irrigation Treatments				**ANOVA (*p*-Value)**
	SNS	**HE**	**AE50**	**AE100**	
N-org (g kg^{-1})	28.70 ± 1.03 ab	31.35 ± 1.63 a	22.26 ± 1.28 c	24.47 ± 0.56 bc	<0.001
N-TOT (g kg^{-1})	38.23 ± 1.66 a	38.24 ± 2.31 a	24.60 ± 1.60 b	28.70 ± 0.59 b	<0.001
P (g kg^{-1})	6.67 ± 0.47 a	4.44 ± 0.28 b	2.88 ± 0.21 c	3.01 ± 0.14 c	<0.001
K (g kg^{-1}) *	67.53 ± 5.23 a	43.58 ± 1.72 b	59.72 ± 3.17 a	13.40 ± 10.37 c	0.004
Ca (g kg^{-1})	43.90 ± 5.26 ab	50.74 ± 3.95 a	21.98 ± 1.64 c	34.72 ± 5.01 bc	0.001
Mg (g kg^{-1})	12.54 ± 0.75	13.45 ± 0.55	14.882 ± 0.55	12.81 ± 0.69	0.060

Compared to other plant groups, the SNS plants also showed higher concentrations of P, K, and Ca (not concerning HE treatment) (Table 2). No significant differences were

recorded among HE, AE50, and AE100 treatments regarding the shoot concentration of macronutrients (Table 2).

Shoot NO_3 concentration did not significantly differ in SNS and HE plants but was significantly lower in AE50 and AE100 plants (Figure 2A). Shoot Na concentration significantly differed across all the irrigation treatments and increased with the salinity and Na level in irrigation water (Figure 2B).

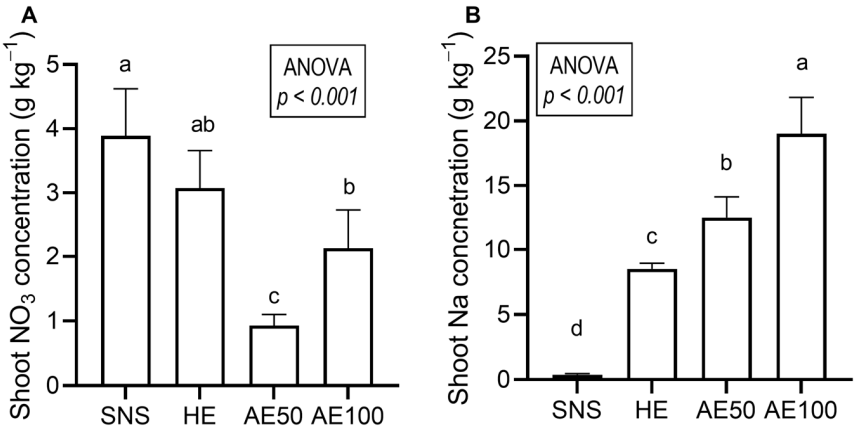

Figure 2. Shoot concentration (on a fresh weight basis) of nitrate (NO_3, (**A**)) and sodium (Na, (**B**)) in *Salicornia europaea* plants grown in pots in a greenhouse and irrigated with standard nutrient solution (SNS) or the effluent from a semi-closed substrate culture of tomato (HE) or a recirculating aquaculture system with Gilthead Sea bream, which was used as such (AE100) or diluted (50:50) with tap water (AE50). Mean values (n = 3; ±SE) flanked by different letters were statistically different for $p \leq 0.05$ (Tuckey's test).

The shoot concentrations of AsA, DAsA, and T-AsA, and total chlorophylls, carotenoids, phenols, and flavonoids, were significantly higher in the SNS plants than in those irrigated with wastewater, without significant differences among HE, AE50, and AE100 treatments (Table 3).

Table 3. Shoot concentration (on a fresh weight basis) of ascorbate and total chlorophylls, carotenoids, phenols, and flavonoids, and total antioxidant capacity (TEAC, DPPH assay) in *Salicornia europaea* plants grown in pots in a greenhouse and irrigated with standard nutrient solution (SNS) or the effluent from a semi-closed substrate culture of tomato (HE) or a recirculating aquaculture system with Gilthead Sea bream, which was used as such (AE100) or diluted (50:50) with tap water (AE50). Means (n = 4; ±SE) flanked by different letters are statistically different for $p \leq 0.05$ (Tuckey's test or Kruskal–Wallis test *). Abbreviations: GAE, gallic acid equivalents; CE, catechin equivalent; TEAC, Trolox equivalent antioxidant capacity.

	Irrigation Treatments				**ANOVA (*p*-Value)**
	SNS	**HE**	**AE50**	**AE100**	
Ascorbate (AsA; mg g^{-1})	0.20 ± 0.02 a	0.14 ± 0.02 b	0.13 ± 0.00 b	0.14 ± 0.01 b	0.007
Dehydroascorbate (DAsA; mg g^{1})	3.20 ± 0.27 a	2.15 ± 0.21 b	2.12 ± 0.06 b	2.26 ± 0.08 b	0.004
Total ascorbate (TAsA; mg g^{-1})	3.40 ± 0.29 a	2.30 ± 0.22 b	2.25 ± 0.07 b	2.40 ± 0.09 b	0.004
Chlorophyll a (Chla; µg g^{-1})	151.97 ± 22.90 a	82.94 ± 6.08 b	93.97 ± 6.21 b	84.28 ± 12.12 b	0.011
Chlorophyll b (Chlb; µg g^{-1}) *	84.06 ± 13.91 a	34.67 ± 2.94 b	35.88 ± 2.61 b	33.43 ± 3.13 b	0.032
Total chlorophylls (TChl; µg g^{-1})	236.03 ± 36.58 a	117.61 ± 8.46 b	129.85 ± 8.57 b	117.71 ± 14.93 b	0.003
Chla/Chlb ratio	1.81 ± 0.07 b	2.39 ± 0.13 ab	2.62 ± 0.17 a	2.52 ± 0.09 a	0.007
Carotenoids (Car; µg g^{-1})	54.23 ± 4.87 a	36.03 ± 3.20 b	37.81 ± 1.69 b	34.20 ± 4.13 b	0.011
Total Phenols (TPC; mg GAE g^{-1}) *	2.36 ± 0.73 a	0.31 ± 0.02 b	0.16 ± 0.01 c	0.15 ± 0.01 c	0.004
Flavonoids (mg CE g^{-1}) *	1.95 ± 0.29 a	0.15 ± 0.03 bc	0.16 ± 0.00 b	0.08 ± 0.02 c	0.007
TEAC (µmol HE g^{-1})	1.82 ± 0.05 a	1.15 ± 0.11 b	0.38 ± 0.03 c	0.13 ± 0.05 d	<0.001

The Chla/Chlb ratio was significantly lower in SNS plants than in the other plant groups (apart from HE), with insignificant differences between HE, AE50, and AE100 treatments (Table 3).

The total antioxidant capacity (TEAC) significantly differed across all the treatments and decreased with increasing water salinity (Table 3).

The shoot concentration of H_2O_2 was significantly greater in the SNS plants than in those irrigated with wastewater, without any significant differences among HE, AE50, and AE100 plants (Table 4). Shoot MDA concentration was significantly lower in AE50 plants than in those irrigated with SNS (Table 4).

Table 4. Concentration (on a FW basis) of hydrogen peroxide (H_2O_2) and malondialdehyde MDA), and the activity of superoxide dismutase (SDO) and catalase (CAT) in the shoots of *Salicornia europaea* plants grown in pots in a greenhouse and irrigated with standard nutrient solution (SNS) or the effluent from a semi-closed substrate culture of tomato or a recirculating aquaculture system with Gilthead Sea bream, which was used as such (AE100) or diluted (50:50) with tap water (AE50). Means (n = 3; ±SE) flanked by different letters are statistically different for $p \leq 0.05$ (Tuckey's test).

	Irrigation Treatments				**ANOVA (*p*-Value)**
	SNS	**HE**	**AE50**	**AE100**	
H_2O_2 (μmol g^{-1})	1.70 ± 0.11 a	1.01 ± 0.08 b	0.83 ± 0.07 b	0.87 ± 0.06 b	<0.001
MDA (nmol g^{-1})	1.99 ± 0.07 a	1.34 ± 0.25 ab	1.03 ± 0.25 b	1.49 ± 0.27 ab	≤0.020
SOD (U/mg protein)	351.11 ± 29.97 a	89.13 ± 2.68 b	47.66 ± 1.47 c	90.92 ± 11.61 b	<0.001
CAT ((U/mg protein)	8.36 ± 1.49 b	20.65 ± 1.81 a	19.11 ± 1.95 a	18.82 ± 1.57 a	<0.001

The activity of SOD significantly differed across the irrigation treatments and decreased with increasing water salinity (Table 4). Diversely, the activity of CAT was markedly lower in the SNS plants than in those irrigated with wastewater, with insignificant differences among HE, AE50, and AE100 treatments.

The first two principal components (PCs) explained a cumulative variance of 86% (Figure 3). The cumulative variance was mostly (78.3%) explained by PC1, which correlated positively with CAT activity, and negatively with SOD activity, the concentration of MDA, H_2O_2, photosynthetic pigments, phenolic compounds, ASA, DAsA, and TEAC. The PC2 explained only 7.7% (Figure 3).

The relationship between the parameters measured in this study is shown by the loadings in Figure 3A. Parameters located close to each other showed strong co-variance. A larger contribution of the parameters to the PCs is indicated by the position furthest away from the origin. On the left half of the loading plot, a cluster with the concentration of MDA, H_2O_2, photosynthetic pigments, and ascorbic acid, and a second cluster with the concentration of phenolic compounds, H_2O_2, and SOD activity, suggests strong co-variance between these variables as well as a strong contribution of these quantities, along with MDA and TEAC to PC1. The CAT also contributed to PC1; however, it was located on the positive side.

The relationship between the analyzed parameters is shown by the scores reported in Figure 3B. Two main groups were distinguished by PC1 and PC2. The negative side of PC1 is constituted by SNS treatment, which showed the highest concentration of pigments, ascorbic acid, phenolic compounds, H_2O_2, TEAC, and MDA, and the highest activity of SOD. The right half of the plot (positive side of PC1) is related to HE, AE50, and AE100 plants, which showed the highest CAT activity.

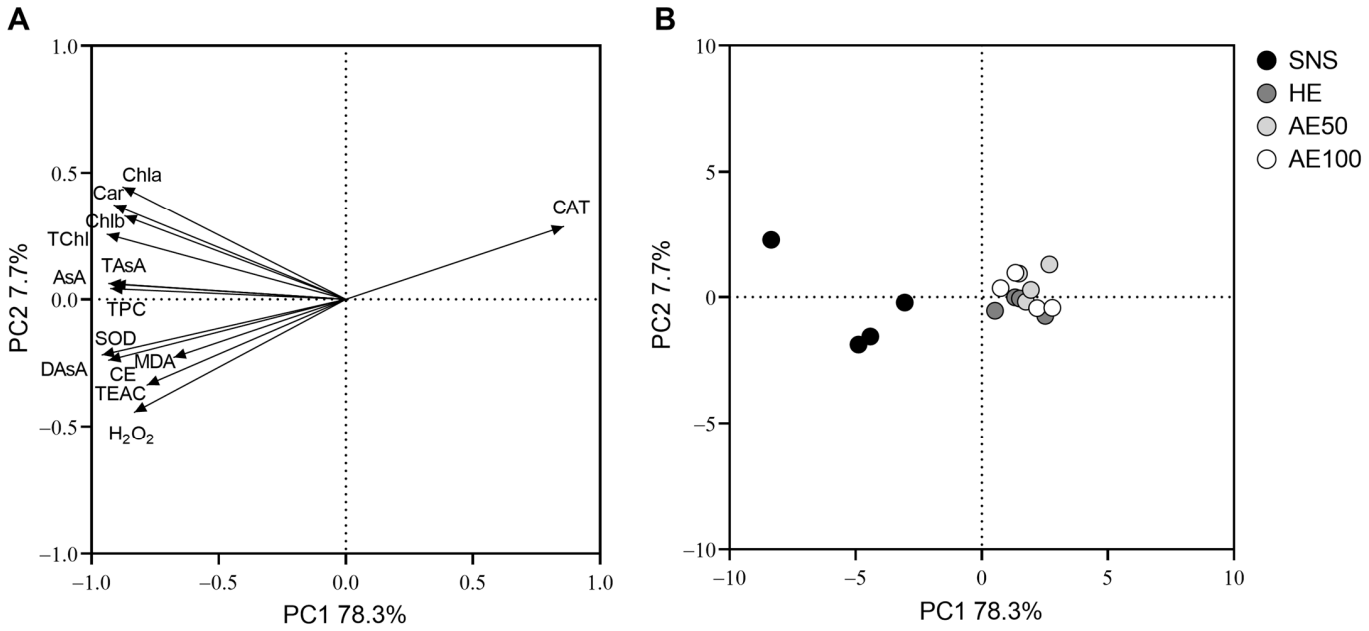

Figure 3. Loading (**A**) and score plots (**B**) for PC1 and PC2 describing the variations of the parameters determined in the shoots of *Salicornia europaea* plants grown in pots in a greenhouse and irrigated with standard nutrient solution (SNS) or the effluent from a semi-closed substrate culture of tomato (HE) or a recirculating aquaculture system with Gilthead Sea bream, which was used as such (AE100) or diluted (50:50) with tap water (AE50). Abbreviations: CE, catechin equivalent; TEAC, Trolox equivalent antioxidant capacity; TChl, total chlorophyll; Car, Carotenoids; Chla, chlorophyll a; Chlb, chlorophyll b; AsA, ascorbic acid; DAsA, dehydroascorbic acid; TAsA, total ascorbic acid; TPC, total phenols; H_2O_2, and hydrogen peroxide; MDA, malondialdehyde; CAT, catalase; SOD, superoxide dismutase.

4. Discussion

4.1. Plant Growth and Mineral Relations

Salicornia europaea tolerates very high salt concentrations (up to 200 mM) in soil and irrigation water [2] and is considered an obligate halophyte (or euhalophyte [9]). However, it is unclear whether it grows better in the presence or absence of salt, since contrasting results were found in greenhouse experiments with *S. europaea* exposed to salinities ranging from zero to up to 1000 mM NaCl. For instance, optimal growth was observed at 10 g L^{-1} NaCl (171 mM) in *S. europaea* grown in stone wool cubes [23]. Cárdenas-Pérez et al. [10] reported that in pot-grown *S. europaea*, the highest fresh and dry biomass production occurred with a concentration of 200 or 400 mM NaCl in the irrigation water; conversely, plant growth was significantly reduced at 0, 800, and 1000 mM NaCl. Similar results were reported by Ushakova et al. [24], Sun et al. [25], and Hulkko et al. [26]. For instance, Hulkko et al. [26] cultivated *S. europaea* in deep water culture with nutrient solution NaCl concentrations of 0, 171, 342, 513, and 684 mM; the highest biomass was obtained at intermediate NaCl levels, while growth was compromised at 0 and 342 mM NaCl.

In our work, *S. europaea* was cultivated in pots under the typical climatic conditions of the fall season in the Mediterranean region, and no significant differences were observed in shoot FW and DW among plants grown with different salinities of irrigation water. Similar results were obtained by other authors in *S. europaea* grown in perlite-filled pots [27], raft systems [28], or sandy soil conditions [29]. In a factorial experiment with *S. europaea* grown in sand, the effect of NaCl (0 or 50 mM) in the nutrient solution depended on the nitrate concentration (2, 14, and 50 mM) [30]. These authors found that NaCl stimulated growth at 2 or 50 mM N-NO_3, while no significant effect of NaCl was observed when the plants were grown with 14 mM N-NO_3 concentration, which is within the typical N-NO_3 levels in hydroponic nutrient solutions [31]. Our results agree with those reported by the

aforementioned authors since the composition of irrigation water did not significantly affect the production of fresh and dry biomass (Figure 1).

These results indicate that *S. europaea* can grow satisfactorily in the absence of high salinity. Moreover, *S. europaea* was confirmed to be a promising crop for the exploitation of saline wastewaters, including the effluents from hydroponics and RAS, as found in previous works (e.g., Fitzner et al. [23]; Quintã et al. [32]; Webb et al. [33]; Chu and Brown [34]).

The effluents used in this work presented the typical defects of semi-closed hydroponic systems (e.g., Massa et al. [35] Puccinelli et al. [5]) and saltwater RAS (e.g., Campanati et al. [36]; Puccinelli et al. [6]) when compared with standard hydroponic solutions: higher salinity and Na concentration; a lower concentration of N (N-NO_3), P, and trace elements apart from B, which was quite high since AE was discharged from a RAS with artificial seawater.

The usage of greenhouse or aquaculture effluents for hydroponic production of fresh vegetables and herbs offers the benefits of saving water and fertilisers and reducing the cost of wastewater depuration and environmental impact due to the emission of nutrients (in particular, nitrate and phosphate) to the environment. In greenhouse horticulture, fertilisation accounts for up to 9% of the total production costs (Martínez-Alvarez et al. [37]; Martínez-Granados et al. [38]). The incidence of fertilisation on the operating costs of commercial crops has recently risen because of the marked increase in the price of fertilisers in the last few years [39]. On the other hand, regulations on the use of agricultural effluents for crop fertigation should be considered. In the European Union, for example, greenhouse drainage water is considered industrial wastewater, and its application for irrigation requires authorization [40].

Our findings also suggest that *S. europaea* could be cultivated hydroponically, with a concentration of several nutrients (e.g., N and P) much lower than those recommended for this growing technology [31]. Indeed, significant differences occurred between the SNS treatments and the other treatments regarding shoot macronutrients (Table 2). These differences were likely due to the lower concentration of these elements and the higher level of Na in HE (not K and Ca), AE50, and AE100 effluents (Table 1). High Na levels in the root zone can reduce the acquisition of K [41] and Ca [42]. In *S. europaea* plants grown in peat-filled pots or water culture, the uptake of K and Ca diminished with increasing NaCl levels in the growing medium [24]. Nevertheless, despite the lower concentration of almost all macronutrients, the plants irrigated with wastewater grew well and healthily, without symptoms of nutrient deficiency, like SNS plants, and biomass production did not differ across the four treatments (Figure 1).

4.2. Shoot Quality

The shoot quality of *S. europaea* was assessed by measuring the concentration of Na, NO_3 (Figure 2), AsA, total photosynthetic pigments, phenols, and flavonoids (Table 3), and the total antioxidant capacity.

High Na consumption increases the risk of cardiovascular disease [43], and NO_3 is also dangerous for human health [44].

In the present work, shoot Na content was much higher in HE, AE50, and AE100 plants than in those irrigated with SNS (Figure 2). However, the daily intake of Na with a serving of 50 g *Salicornia* shoots with the highest Na content was lower than the allowed daily intake for adults (2 g d^{-1}; [43]).

Many leafy greens are among the primary sources of NO_3 for humans, and, hence, the European Commission has imposed limits on the NO_3 levels in some crop species, such as lettuce, spinach, and rocket salad [45]. In wastewater irrigation treatments, the shoot NO_3 level was lower than the maximum level laid down for autumn-grown spinach by the European Union (3.5 g NO_3 kg^{-1} FW), while it was notably higher in SNS plants (Figure 2). *Salicornia* is generally consumed cooked, and cooking has been found to reduce the NO_3 level in vegetables [46]; therefore, the risk of an excessive NO_3 intake with sporadic *Salicornia* consumption is minimized. The lower NO_3 concentration of AE50 and AE100 shoots was probably the result of high NaCl in irrigation water (Table 1). It is known that

salinity decreases the root uptake and leaf accumulation of NO_3 because of the antagonistic interaction between NO_3 and chloride [47].

The nutraceutical value of fruits and vegetables is generally associated with their content of minerals and antioxidant molecules, such as carotenoids, flavonoids, and phenols. These molecules and a set of antioxidant enzymes protect plants against the production of reactive oxygen species (ROS) induced by many abiotic and biotic stressors [48]. For instance, SOD and CAT play a fundamental role in eliminating superoxide (O_2^-) and (H_2O_2) [49]; SOD catalyzes the conversion of the highly toxic O_2^- to H_2O_2, which, in turn, is detoxified by CAT and other enzymes (e.g., APX). A low amount of ROS triggers plant adaptation to stress conditions, while an excess generation of ROS produces severe cell damage [50].

Salicornia europaea usually grows efficiently in salt soils thanks to the osmotic adjustment resulting from the uptake of salt ions [9,51]. In this work, low irrigation water salinity induced moderate oxidative stress in *S. europaea,* as indicated by the significant increase in the shoot concentration of H_2O_2 and antioxidant metabolites (i.e., AsA, carotenoids, phenols, and flavonoids) in SNS plants. An alteration in the ion imbalance and osmotic adjustment may be the cause of oxidative stress that occurs in SNS plants. The increase in the shoot content of H_2O_2 was consistent with the increased SOD activity and a parallel decrease in CAT activity.

Ascorbic acid (AsA) is one of the most powerful agents for scavenging ROS and contributes to promoting several physiological functions in plants, such as the reduction of H_2O_2 in the chloroplast, producing DAsA [52]. In this work, the higher level of DAsA found in SNS plants was likely to protect them against oxidative stress induced by low salinity. Our results are in contrast with those reported by several authors, who observed oxidative stress induced by the presence of NaCl, not by its absence, in halophyte species including *S. europaea* [27]. In agreement with our findings, however, Ghanem et al. [29] reported that in *S. europaea*, fresh and dry shoot biomass was not affected by the NaCl salinity of irrigation water in a range between 0 and 400 mM, while the shoot concentration of total carotenoids and flavonoids was notably greater in plants grown in the absence of NaCl than in salt-treated plants.

A stressor of a physical, chemical, or biological nature may have a positive (eustress) or negative (distress) effect on plants [53]. In this work, a low salt concentration appeared as a eustress factor that improved the quality of *S. europaea* shoots without adversely affecting crop yield.

5. Conclusions

Our results suggest that *S. europaea* can be cultivated using wastewater with moderate to high salt concentrations discharged from greenhouse hydroponic crops or recirculation aquaculture systems. We also found that salt is not strictly required for the growth of this plant species; however, a low salt concentration in irrigation water can result in moderate oxidative stress that induces an improvement in shoot nutraceutical quality.

The adaptation of obligate halophytes to tolerate salinity needs further research to individuate the activation of specific genes in conditions of stressful or non-stressful salt concentrations in the growing medium.

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