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NaCl Improves *Suaeda salsa* Aniline Tolerance in Wastewater

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Abstract: Halophytes have been studied as a model for morphological traits of adaptation to saline environments. However, little information has been given on plant growth, chlorophyll fluorescence responses, and change of ion content in halophytes grown in an aniline–salinity coexistent environment. This study hypothesized that aniline could induce alterations in plant growth, chlorophyll fluorescence, and ion content in *Suaeda salsa*, but salinity could promote the tolerance of halophytes to aniline. A 6 (aniline) × 3 (NaCl) factorial experiment (for a total of 18 treatments) was conducted to test the above hypothesis. After 30 d of cultivation, roots and shoots were harvested separately to analyze the effects of salinity on the seedling growth under aniline stress. Biomass accumulation was inhibited by aniline treatment, and the inhibition was significantly alleviated by 200 mM NaCl. The change in chlorophyll fluorescence in leaves with aniline stress was moderated by the addition of NaCl. The removal efficiency of aniline was significantly enhanced by moderate salinity. Aniline stress decreased the accumulation of Mg^{2+} , but various concentrations of NaCl increased the accumulation of Mg^{2+} , especially with 200 mM NaCl in both roots and shoots. Both aniline and salinity decreased the content of Ca^{2+} . There was a negative correlation between the K^+ and NaCl concentrations and between the Cl^- and aniline concentrations. Our results indicated that *Suaeda salsa* may be suitable for the remediation of salinity and aniline-enriched wastewater.

Keywords: halophytes; aniline; salinity; chlorophyll fluorescence

1. Introduction

Aniline ($C_6H_5NH_2$) is an important intermediate in the organic and fine chemical industry [1]. It is typically used for biochemical treatment of azo dye wastewater containing benzidine [2]. This is possibly one of the most important environmental issues [3]. Aniline discharged into water has strong toxicity and inhibits the growth of aquatic animals and plants [4]. It can directly or indirectly harm human health by migration through the food chain [5]. Plants show high sensitivity to aniline according to morphological and physiological aspects [6]. Aniline is also easily adsorbed or oxidized by soil into secondary intermediate products that are more toxic and difficult to degrade [7]. Therefore, aniline is listed as one of the serious organic pollutants that need to be strictly controlled in the environment [8]. Technologies have been proposed to remedy aniline-polluted wastewater [9,10], and plants are effective, convenient, and sensitive, thereby making them ideal indicator organisms for environmental pollutants and gene toxicity effects [11].

However, nearly 10% of dyestuffs in printing and dyeing wastewater are discharged into the environment as effluent after dying and processing [12]. They contain a large amount of salts and ultimately affect the well-being of aquatic ecosystems [13,14]. The damage effected by salt stress on glycophytes is manifested mainly in plant growth [15], limiting photosynthesis [16], ion toxicity, and osmotic stress by changing all aspects of plant physiology [17]. The mineral disturbance induced by the interaction between microelements and essential elements is also one of the causes of toxicity in plants [18]. However, halophytes can grow normally and are able to complete their seed-to-seed cycle at concentrations of 200 mM NaCl or higher [19,20]. Halophyte is a very promising phytoresource with many fundamental and potential uses [21].

Salinity and aniline in printing and dyeing wastewater place further constraints on phytoremediation. In addition to aniline tolerance, the plants used for this purpose must also bear the ion and osmotic stress of high NaCl loads. *Suaeda salsa* (*S. salsa*) is an annual halophyte that is widely distributed in China [22]. It was demonstrated that *S. salsa* can thrive in saline–alkali land [23] and reduce the soil salt content. This suggests that *S. salsa* may be a viable phytoremediator of saline–alkali land [24]. This species was studied as a model of morphological characteristics adapted to saline environments [25].

The selection of *S. salsa* was based on the idea that the cross-tolerance of halophytes is relatively straightforward [26]. Cross-tolerance refers to the phenomenon that the resistance of plants to stress causes the resistance to another stress. High concentrations of aniline in printing and dyeing wastewater resources may have a potentially adverse influence on the growth of *S. salsa*. However, studies indicating the biological physiology of aniline in halophytes have rarely been conducted. In this research, we used *S. salsa* as a candidate to investigate its cross-tolerance to aniline and salinity. This research provides not only a scientific basis for the evaluation of the toxic effects of aniline on halophytes, but also a technical reference for the adaptive strategy and mechanism of wastewater polluted by aniline and salinity.

2. Materials and Methods

2.1. Plant Growth Conditions

The experiment was performed in the greenhouse of Shaanxi University of Science and Technology with temperatures of 26 ± 2 °C/ 20 ± 2 °C in the day and night, respectively, and a light/dark period of 16 h/8 h. The seeds of the halophyte *S. salsa* were collected from saline soils in Fukang, Xinjiang ($44^{\circ}09'56''$ N, $87^{\circ}50'55''$ E). The seeds of *S. salsa* were randomly selected for cultivation and germinated in an incubator at 25 °C. When the seedlings reached a height of 6–8 cm, seedlings with uniform size were transplanted into clean culture bottles with 1/4 Hoagland's solution (200 mL) for cultivation.

2.2. Experimental Design

The stress treatments were started 1 week after the transplantation. The basic irrigation medium was 1/4-Hoagland's solution. Salt was supplied with sodium chloride (NaCl) at 0, 100, and 200 mM, the concentration of NaCl solution was increased by 50 mM every day. After 2 weeks of NaCl treatment, 0, 1, 2, 4, 6, and 8 mg·L^{−1} of aniline (Tianjin Chemical Reagent Factory, Tianjin, China) was added, and then the cultures were incubated for 2 weeks before harvesting. Throughout the process, the exposure solution was replaced daily and completely shielded from light. The experiment was a 3 (NaCl) × 6 (aniline) factorial combination, making a total of 18 treatments, four biological replicates. Each treatment was performed in quadruplicate. The light intensity of the artificial climate chamber (RZX-500C Intelligent Artificial Climate Chamber, Ningbo Jiangnan Instrument Factory) was 12,000 lx.

2.3. Plant Growth Measurements

Plants were harvested after washing with deionized water [27]. The plants were divided into root and shoot parts. The dry weight (DW) of the plant was measured after oven-drying the samples at 70 °C for 48 h.

2.4. Shoot and Root Elemental Analysis

Dry plant material was ground into fine powder in an agate mortar, and 0.05 g of powder sample was digested using a mixture of HNO₃:H₂SO₄:HClO₄ (10.0:1.0:0.5; v/v/v) for 2.5 h at 110 °C. After cooling, the digestion products were filtered and diluted with distilled water to a total volume of 50 mL. The total concentrations of Na, K, Ca, and Mg were determined by atomic absorption spectrometry (ZEE nit 700P, Analytik Jena AG, Jena, Germany). The concentration of Cl was determined by ion chromatography IC (940 Professional IC Vario, Metrohm, Herisau, Switzerland) [28].

To determine the aniline in water (collected under the concentration of aniline 1, 2, 4, 6, and 8 mg·L⁻¹) by HPLC (515, Waters, USA) [29], 900 µL of the sample was withdrawn, immediately filtered with a 0.22 µm filter membrane, and collected in an HPLC vial mixed with 100 µL of acetonitrile to obtain the liquid to be measured. An HPLC 2478 dual-wavelength UV detector was used to determine aniline in the sample. The chromatographic conditions included a wavelength of 240 nm, mobile phase of methanol:water (35:65; v/v), mobile phase pH of 3.0, flow rate of 0.8 mL·min⁻¹, and injection volume of 10 µL. Each sample was measured three times (Supplemental Figures S1 and S2).

The removal efficiency (RE) of aniline was determined by applying Equation (1), as follows [30]:

$$\%RE = \frac{(C_0 - C_f)}{C_0} \times 100, \quad (1)$$

where RE (%) is the RE, C₀ (mg·L⁻¹) is the initial concentration (1, 2, 4, 6, and 8 mg·L⁻¹ aniline), and C_f (mg·L⁻¹) is the final concentration (the remaining concentration of aniline in the culture).

2.5. Chlorophyll Fluorescence and Leaf Pigment Content Analysis

Determination of leaf chlorophyll (Chl) fluorescence was made by pulse–amplitude–modulation fluorometer (PAM 2100, H. Walz, Effeltrich, Germany). Before measuring the Chl fluorescence parameters, leaves were placed in a dark-adapted state for 30 min using light exclusion clips. After irradiating the measuring light (1 µmol·m⁻²·s⁻¹) and saturating illuminations (3000 µmol·m⁻²·s⁻¹), the minimum Chl fluorescence yield in the dark-adapted state (F₀) and maximum Chl fluorescence yield in the dark-adapted state (F_m) were measured. After irradiating the light (150 µmol·m⁻²·s⁻¹) and saturating illuminations, we measured the minimum Chl fluorescence yield in the light-adapted state (F₀'), maximum Chl fluorescence yield in the light-adapted state (F_m'), and steady-state Chl fluorescence yield in the light-adapted state (F_s). Using these parameters, we calculated the maximum photosystem II (PSII) photochemical efficiency (F_v/F_m = (F_m – F₀)/F_m) and quantum yield of PSII electron transport (ΦPSII = (F_m' – F_s)/F_m') [31]. The Chl-*a*, Chl-*b*, and total Chl were extracted based on the method of Lichtenthaler (1987) [32].

2.6. Statistical Analyses

Statistical analyses were conducted with analysis of variance (ANOVA) using the statistical software package SPSS 17.0 (SPSS Inc, version 17.0). The least significant difference (LSD) test was applied at 5% probability for the ranking and appraisal of treatment means. The normality and homogeneity of variances were tested before analysis. For the ion content, each treatment group (Aniline alone, Aniline + 100 mM NaCl, and Aniline + 200 mM NaCl) was separately compared with control. Redundancy analysis (RDA) in the CANOCO package was used for evaluation, and the parametric test was replaced by the Monte Carlo test to evaluate the responses of all parameters.

3. Results

3.1. Alleviation of the Effects Caused by Aniline on the Growth of *S. salsa* by Salinity

S. salsa, as a representative halophyte, was used to investigate the toxicity of aniline. As shown in Figure 1, 1–8 mg·L^{−1} of aniline significantly ($p = 0.003$) reduced the DW of shoots, and 4–8 mg·L^{−1} of aniline reduced the DW of roots; however, 1–2 mg·L^{−1} of aniline increased the DW of roots. Therefore, the stress of aniline on roots showed the phenomenon of “low promotion and high suppression” (Figure 1). Specifically, the root and shoot DW decrease under 8 mg·L^{−1} of aniline was about 9% and 25%, compared to the control under non-aniline stress (Figure 1). Furthermore, 200 mM NaCl increased the root DW by 59% and shoot DW by 27%, compared with those of the control under non-aniline stress. There were significant interactions between the effects of NaCl and aniline on the root ($p = 0.008$) and shoot ($p = 0.05$) DW according to the ANOVA (Table S1). The DW of roots under the combined stresses of aniline and NaCl was higher than that under individual aniline stress ($p = 0.003$), thereby indicating that NaCl alleviated the decrease in root DW caused by aniline (1–8 mg·L^{−1}). There were also significant interactions between the effects of NaCl and aniline on shoot DW ($p = 0.001$) (Table S1). The results suggested that 200 mM NaCl alleviated the toxicity of aniline. The interactions between aniline and NaCl showed a greater effect on *S. salsa* shoot and root growth (Figure 1).

Figure 1. The effect of NaCl on the shoot and root dry weight (DW) (mg) of *S. salsa* under various aniline stress. A, aniline; N, NaCl; * means with different uppercase letters indicate significant difference ($p < 0.05$) between NaCl treatments at a particular aniline level. ** Means with different lowercase letters are significantly different ($p < 0.01$) between aniline treatments at a particular salinity level. *** Means denoted by the different letters are significantly different at a $p < 0.001$ according to the LSD test.

3.2. Increase in the Removal Efficiency of Aniline in *S. Salsa* Seedlings by Salinity

Various initial concentrations (1, 2, 4, 6, and 8 mg·L^{−1}) of aniline solution were prepared to investigate the effect of the initial concentration on aniline removal efficiency (RE) under 0, 100,

and 200 mM NaCl treatments. As shown in Figure 2, with the treatment of 0 mM NaCl, the RE of aniline gradually decreased with the increase in its initial concentration from the highest efficiency (35.1%) with the 1 mg·L⁻¹ aniline treatment to the lowest efficiency (7.3%) with the 8 mg·L⁻¹ aniline treatment. The regression analysis demonstrated a relationship between the RE (RE%) (y) of plants of treated groups (aniline) and the initial concentration (x) ($R^2 = 0.906$) (Figure 2, Figure S3a). At 100 mM NaCl, the RE of 1 mg·L⁻¹ of aniline was promoted by 15.8% compared with that of the non-NaCl treatment ($R^2 = 0.982$) (Figure 2, Figure S3a). With the supplementation of 200 mM NaCl, the RE of 1 mg·L⁻¹ of aniline was increased by 32.46% compared with that of the non-NaCl treatment (Figure 2, Figure S3b) ($R^2 = 0.995$). The results indicated that moderate NaCl can significantly enhance the RE of aniline in wastewater.

Figure 2. Effect of initial concentration on removal efficiency of aniline under different NaCl culture media.

3.3. Salinity Moderation of the Decrease in Leaf Pigment Contents Due to Aniline

Chl, as the important growth indicator, was analyzed with the application of NaCl and aniline. As shown in Figure 3a, the significant differences in Chl-*a* content under 1 mg·L⁻¹ aniline were caused by 200 mM NaCl, compared with 0 and 100 mM NaCl. As the aniline content increased, the Chl-*a* content decreased, and NaCl significantly alleviated this effect. With the application of 200 mM NaCl, the content of Chl-*a* increased by 9.60%, 10.80%, 18.03%, and 34.80%, under 2, 4, 6, and 8 mg·L⁻¹, respectively, compared with that of the aniline control (Figure 3a). There was no significant effect on the content of Chl-*b* under 1 and 2 mg·L⁻¹ aniline treatment. The Chl-*b* content decreased with the increase in aniline, and NaCl also significantly alleviated this decrease under 4–8 mg·L⁻¹ aniline (Figure 3b). With the application of 200 mM NaCl, the content of Chl-*b* increased by 2.90%, 6.60%, 9.60%, and 18.13%, under 2, 4, 6, and 8 mg·L⁻¹, respectively, compared with that of the aniline control (Figure 3b). The ratio of Chl *a/b* did not significantly decrease with the 1, 2, and 4 mg·L⁻¹ mg·L⁻¹

aniline treatments. With the application of 200 mM NaCl, the ratio of Chl *a/b* increased by 8.2% and 13.4%, under 6 and 8 mg·L⁻¹, respectively, compared with that of the aniline treatment (Figure 3c).

Figure 3. The effect of NaCl on the chlorophyll content under aniline stress. (a) The content of chlorophyll (Chl-*a*), (b) the content of chlorophyll (Chl-*b*), (c) the ratio of Chl *a/b*. For each parameter, the data are the means $\pm$ SE of five replications, with standard errors indicated by the vertical bars. *** Means denoted by the different letters are significantly different at a $p < 0.001$ according to the LSD test.

3.4. Photosystem II Parameters Altered by Aniline and Salinity

To determine whether the NaCl and aniline treatments affected the efficiency of photosynthesis, we measured the maximal efficiency of photochemistry of PSII (F_v/F_m) and Φ PSII. NaCl treatment had very little effect on F_v/F_m (Figure 4a). This suggested that the PSII of *S. salsa* seedlings was tolerant to NaCl, and NaCl stress had no effect on PSII photochemistry in dark-adapted leaves. However, with the 4, 6, and 8 mg·L⁻¹ aniline treatments, the F_v/F_m of the PSII was significantly reduced by 41.35%, 44.40%, and 47.02%, respectively, compared with that of the control (Figure 4a). Without aniline stress, NaCl showed no effect on F_v/F_m , but under the stress of high concentrations of aniline (4, 6, and 8 mg·L⁻¹), 200 mM NaCl displayed a positive promoting effect on F_v/F_m (Figure 4a). The results showed that NaCl counteracted the aniline-induced inhibition of the photochemical activity of PSII.

Figure 4. The effect of NaCl on chlorophyll fluorescence parameters of *S. salsa* under aniline stress. (a) F_v/F_m ; (b) Φ PSII. For each parameter, the data are the means $\pm$ SE of five replications, with standard errors indicated by the vertical bars. Means denoted by the different letters are significantly different at a $p < 0.05$ according to LSD test.

Φ PSII is the product of F_v/F_m , which reflects the actual photochemical reaction efficiency. Φ PSII increased by 5.2% and 12.3% under the 100 mM and 200 mM NaCl treatments (Figure 4b), respectively, which indicated that photosynthetic electron transport was increased under NaCl treatment. Φ PSII

was significantly reduced under aniline stress in a dose-dependent manner. Φ PSII was the lowest under 0 mM NaCl treatment and 8 mg·L⁻¹ aniline stress. The decrease in the Φ PSII value caused by 8 mg·L⁻¹ of aniline was 55.20%, but after the supplementation of 200 mM NaCl, the observed decrease was only approximately 20.73%. These results indicated NaCl alleviated the decrease in Φ PSII due to aniline stress.

The photochemical quenching coefficient (qP) measures the proportion of PSII reaction centers capable of photochemistry [33]. There was no significant effect on qP under the 1 mg·L⁻¹ aniline treatment. The qP decreased with the increase in aniline, and 200 mM NaCl significantly alleviated the decrease by 8.90%, 6.40%, 16.80%, and 22.51%, under 2, 4, 6, and 8 mg·L⁻¹, respectively, compared with that of the control (Figure S4). These results indicated that the application of NaCl increased the quantum yield and protected the photosynthetic system from aniline damage.

3.5. Cl⁻ Concentration in *S. salsa* Tissues

Both the root and shoot Cl⁻ concentrations were increased by the individual effects of NaCl (Figure 5c, Figure 6c). Specifically, 200 mM NaCl increased the root and shoot Cl⁻ concentrations by 82.05% and 80.80%, respectively, and 100 mM NaCl increased the root and shoot Cl⁻ concentrations by 66.91% and 61.56%, respectively. Furthermore, 4–8 mg·L⁻¹ of aniline significantly decreased the shoot Cl⁻ concentrations under 100 mM NaCl, but in the roots, 2, 4, 6, and 8 mg·L⁻¹ of aniline significantly decreased the Cl⁻ concentrations under the 100 mM NaCl treatment ($p = 0.001$). In the treatment with 200 mM NaCl, aniline significantly decreased the shoot Cl⁻ concentrations, and decreased the shoot Cl⁻ concentrations under 8 mg·L⁻¹ aniline (Figure 5c, Figure 6c). These results indicated that aniline can inhibit the accumulation of Cl⁻ in *S. salsa* seedlings.

Figure 5. Effect of NaCl on shoot elemental Na⁺ (a), K⁺ (b), Cl⁻ (c), K⁺/Na⁺ (d), Ca²⁺ (e), and Mg²⁺ (f) concentrations (mmol·g⁻¹ DW) of *S. salsa* exposed to aniline. For each parameter, the data are the means $\pm$ SE of five replications, with standard errors indicated by the vertical bars. Means denoted by the different letters are significantly different at a $p < 0.05$ according to the LSD test, the different letters indicate statistically significant differences within different NaCl treatments.

Figure 6. Effect of NaCl on root elemental Na^+ (a), K^+ (b), Cl^- (c), K^+/Na^+ (d), Ca^{2+} (e) and Mg^{2+} (f) concentrations ($\text{mmol}\cdot\text{g}^{-1}$ DW) of *S. salsa* exposed to aniline. For each parameter, the data are the means $\pm$ SE of five replications, with standard errors indicated by the vertical bars. Means denoted by the different letters are significantly different at a $p < 0.05$ according to the LSD test, the different letters indicate statistically significant differences within different NaCl treatments.

3.6. Aniline Inhibition of Na^+ Absorption and Change in K^+/Na^+ in *S. salsa* Seedlings

The NaCl supply significantly increased the root and shoot Na^+ concentrations ($p < 0.01$); Na^+ increased by 34.68% and 195.70% in the shoots and by 59.90% and 86.36% in the roots with a supply of 100 mM and 200 mM NaCl, respectively (Figure 5a, Figure 6a). In shoots, 1–2 $\text{mg}\cdot\text{L}^{-1}$ of aniline showed no effect on the Na^+ content, while 6 $\text{mg}\cdot\text{L}^{-1}$ and 8 $\text{mg}\cdot\text{L}^{-1}$ of aniline clearly decreased the Na^+ content under the treatments of 100 mM and 200 mM NaCl (Figure 5a). However, aniline had no effect on the content of Na^+ in the roots (Figure 6a). In addition, the increments/decrements caused by NaCl were less with 8 $\text{mg}\cdot\text{L}^{-1}$ of aniline than those with 1–2 $\text{mg}\cdot\text{L}^{-1}$ and 4–6 $\text{mg}\cdot\text{L}^{-1}$ of aniline (Figure 6), thereby indicating that 8 $\text{mg}\cdot\text{L}^{-1}$ of aniline inhibited NaCl absorption. NaCl significantly ($p < 0.01$) decreased the root K^+/Na^+ ratio in comparison with that caused by individual aniline stress, especially for the 8 $\text{mg}\cdot\text{L}^{-1}$ aniline treatment.

There was no significant effect on shoot K^+ concentrations under the 1 $\text{mg}\cdot\text{L}^{-1}$, 2 $\text{mg}\cdot\text{L}^{-1}$, and 4 $\text{mg}\cdot\text{L}^{-1}$ aniline treatments. With the application of 200 mM NaCl, the K^+ concentrations decreased by 23.4%, 17.5%, and 15%, under 4, 6, and 8 $\text{mg}\cdot\text{L}^{-1}$, respectively, compared with no NaCl within corresponding aniline concentration. There was no significant effect on the root K^+ concentrations under the 1, 2, and 4 $\text{mg}\cdot\text{L}^{-1}$ aniline treatments, and the root K^+ concentrations decreased with the increase in aniline (Figure 6b). With the application of 200 mM NaCl, the K^+ concentrations increased by 11.17% and 9.05%, under 6 and 8 $\text{mg}\cdot\text{L}^{-1}$, respectively, compared with no NaCl within corresponding aniline concentration. There were interactions between the effects of NaCl and aniline on the shoot and root Na^+ ($p < 0.01$), K^+ ($p < 0.01$), and K^+/Na^+ ratio ($p < 0.01$) (Table S1). In addition, the K^+/Na^+ ratio reached homeostasis with NaCl supplementation under aniline stress (Figure 5a, Figure 6a).

3.7. NaCl Alleviation of the Decrease in Ca^{2+} and Mg^{2+} Caused by Aniline in *S. salsa*

Ca^{2+} and Mg^{2+} are essential elements for plant growth. The intracellular Ca^{2+} concentration can regulate ion transport across the membrane via its role in cell wall and membrane integrity [34]. Aniline stress significantly influenced the shoot and root Ca^{2+} concentrations, which shows that 4–8 $\text{mg}\cdot\text{L}^{-1}$ aniline affects the shoot and 6–8 $\text{mg}\cdot\text{L}^{-1}$ aniline affects the Ca^{2+} concentration of *S. salsa* root (Figure 5e, Figure 6e). With the application of 200 mM NaCl, the shoot Ca^{2+} concentration decreased by 4%, compared with that of the non-NaCl treatment ($p > 0.05$) (Figure 5e). Nevertheless, there was no significant effect on the root Ca^{2+} concentration under the 1 $\text{mg}\cdot\text{L}^{-1}$ aniline treatment. The root Ca^{2+} concentration decreased with the increase in aniline, but 200 mM NaCl increased the root Ca^{2+} concentration by 1.60%, 11.10%, 12.03%, and 11.48%, under 2, 4, 6, and 8 $\text{mg}\cdot\text{L}^{-1}$, respectively, compared with that of the control. The results suggested that 200 mM NaCl alleviated the Ca^{2+} reduction in the shoots and roots of *S. salsa* seedlings under high aniline (8 $\text{mg}\cdot\text{L}^{-1}$) concentration.

Mg in tissues is closely related to the salt tolerance of halophytes. The direct contribution of Mg indicates the succulent characteristics of *S. salsa* cells, which may be used as an isolation pool for salt ions [35]. Both the shoot and root Mg^{2+} concentrations increased with NaCl supply (100 mM and 200 mM) (Figure 5f, Figure 6f). The 8 $\text{mg}\cdot\text{L}^{-1}$ aniline treatment decreased the shoot Mg^{2+} concentration by 15.24%, 7.86%, and 1.23% under the 0, 100, and 200 mM NaCl treatments, respectively (Figure 5f), and also decreased the root Mg^{2+} concentration by 4.72%, 4.45%, and 2.73% under the 0, 100, and 200 mM NaCl treatments, respectively (Figure 6f). The results indicated that aniline stress inhibited Mg^{2+} accumulation in *S. salsa* and the supply of NaCl alleviated the inhibition of the Mg^{2+} concentration caused by aniline.

3.8. Ordination Analysis

Redundancy analysis (RDA) is used to detect the relationship between environmental factors and samples, and important environmental driving factors affecting sample distribution can be obtained [35–37]. Figure 7 indicates the relationship between visual response and explanatory variables (aniline and NaCl) (Figure 7a,b), which is based on a linear model. The Monte Carlo test with forward selection of variables was conducted, and highly significant conditional effects of all explanatory variables ($p = 0.002$) were indicated, with NaCl showing the strongest effect, followed by salinity on root and shoot ($F = 85.45$ and 70.85 , respectively). The response variables were divided into variables related to plant growth (including root DW and shoot DW) and ion concentrations in shoots and roots (including Cl^- , K^+ , Na^+ , K^+/Na^+ , Ca^{2+} , and Mg^{2+}).

There were several positive and negative trends in the relationships between the ion balance-related variables and explanatory variables. The K^+ and Na^+ concentrations in the plants had the strongest response to NaCl (Figure 7a,b). There was a significantly positive correlation between the Na^+ concentrations and NaCl, while a negative correlation between the K^+ concentrations and NaCl was also observed, but was not significant. The K^+/Na^+ ratio was also negatively correlated with NaCl. The Cl^- concentrations were negatively correlated with aniline, and most correlations were not significant. The Ca^{2+} concentrations were negatively correlated with aniline and Mg^{2+} was negatively correlated with NaCl and aniline, but most correlations were not significant. Meanwhile, there was a strong correlation between plant growth and NaCl.

The ANOVA rendered similar results to those of the RDA (Table S1). The results indicated that the elemental composition of *S. salsa* is related to salt-tolerance categories, and this relationship is mainly driven by elements directly related to salinity, such as Na, Cl, Mg, K, and Ca.

Figure 7. Response variables–environmental variables biplot of the redundancy analysis procedure. Response variables: R, root; R D, root dry weight (a); S, Shoot; S D, shoot dry weight (b); Na, Na⁺ concentration; K, K⁺ concentration; K/Na, K⁺/Na⁺ ratio; Ca, Ca²⁺ concentration; Mg, Mg²⁺ concentration; environmental variables: A, aniline; S, salinity. Partial effects of all explanatory variables were highly significant; (a) A, and S explained 12.87%, and 85.4% of data variability, respectively. (b) Partial effects of all explanatory variables were highly significant; A and S explained 28.1% and 70.8% of data variability, respectively.

4. Discussion

4.1. *S. salsa* Absorbance and Utilization of Salinity of Wastewater and Removal of Aniline

NaCl inhibited the growth of glycophytes, and the damage mainly included the comprehensive response of osmotic stress, ion toxicity, and mineral nutrient deficiency [34,38–40]. Halophytes can grow normally and are able to complete their seed-to-seed cycle at 200 mM NaCl or higher [19]. For most halophytes, low salinity promotes plant growth, but if the NaCl level reaches higher than 200–400 mM, halophytes will die [24,41]. In this study, optimal growth of *S. salsa* occurred at 200 mM NaCl. This suggested that the salt tolerance of *S. salsa* is extremely broad, and *S. salsa* is more adapted to high salinity conditions. The 200 mM NaCl treatment significantly promoted the growth of *S. salsa* (Figure 1), the increase in these indicators of the root system affected the growth of *S. salsa*. This may mainly be attributed to the relationship between Na⁺ and Cl[−], which can clearly induce leaf succulence of the species [42].

Aniline is often detected in aquatic environments with higher concentrations in aquatic organisms owing to its strong lipophilic properties and low water solubility [8,43]. The environmental fate of aniline is mostly dependent on the molecular size. Generally, the octanol–water partition coefficients of aniline range from 0.5–3.0 (log K_{OW} = 0.5–3.0). In this study, *S. salsa* removed aniline and salt improved the RE (Figure 2) because of the lower lipophilicity and lower metabolic ability of the plants [44,45]. This may have happened because the vacuole isolation of halophytes is not only limited to salt ions, but also extended to a kind of toxic ions and organic pollutants [46,47]. The strong subcellular isolation of toxic ions and compounds by *S. salsa* is an important feature of its salt tolerance and stress of organic pollutants [48,49]. It has been reported that phenanthrene and pyrene are transported via the apoplastic pathway [50]. The transportation of organic pollutants in plant phloem and xylem depends on the size of the molecule. It can be speculated that, because halophytes partition salt ions more efficiently than glycophytes, theoretically they can isolate any other toxic ions or compounds.

4.2. Moderate Salinity Enhancement of Protection of Photosystem II under High Aniline Stress in *S. salsa*

Chl-*a* is a critical component for light-harvesting and electron transfer in photosynthesis, which can regulate light absorption, transition, and distribution. A previous study indicated that NaCl increases the content of Chl and the Chl *a/b* ratio, which is the positive response of halophytes to the NaCl

environment [51]. A higher Chl content resulted in higher photochemical efficiency of PSII and then higher production (Figure 3, Figure 4). Chl *a/b* reflects the stacking extent of the thylakoid membrane, and the increase is often interpreted as a sign of less stacking [52]. In this study, aniline significantly reduced the content of Chl-*a*, and with the lower Chl *a/b* ratio, the extent of thylakoid stacking and the content of the light harvesting complex were greater. However, with the supply of 200 mM NaCl, the harvested light energy can be used adequately and it is more difficult for photoinhibition to occur.

PSII is believed to play a key role in leaf photosynthesis in responses to environmental perturbations [53]. In order to investigate if NaCl can counteract the aniline-induced inhibition of the photochemical activity of PSII, we detected the maximum PSII photochemical efficiency (F_v/F_m). Aniline decreased F_v/F_m by 47.1%, but the 200 mM NaCl treatment alleviated this decrease by 19.9% (Figure 4a). There were differences in F_v/F_m with different levels of aniline stress, and non-stress further indicated that aniline stress induced the increase in susceptibility of PSII to photoinhibition. However, no changes in F_v/F_m were observed between the 200 mM NaCl-treated and non-stressed plants measured at predawn. For *S. salsa*, the unsaturated fatty acids in membrane lipids protect the photosynthesis system against stress by alleviating the photoinhibition of PSII and PSI; meanwhile, high salinity can increase the unsaturated fatty acids content of membrane lipids [51,54].

We observed a significant decrease in Φ PSII at the $8 \text{ mg} \cdot \text{L}^{-1}$ aniline level (Figure 4b), which indicated the impairment of the light-harvesting complex of Φ PSII [55], thereby resulting in the decrease in heat dissipation depending on the xanthophyll cycle [56] and significant damage to the photosynthetic system of PSII in plants. We also noticed that the treatment of NaCl as high as 200 mM increased Φ PSII by only approximately 14.76%, thereby suggesting that PSII is very tolerant to NaCl. *S. salsa* has effective mechanisms to protect the photosystem under NaCl stress [57]. A possible explanation for this may be that phosphatidylglycerol (PG) is an integral component of photosynthetic membranes. It is important for both development and functioning of the photosynthesis apparatus [58,59]. For *S. salsa*, PG increased at higher salinity. Maintenance of PSII function and photosynthetic composition under salinity stress should be viewed as an important strategy for the halophyte *S. salsa* to grow in saline environments [22].

Zhang et al. [60] used transcriptome analysis to assess the salt stress responses in *S. salsa* leaves, identified different types of candidate genes associated with photosynthesis (PSB33 and ABA4). Although the effect of aniline on Chl fluorescence in *S. salsa* leaves has not been reported, it has been confirmed that changes in Chl fluorescence can reflect stress [61]. The decrease in F_v/F_m , Φ PSII, and qP indicated that the photoactivation of PSII was inhibited by aniline, which resulted in the restriction of electron transport from PSII to PSI and the destruction of the structural integrity of the thylakoid membrane [51].

4.3. Enhancement of Excretion of Toxic Molecules in *S. salsa* by Moderate Salinity

Na is not an essential element for most plant species, but it is necessary for osmotic regulation and the optimum growth of halophytes [62]. Our results indicated that aniline inhibited the absorption of Na by *S. salsa*, which was counteracted by the supplementation of 200 mM NaCl (Figure 5a, Figure 6a). *S. salsa* with 200 mM NaCl grew well under aniline stress and had high Na concentrations in their leaves (Figure 1, Figure 5a). Halophytes have the ability to retain high Na concentrations in their shoots via Na compartmentalization in the leaf cell vacuoles or by withdrawing it from cells by glands and bladders [63]. It was reported that Na (in the vacuoles) associated with compatible solutes, such as proline and mannitol, can conduce to the osmoregulation in halophytes. The physiological role of Na in succulent halophytes may be associated with the Na^+/H^+ antiporter activity, which is able to participate in Na compartmentalization in vacuoles for osmoregulation [64]. In order to maintain the normal growth of *S. salsa*, the contents of Na^+ and K^+ and the ratio of Na^+/K^+ in cells are important indexes of plant salt tolerance [65].

NaCl has a certain regulatory effect on the adverse environment of halophytes [66,67]. We also observed that the K^+ contents in the shoots and roots of *S. salsa* decreased with the increase in NaCl

concentration, even when exposed to $8 \text{ mg}\cdot\text{L}^{-1}$ of aniline stress (Figure 5b, Figure 6b). This indicated that the addition of NaCl decreased the absorption of K in *S. salsa*. Na was found to play an important role in osmotic regulation by replacing K under high salinity. It has been reported that halophytes prefer to keep low cation balances ($[(\text{K}^+) + [\text{Ca}^{2+}] + [\text{Mg}^{2+}]]/[\text{Na}^+]$), while this ratio may be higher in glycophytes [64,68]. However, the salt glands of *S. salsa* control salinity accumulation in plant tissues [62]. Thus, salt excretion is a phenomenon by which halophytes discharge excess salt reaching their leaves [69] and the high selectivity contributes to maintaining a suitable K^+/Na^+ ratio. However, excess ions such as K^+ , Mg^{2+} , and Ca^{2+} can be excreted [70]. It has been reported that salt glands and trichomes on the leaf surfaces of some halophytes can drain excess metals as a possible detoxification mechanism [27]. Zhang et al. [60] reported that potassium transporters (such as KEA3) could regulate balance excess Na^+ and Cl^- ions in the vacuole.

4.4. Increase in Resistance to Aniline by Moderate Salinity Regulating Nutrient Homeostasis in *S. salsa*

Nutrient homeostasis is one of the major factors of the survival of halophytes under stress [71]. In this study, *S. salsa* was exposed to a range of aniline concentrations and was more able to maintain its nutrient homeostasis with NaCl (NaCl 200 mM + aniline) than with aniline treatment alone. The effect of NaCl was shown to alleviate the inhibition of aniline toxicity on accumulation of Ca^{2+} and Mg^{2+} (Figure 5e,f, Figure 6e,f), and NaCl was negatively correlated with Ca^{2+} and Mg^{2+} accumulation (Figure 7). Our findings are in agreement with those of several previous reports on the incompatibility of Ca and Na in plant cells under salinity [72]. The uptake competition of Na^+ with Ca^{2+} and Mg^{2+} is induced by NaCl [73]. This is probably due to its higher Ca^{2+} and K^+ selectivity [64]. Meanwhile, the K^+ selectivity in the roots (Figure 6b) indicated that the roots have a regulation mechanism for efficient absorption of K^+ [74]. The transport of the polycyclic aromatic hydrocarbon phenanthrene into the root cells is coupled with K^+ influx and H^+ efflux [75]. This is a new perspective on the role of nutrient homeostasis in plant tolerance to organic pollutants, particularly in halophytes. Mg in the leaves of halophytes plays a major role in osmotic regulation and water uptake in saline soils. The Mg concentration of some halophytes has been shown to achieve similar levels to those of Na [76].

There is a strong relationship between the ion content of plants growing with both salinity and aniline and cross-tolerance. In this study, the halophyte *S. salsa*, which has succulent organs or salt glands, was characterized by high Na and Cl and low K concentrations in the leaves. Supplementation of 200 mM NaCl can not only assist *S. salsa* growth, but also alleviate the accumulation strategy of ions under aniline stress.

5. Conclusions

Halophytes tolerate abiotic stresses through cross-tolerance mechanisms. Moderate salinity alleviated aniline toxicity in the plants by protection of PSII, maintenance of ionic homeostasis, and increase in biomass accumulation. Salinity also enhanced the Na^+ and Cl^- uptake in plants under aniline stress and the nutrient uptake efficiency. Therefore, the present study is valuable for understanding the cross-tolerance of halophytes to salinity and organic pollutants, and is a new attempt at the phytoremediation of aniline wastewater by halophytes. This study provides important information about the toxic effects of aniline on halophytes in saline environments.

Supplementary Materials: The following are available online at <http://www.mdpi.com/2071-1050/12/18/7457/s1>, Figure S1: Determination of aniline a: $0 \text{ mg}\cdot\text{L}^{-1}$, b: $1 \text{ mg}\cdot\text{L}^{-1}$, c: $2 \text{ mg}\cdot\text{L}^{-1}$, d: $4 \text{ mg}\cdot\text{L}^{-1}$, e: $6 \text{ mg}\cdot\text{L}^{-1}$, f: $8 \text{ mg}\cdot\text{L}^{-1}$ by HPLC. Figure S2: Determination of aniline a: standard curve of aniline ($Y = 45020.502X - 3488.433$, $R^2 = 0.999$). b: Measurement concentration and configuration concentration of aniline (mean $\pm$ SE of three replications). Figure S3: Effect of NaCl on aniline removal efficiency (a) 0 mM NaCl, (b) 100 mM NaCl, (c) 200 mM NaCl. Figure S4: Effect of NaCl on chlorophyll fluorescence parameters qP of *S. salsa* under aniline stress. For each parameter, the data are the means $\pm$ SE of five replications, with standard errors indicated by the vertical bars. Means denoted by the different letters are significantly different at a $p < 0.05$ according to LSD test. Table S1: Results of ANOVA of aniline, salt on growth parameters

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References

- Gogoi, A.; Mazumder, P.; Tyagi, V.K.; Chaminda, G.T.; An, A.K.; Kumar, M. Occurrence and fate of emerging contaminants in water environment: A review. *Groundwater for Sustainable Development* **2018**, *6*, 169–180. [[CrossRef](#)]
- Wang, Z.; Zhao, W.Y.; Xu, L.W.; Mu, Q. Analysis of Genotoxicity and Characterization of Dissolved Organic Matter in the Aniline Industrial Wastewater. *Adv. Mater. Res.* **2014**, *1010–1012*, 472–477. [[CrossRef](#)]
- Dom, N.; Knapen, D.; Benoot, D.; Nobels, I.; Blust, R. Aquatic multi-species acute toxicity of (chlorinated) anilines: Experimental versus predicted data. *Chemosphere* **2010**, *81*, 177–186. [[CrossRef](#)] [[PubMed](#)]
- Cravedi, J.P.; Gillet, C.; Monod, G. In vivo metabolism of pentachlorophenol and aniline in arctic charr (*Salvelinus alpinus* L.) larvae. *Bull. Environ. Contam. Toxicol.* **1995**, *54*, 711–716. [[CrossRef](#)]
- Zhang, X.; Song, J.; Ji, W.; Xu, N.; Gao, N.; Zhang, X.; Yu, H. Phase-selective gelators based on closed-chain glucose derivatives: Their applications in the removal of dissolved aniline/nitrobenzene, and toxic dyes from contaminated water. *J. Mater. Chem. A* **2015**, *3*, 18953–18962. [[CrossRef](#)]
- Xie, H.C.; Li, C.R.; Li, J.H.; Wang, L. Phytoremediation of wastewater containing azo dye by sunflowers and their photosynthetic response. *Acta Ecol. Sin.* **2012**, *32*, 240–243. [[CrossRef](#)]
- Chen, H.; Zhuang, R.; Yao, J.; Wang, F.; Qian, Y.; Masakorala, K. Short-term effect of aniline on soil microbial activity: A combined study by isothermal microcalorimetry, glucose analysis, and enzyme assay techniques. *Environ. Sci. Pollut. Res.* **2014**, *21*, 674–683. [[CrossRef](#)]
- Yan, F.; Liu, T.; Jia, Q.; Wang, Q. Multiple toxicity endpoint–structure relationships for substituted phenols and anilines. *Sci. Total Environ.* **2019**, *663*, 560–567. [[CrossRef](#)]
- Nie, C.; Ao, Z.; Duan, X.; Wang, C.; Wang, S.; An, T. Degradation of aniline by electrochemical activation of peroxydisulfate at MWCNT cathode: The proofed concept of nonradical oxidation process. *Chemosphere* **2018**, *206*, 432–438. [[CrossRef](#)]
- Li, Y.; Zhang, Q.; Li, M.; Sang, W.; Wang, Y.; Wu, L. Bioaugmentation of sequencing batch reactor for aniline treatment during start-up period: Investigation of microbial community structure of activated sludge. *Chemosphere* **2020**, *243*, 125426. [[CrossRef](#)]
- Pullagurala, V.L.R.; Rawat, S.; Adisa, I.O.; Hernandez-Viezas, J.A.; Peralta-Videa, J.R.; Gardea-Torresdey, J.L. Plant uptake and translocation of contaminants of emerging concern in soil. *Sci. Total Environ.* **2018**, *636*, 1585–1596. [[CrossRef](#)] [[PubMed](#)]
- Wang, J.; Bi, L.; Ji, Y.; Ma, H.; Yin, X. Removal of humic acid from aqueous solution by magnetically separable polyaniline: Adsorption behavior and mechanism. *J. Colloid Interface Sci.* **2014**, *430*, 140–146. [[CrossRef](#)] [[PubMed](#)]
- Kariminiaae-Hamedani, H.R.; Sakurai, A.; Sakakibara, M. Decolorization of synthetic dyes by a new manganese peroxidase-producing white rot fungus. *Dyes Pigments* **2007**, *72*, 157–162. [[CrossRef](#)]
- Tabagari, I.; Kurashvili, M.; Varazi, T.; Adamia, G.; Gigolashvili, G.; Pruidze, M.; Niemsdorff, P. Application of *Arthrospira (Spirulina) platensis* against Chemical Pollution of Water. *Water* **2019**, *11*, 1759. [[CrossRef](#)]

15. Lin, J.; Wang, Y.; Sun, S.; Mu, C.; Yan, X. Effects of arbuscular mycorrhizal fungi on the growth, photosynthesis and photosynthetic pigments of *Leymus chinensis* seedlings under salt-alkali stress and nitrogen deposition. *Sci. Total Environ.* **2017**, *576*, 234–241. [[CrossRef](#)]
16. Fatma, M.; Asgher, M.; Masood, A.; Khan, N.A. Excess sulfur supplementation improves photosynthesis and growth in mustard under salt stress through increased production of glutathione. *Environ. Exp. Bot.* **2014**, *107*, 55–63. [[CrossRef](#)]
17. Chen, T.H.H.; Murata, N. Glycinebetaine protects plants against abiotic stress: Mechanisms and biotechnological applications. *Plant Cell Environ.* **2011**, *34*, 1–20. [[CrossRef](#)]
18. Rocha, D.N.; Martins, M.A.; Soares, J.; Vaz, M.G.M.V.; de Oliveira Leite, M.; Covell, L. Combination of trace elements and salt stress in different cultivation modes improves the lipid productivity of *Scenedesmus* spp. *Bioresour. Technol.* **2019**, *289*, 121644. [[CrossRef](#)]
19. Kefu, Z.; Hai, F.; Ungar, I.A. Survey of halophyte species in China. *Plant Sci.* **2002**, *163*, 491–498. [[CrossRef](#)]
20. Flowers, T.J.; Colmer, T.D. Plant salt tolerance: Adaptations in halophytes. *Ann. Bot.* **2015**, *115*, 327–331. [[CrossRef](#)]
21. Wang, H.L.; Wang, L.; Tian, C.Y.; Huang, Z.Y. Germination dimorphism in *Suaeda acuminata*: A new combination of dormancy types for heteromorphic seeds. *S. Afr. J. Bot.* **2012**, *78*, 270–275. [[CrossRef](#)]
22. Song, J.; Shi, G.; Xing, S.; Yin, C.; Fan, H.; Wang, B. Ecophysiological responses of the euhalophyte *Suaeda salsa* to the interactive effects of salinity and nitrate availability. *Aquat. Bot.* **2009**, *91*, 311–317. [[CrossRef](#)]
23. Tian, C.; Feng, G.; Li, X.; Zhang, F. Different effects of arbuscular mycorrhizal fungal isolates from saline or non-saline soil on salinity tolerance of plants. *Appl. Soil Ecol.* **2004**, *26*, 143–148. [[CrossRef](#)]
24. Song, J.; Wang, B. Using euhalophytes to understand salt tolerance and to develop saline agriculture: *Suaeda salsa* as a promising model. *Ann. Bot.* **2015**, *115*, 541–553. [[CrossRef](#)] [[PubMed](#)]
25. Ben Hamed, K.; Chibani, F.; Abdelly, C.; Magne, C. Growth, sodium uptake and antioxidant responses of coastal plants differing in their ecological status under increasing salinity. *Biologia* **2014**, *69*, 193–201. [[CrossRef](#)]
26. Shiri, M.; Rabhi, M.; El Amrani, A.; Abdelly, C. Cross-tolerance to abiotic stresses in halophytes: Application for phytoremediation of organic pollutants. *Acta Physiol. Plant* **2015**, *37*, 209. [[CrossRef](#)]
27. Tipirdamaz, R.; Gagneul, D.; Duhazé, C.; Aïnouch, A.; Monnier, C.; Özkum, D. Clustering of halophytes from an inland salt marsh in Turkey according to their ability to accumulate sodium and nitrogenous osmolytes. *Environ. Exp. Bot.* **2006**, *57*, 139–153. [[CrossRef](#)]
28. Song, J.; Fan, H.; Zhao, Y.; Jia, Y.; Du, X.; Wang, B. Effect of salinity on germination, seedling emergence, seedling growth and ion accumulation of a euhalophyte *Suaeda salsa* in an intertidal zone and on saline inland. *Aquat. Bot.* **2008**, *88*, 331–337. [[CrossRef](#)]
29. Jia, J.; Huang, C.; Bai, J.; Zhang, G.; Zhao, Q.; Wen, X. Effects of drought and salt stresses on growth characteristics of euhalophyte *Suaeda salsa* in coastal wetlands. *Phys. Chem. Earth* **2018**, *103*, 68–74. [[CrossRef](#)]
30. Sarafraz-Yazdi, A.; Es'Haghi, Z. Comparison of hollow fiber and single-drop liquid-phase microextraction techniques for HPLC determination of aniline derivatives in water. *Chromatographia* **2006**, *63*, 563–569. [[CrossRef](#)]
31. Lichtenthaler, H.; Buschmann, C.; Knapp, M. How to correctly determine the different chlorophyll fluorescence parameters and the chlorophyll fluorescence decrease ratio Rfd of leaves with the PAM fluorometer. *Photosynthetica* **2005**, *43*, 379–393. [[CrossRef](#)]
32. Lichtenthaler, H.K. Chlorophyll fluorescence signatures of leaves during the autumnal chlorophyll breakdown. *J. Plant Physiol.* **1987**, *131*, 101–110. [[CrossRef](#)]
33. Song, P.; Zhang, L.; Li, Q. Response of photosynthetic apparatus of *Isochrysis galbana* to different nitrogen concentrations. *Bioresour. Technol. Rep.* **2018**, *4*, 74–79. [[CrossRef](#)]
34. Sghaier, D.B.; Duarte, B.; Bankaji, I.; Caçador, I.; Sleimi, N. Growth, chlorophyll fluorescence and mineral nutrition in the halophyte *Tamarix gallica* cultivated in combined stress conditions: *Arsenic and NaCl*. *J. Photochem. Photobiol. B Biol.* **2015**, *149*, 204–214. [[CrossRef](#)]
35. Gao, B.; Song, J.; Liu, J.; Sui, N.; Fan, H.; Wang, B. Effects of salt stress on photosynthesis and ion accumulation patterns of *Suaeda salsa* under different habitats. *Acta Phytoecol. Sin.* **2010**, *34*, 671–677. [[CrossRef](#)]
36. Zhang, Y.; Thas, O. Constrained ordination analysis in the presence of zero inflation. *Stat. Model.* **2012**, *12*, 463–485. [[CrossRef](#)]

37. Ter Braak, C.J.; Šmilauer, P. Topics in constrained and unconstrained ordination. *Plant Ecol.* **2015**, *216*, 683–696. [[CrossRef](#)]
38. Gorcek, Z.; Erdal, S. Lipoic acid mitigates oxidative stress and recovers metabolic distortions in salt-stressed wheat seedlings by modulating ion homeostasis, the osmo-regulator level and antioxidant system. *J. Sci. Food* **2015**, *95*, 14. [[CrossRef](#)]
39. Barnawal, D.; Bharti, N.; Pandey, S.S.; Pandey, A.; Chanotiya, C.S.; Kalra, A. Plant growth-promoting rhizobacteria enhance wheat salt and drought stress tolerance by altering endogenous phytohormone levels and TaCTR1/TaDREB2 expression. *Physiol. Plantarum.* **2017**, *161*, 502–514. [[CrossRef](#)]
40. Xu, Q.; Burgess, P.; Xu, J.; Meyer, W.; Huang, B. Osmotic stress-and salt stress-inhibition and gibberellin-mitigation of leaf elongation associated with up-regulation of genes controlling cell expansion. *Environ. Exp. Bot.* **2016**, *131*, 101–109. [[CrossRef](#)]
41. Colmer, T.D.; Pedersen, O.; Wetson, A.M.; Flowers, T.J. Oxygen dynamics in a salt-marsh soil and in *Suaeda maritima* during tidal submergence. *Environ. Exp. Bot.* **2013**, *92*, 73–82. [[CrossRef](#)]
42. Qiu, N.; Chen, M.; Guo, J.; Bao, H.; Ma, X.; Wang, B. Coordinate up-regulation of V-H⁺-ATPase and vacuolar Na⁺/H⁺ antiporter as a response to NaCl treatment in a C₃ halophyte *Suaeda salsa*. *Plant Sci.* **2007**, *172*, 1218–1225. [[CrossRef](#)]
43. Fu, L.; Li, J.J.; Wang, Y.; Wang, X.H.; Wen, Y.; Qin, W.C. Evaluation of toxicity data to green algae and relationship with hydrophobicity. *Chemosphere* **2015**, *120*, 16–22. [[CrossRef](#)] [[PubMed](#)]
44. Ahmadi, S.; Igwegbe, C.A. Adsorptive removal of phenol and aniline by modified bentonite: Adsorption isotherm and kinetics study. *Appl. Water Sci.* **2018**, *8*, 170. [[CrossRef](#)]
45. Yang, B.; Wang, Y.; Liu, Z.; Liu, J.; Cai, J. Optimum removal conditions of aniline compounds in simulated wastewater by laccase from white-rot fungi. *J. Environ. Health Sci.* **2019**, *17*, 135–140. [[CrossRef](#)]
46. Hossain, M.A.; Piyatida, P.; da Silva, J.A.T.; Fujita, M. Molecular mechanism of heavy metal toxicity and tolerance in plants: Central role of glutathione in detoxification of reactive oxygen species and methylglyoxal and in heavy metal chelation. *J. Botany* **2012**, *2012*, 1–37. [[CrossRef](#)]
47. Tissut, M.; Raveton, M.; Ravanel, P. Ecoremediation. Cooperation between plants and soil microorganisms, molecular aspects and limits. In *Soil and Water Pollution Monitoring, Protection and Remediation*; Springer: Berlin/Heidelberg, Germany, 2006; pp. 489–504. ISBN 978-1-4020-4728-2.
48. Barhoumi, Z.; Djebali, W.; Abdelly, C.; Chaïbi, W.; Smaoui, A. Ultrastructure of *Aeluropus littoralis* leaf salt glands under NaCl stress. *Protoplasma* **2008**, *233*, 195–202. [[CrossRef](#)]
49. Smaoui, A.; Barhoumi, Z.; Rabhi, M.; Abdelly, C. Localization of potential ion transport pathways in vesicular trichome cells of *Atriplex halimus* L. *Protoplasma* **2011**, *248*, 363–372. [[CrossRef](#)]
50. Su, Y.H.; Zhu, Y.G. Transport mechanisms for the uptake of organic compounds by rice (*Oryza sativa*) roots. *Environ. Pollut.* **2006**, *148*, 94–100. [[CrossRef](#)]
51. Sui, N.; Li, M.; Li, K.; Song, J.; Wang, B.S. Increase in unsaturated fatty acids in membrane lipids of *Suaeda salsa* L. enhances protection of photosystem II under high salinity. *Photosynthetica* **2010**, *48*, 623–629. [[CrossRef](#)]
52. Zhang, H.; Hu, H.; Zhang, X.; Wang, K.; Song, T.; Zeng, F. Detecting *Suaeda salsa* L. chlorophyll fluorescence response to salinity stress by using hyperspectral reflectance. *Acta Physiol. Plant* **2012**, *34*, 581–588. [[CrossRef](#)]
53. Rozentsvet, O.; Nesterov, V.; Bogdanova, E.; Kosobryukhov, A.; Subova, S.; Semenova, G. Structural and molecular strategy of photosynthetic apparatus organisation of wild flora halophytes. *Plant Physiol.* **2018**, *129*, 213–220. [[CrossRef](#)]
54. Ghassemi-Golezani, K.; Farhangi-Abriz, S. Changes in Oil Accumulation and Fatty Acid Composition of Soybean Seeds under Salt Stress in Response to Salicylic Acid and Jasmonic Acid. *Russ. J. Plant Physiol.* **2018**, *65*, 229–236. [[CrossRef](#)]
55. Kalaji, H.M.; Govindjee Bosa, K.; Kościelniak, J.; Żuk-Golaszewska, K. Effects of salt stress on photosystem II efficiency and CO₂ assimilation of two Syrian barley landraces. *Environ. Exp. Bot.* **2011**, *73*, 64–72. [[CrossRef](#)]
56. Yin, Y.; Li, S.; Liao, W.; Lu, Q.; Wen, X.; Lu, C. Photosystem II photochemistry, photoinhibition, and the xanthophyll cycle in heat-stressed rice leaves. *J. Plant Physiol.* **2009**, *167*, 959–966. [[CrossRef](#)] [[PubMed](#)]
57. Lu, C.; Qiu, N.; Lu, Q.; Wang, B.; Kuang, T. Does salt stress lead to increased susceptibility of photosystem II to photoinhibition and changes in photosynthetic pigment composition in halophyte *Suaeda salsa* grown outdoors? *Plant Sci.* **2002**, *163*, 1063–1068. [[CrossRef](#)]
58. Rozentsvet, O.; Nesterov, V.; Bogdanova, E. Structural, physiological, and biochemical aspects of salinity tolerance of halophytes. *Russ. J. Plant Physiol.* **2017**, *64*, 464–477. [[CrossRef](#)]

59. Ivanov, A.; Allakhverdiev, S.; Huner, N.; Murata, N. Genetic decrease in fatty acid unsaturation of phosphatidylglycerol increased photoinhibition of photosystem I at low temperature in tobacco leaves. *BBA Bioenerg.* **2012**, *1817*, 1374–1379. [[CrossRef](#)] [[PubMed](#)]
60. Zhang, X.; Yao, Y.; Li, X.; Zhang, L.; Fan, S. Transcriptomic analysis identifies novel genes and pathways for salt stress responses in *Suaeda salsa* leaves. *Sci. Rep.* **2020**, *10*, 4236. [[CrossRef](#)] [[PubMed](#)]
61. Banks, J.M. Chlorophyll fluorescence as a tool to identify drought stress in *Acer* genotypes. *Environ. Exp. Bot.* **2018**, *155*, 118–127. [[CrossRef](#)]
62. Chenchouni, H. Edaphic factors controlling the distribution of inland halophytes in an ephemeral salt-lake “Sabkha ecosystem” at North African semi-arid lands. *Sci. Total Environ.* **2017**, *575*, 660–671. [[CrossRef](#)] [[PubMed](#)]
63. Dassanayake, M.; Larkin, J.C. Making plants break a sweat: The structure, function, and evolution of plant salt glands. *Front. Plant Sci.* **2017**, *8*, 406. [[CrossRef](#)] [[PubMed](#)]
64. Mori, S.; Suzuki, K.; Oda, R.; Higuchi, K.; Maeda, Y.; Yoshida, M. Characteristics of Na⁺ and K⁺ absorption in *Suaeda salsa* (L.) Pall. *Soil Sci. Plant Nutr.* **2011**, *57*, 377–386. [[CrossRef](#)]
65. Guo, J.R.; Wang, B.S. Effects of NaCl treatments on flower number, Na⁺ and K⁺ contents of *Suaeda salsa*. *Plant Physiol. J.* **2014**, *50*, 861–866. [[CrossRef](#)]
66. Feng, Z.T.; Deng, Y.Q.; Zhang, S.C.; Liang, X.; Yuan, F.; Hao, J.L. K⁺ accumulation in the cytoplasm and nucleus of the salt gland cells of *Limonium bicolor* accompanies increased rates of salt secretion under NaCl treatment using NanoSIMS. *Plant Sci.* **2015**, *238*, 286–296. [[CrossRef](#)]
67. Zhao, S.; Zhou, N.; Zhao, Z.Y.; Zhang, K.; Wu, G.H.; Tian, C.Y. Isolation of endophytic plant growth-promoting bacteria associated with the halophyte *Salicornia europaea* and evaluation of their promoting activity under salt stress. *Curr. Microbiol.* **2016**, *73*, 574–581. [[CrossRef](#)]
68. Kudo, N.; Fujiyama, H. Responses of Halophyte *Salicornia bigelovii* to Different Forms of Nitrogen Source. *Pedosphere* **2010**, *20*, 311–317. [[CrossRef](#)]
69. Labidi, N.; Ammari, M.; Mssedi, D.; Benzerti, M.; Snoussi, S.; Abdelly, C. Salt excretion in *suaeda fruticosa*. *Acta Biol. Hung.* **2010**, *61*, 299–312. [[CrossRef](#)]
70. Sobrado, M.A.; Greaves, E.D. Leaf secretion composition of the mangrove species *Avicennia germinans* (L.) in relation to salinity: A case study by using total-reflection X-ray fluorescence analysis. *Plant Sci.* **2000**, *159*, 1–5. [[CrossRef](#)]
71. Manousaki, E.; Kalogerakis, N. Halophytes-an emerging trend in phytoremediation. *Int. J. Phytorem.* **2011**, *13*, 959–969. [[CrossRef](#)]
72. Rabhi, M.; Ferchichi, S.; Jouini, J.; Hamrouni, M.H.; Koyro, H.W.; Ranieri, A.; Smaoui, A. Phytodesalination of a salt-affected soil with the halophyte *Sesuvium portulacastrum* L. to arrange in advance the requirements for the successful growth of a glycophytic crop. *Bioresour. Technol.* **2010**, *101*, 6822–6828. [[CrossRef](#)]
73. Reginato, M.A.; Turcios, A.E.; Luna, V.; Papenbrock, J. Differential effects of NaCl and Na₂SO₄ on the halophyte *Prosopis strombulifera* are explained by different responses of photosynthesis and metabolism. *Plant Physiol. Biochem.* **2019**, *141*, 306–314. [[CrossRef](#)] [[PubMed](#)]
74. Zhao, K.F.; Song, J.; Fan, H.; Zhou, S.; Zhao, M. Growth response to ionic and osmotic stress of NaCl in salt-tolerant and salt-sensitive maize. *J. Integr. Plant Biol.* **2010**, *52*, 468–475. [[CrossRef](#)]
75. Guan, X.; Zhao, C.; Liu, X.; Zhang, H. Hyperbranched polymers containing stereocontorted cores as on-line solid-phase microextraction adsorbent for polycyclic aromatic hydrocarbons. *J. Chromatogr. A* **2013**, *1302*, 28–33. [[CrossRef](#)] [[PubMed](#)]
76. Matinzadeh, Z.; Akhiani, H.; Abedi, M.; Palacio, S. The elemental composition of halophytes correlates with key morphological adaptations and taxonomic groups. *Plant Physiol. Biochem.* **2019**, *141*, 259–278. [[CrossRef](#)] [[PubMed](#)]