Influence of Chloride- and Soda-Dominated Salinity on Physiological and Biochemical Aspects of Halophytes with Different Strategies of Salt Metabolism

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Abstract. The authors study the effect of NaCl and NaHCO$_3$ at concentrations of 1 M on the physiological and biochemical state of Salicornia perennans and Artemisia santonica for 24 hours. In our experiment, we evaluated: the accumulation of Na in the aerial part of plants, the stress index is lipid peroxidation (LPO); the state of membranes – by the composition and content of lipids and proteins, the level of photosynthetic pigments. The euhalophyte S. perennans accumulated Na on average 30% more than the glycohalophyte A. santonica. The addition of NaCl and NaHCO$_3$ to the root environment promoted an increase in LPO in S. perennans by 2.4 times as compared to the control. In the case of A. santonica, LPO concentration increased by 1.2 times, but only when NaHCO$_3$ was added to the soil. In addition, NaCl and NaHCO$_3$ negatively affected plant lipids and proteins. Thus, in S. perennans plants, NaCl contributed to a decrease in phosphlipids by 34%, and NaHCO$_3$ in glycolipids by 22% in comparison with the control. The quantitative content of the sum of water-soluble and membrane-bound proteins in the studied plants decreased by 10–36%.

Chloride and soda-dominated salinity caused a decrease in the proportion of chlorophyll $a$ and carotenoids only in S. perennans. The authors concluded that the response of the photosynthetic system and membrane complexes to various types of salinity in euhalophyte and glycohalophyte was different.

1. Introduction

Excessive content of mineral salts in the soil is one of the factors limiting the growth and productivity of plants [1]. Soil salinity can be sulfate, chloride or soda-dominated, etc. It depends on the accumulation of different types of salts in the soil. As a rule, Na$^+$, sometimes Mg$^{2+}$ and Ca$^{2+}$, prevail among cations in saline soils. Anions such as Cl$^-$ and SO$_4^{2-}$ make the greatest contribution to soil salinity; salinization of carbonate type also occurs [2]. For plants, the most toxic salinity is soda or carbonate, i.e., with a high concentration of sodium bicarbonate (NaHCO$_3$), sodium carbonate (Na$_2$CO$_3$) or other sodium salts. Plant oppression under soda salinity begins when the content of the bicarbonate anion HCO$_3$ is more than 0.08%, and exchangeable Na$^+$ is 10–15% of the absorption capacity [3]. Soda - dominated salinity of soils adversely affects not only plants, but also causes soil dispersion, lowers water permeability, reduces porosity, and impairs its physical properties.

Halophytes are plants of saline soils, salt marshes, solonetzes, resistant to increased salt concentration due to the presence of a number of hereditarily fixed morphological and physiological
features [4]. Halophytes differ in the type of salt accumulation depending on salt tolerance [5]. Plants of the genus Salicornia (Amaranthaceae) and Artemisia (Asteraceae) are typical representatives of various saline ecotopes [6]. A specific feature of glycohalophytes is their ability to limit the intake of salts due to the low permeability of root cell membranes for inorganic ions [7]. At the same time, euhalophytes are able to carry out their vital activity in an extremely saline environment, accumulating high concentrations of Na⁺ and Cl⁻ in the cells of leaves and stems [8]. Many species of the genus Salicornia successfully grow when the NaCl concentration ranges from 200 to 1000 mM [9-11].

It is known that the effectiveness of defense mechanisms against an excess of salt is largely associated with the state of the membranes providing the functionality of protein complexes [12]. It was found that changes in the unsaturation of fatty acids (FA), the content and composition of glycolipids (GL), phospholipids (PL), and sterol components (ST) of membranes are important for the salt tolerance of halophytes [13, 14]. At the same time, lipid peroxidation (LPO), induced by the formation of reactive oxygen species (ROS), leads to a change in conformation and denaturation of structural and enzymatic proteins [15]. LPO is one of the earliest plant responses to damaging factors, including salinity [16]. In addition, numerous studies have shown that the photosynthetic apparatus of halophytes is sensitive to salt stress [17]. A high content of NaCl can cause a decrease in photosynthetic pigments, a weakening of the interaction of protein-pigment-lipid complexes, or an increase in the activity of the chlorophyllase enzyme [18].

However, halophytic plants are able to complete their full life cycle even in such extreme conditions for other plant species. Therefore, it is of scientific interest both to compare the effects of chloride and soda-dominated salinity on halophytes and the response of plants to this type of impact.

The aim of our research is to study the effect of chloride and soda salinity on physiological and biochemical aspects of halophytes with different strategies of salt metabolism.

2. Materials and Methods

**Plant material.** We selected seeds from wild plants *Salicornia perennans* Willd. and *Artemisia santonica* L. in natural conditions of growth in September 2018 in the area of Lake Elton (Russia) (49°07'N, 46°50'E). We germinated seeds in distilled water for 1–3 days and sowed them into vessels with sand (3 kg). Watering was carried out with Robinson’s nutrient solution [19] 1–2 times a week until the soil was fully water-absorbing. The light intensity was 1200 µmol / m² s⁻¹; the photoperiod was 10 hours of light / 14 hours of darkness at a constant day / night air temperature of 20–22 °C. When the plants reached the age of three months, we divided them into two groups: control and experiment. In the experimental variants, 1 M NaCl or NaHCO₃ was added to the soil once. The exposure time was 1 day. Then the aboveground mass of plants was cut and used for analysis. The experiments were repeated three times. For each experiment with salt or soda, there were 3 vessels with plants. Biological samples of 0.5–2 g of wet weight were made from the aerial mass of leaves (shoots in the case of *S. perennans*).

**Water content of tissues.** Water content in plant leaves was calculated as % of fresh weight (FW) after determining dry weight (DW) according to the formula: W = (FW–DW)/FW×100%. DW was measured after drying plants at 60 °C.

**Lipid peroxidation.** We determined the LPO intensity by the content of malondialdehyde (MDA) after the reaction with thiobarbituric acid using a spectrophotometer (PromEcoLab PE-3000 UF, Russia) [8], and expressed it in µM/g DW.

**Isolation and analysis of lipids.** We extracted lipids with a mixture of chloroform and methanol (1:2) with simultaneous mechanical destruction of tissues [20]. Lipids were separated by thin layer chromatography [21]. We determined the amount of PL, GL, and ST by densitometric method using the program (Lenkhrom Denskan-04, Russia). Chromatograms were analyzed in the mode of parabolic approximation by calibration dependences using phosphatidylcholine, monogalactosyldiacylglycerol, and cholesterol as standards. We carried out the methanalysis of FA by boiling in a 5% HCl solution in methanol. The resulting esters were analyzed on a chromatograph (Khromatek Kristall 5000.1, Russia) in isothermal mode using a capillary column 105 m long and 0.25 mm in diameter (RESTEK,
USA). Column temperature was 180 °C, evaporator and detector – 260 °C, carrier gas (helium) flow rate – 2 ml / min. FAs were identified by retention time using the standard 37 Comp. FAME Mix (Supelco, USA). The unsaturation index (UI) was used to assess FA unsaturation. $UI = \sum P_j n/100$, where $P_j$ is the FA content (% of the total FA) and $n$ is the number of double bonds in each acid.

**Protein content.** We determined the amount of water-soluble (WP) and membrane-bound protein (MP) using the Lowry method [22].

**Pigment analysis.** We determined the content of pigments spectrophotometrically in an acetone extract (90%) at $\lambda$ - 662, 645 and 470 nm. The content chlorophylls (Chl) $a$, $b$ and carotenoids (Car) were calculated accordance with the recommendations of Lichtenthaler [23].

**Statistics.** Results are presented as averages, for to estimate the scatter of the obtained values used the relative standard deviation (RSD). We used analysis of variance (One-way ANOVA) to compare the means. The calculations were performed using Statistica 6.0 for Windows, Past 3, and Microsoft Excel 2003.

3. Results and Discussion

The sodium content in the soil in the control was no more than 0.1 mg/g of DW of the soil (figure 1A). The sodium concentration in the leaves of the control $S. \text{perennans}$ plants was 66 mg/g of DW, $A. \text{santonica}$ – 18 mg/g (figure 1B). Such a difference in the accumulation of this element can be explained by the strategy of salt accumulation of the studied plants. Thus, in the natural environment $S. \text{perennans}$ accumulates salts in photosynthetic organs, while $A. \text{santonica}$ is mainly a salt-excluding species, consequently less sodium reaches the leaves. After the introduction of NaCl and NaHCO$_3$ into the soil, the sodium content in it increased to 1.5–2.2 mg/g DW. At the same time, the concentration of this element remained at the same high level in the leaves of $S. \text{perennans}$ plants, while in $A. \text{santonica}$ it increased by 1.4–2 times. However, in general, $S. \text{perennans}$ plants accumulated sodium 2–3 times higher than $A. \text{santonica}$ (figure 1B). We found no differences in salt accumulation under chloride or soda-dominated salinity for $S. \text{perennans}$. With regard to $A. \text{santonica}$, we found that, in case of soda-dominated salinity, the sodium level in the leaves was 44% higher than in the plants under chloride-dominated salinity.

![Figure 1](image.png)

**Figure 1.** Sodium content in soil and in leaves of halophytes before and after 1 day exposure to NaCl and NaHCO$_3$. Sp – $S. \text{perennans}$, As – $A. \text{santonica}$. The RSD is 10–15%. Different letters indicate significant differences between the variants of the experiments.

The nature of the toxic effect of ions has not been sufficiently studied. For example, practically nothing is known about the targets for chlorine toxicity. However, NaCl in high concentrations can disintegrate cell membranes, suppress the activity of enzymes due to a violation of the hydrophobic-electrostatic balance of forces that support the structure of proteins [2]. Even less is known about soda salinity.
In our experiments, we found that NaCl negatively affects the water content in both plant species; NaHCO₃ caused a negative effect only in *S. perennans* (table 1). The stress level, assessed by LPO indicator, increased equally in experiments with *S. perennans* under chloride and soda salinity. In experiments with *A. santonica*, only under soda salinity.

GL are the main structural components of chloroplast membranes. PL are predominantly localized in non-plastid cell membranes – plasmalemma, ER, tonoplast, etc. With regard to *S. perennans*, we found that the exposure to NaHCO₃ caused the GL content to decrease by 22% in comparison with control plants. On the contrary, NaCl negatively affected the PL content in *S. perennans*. For *A. santonica*, chloride and soda salinity did not affect the GL content, but the PL content decreased by 30% in comparison with the control.

STs are a structural component of membranes in addition to glycerolipids. They are precursors of plant hormones brassinosteroids, which regulate plant growth and development [24]. STs are directly involved in transmembrane signal transduction into the cell through the formation of specific lipid microdomains in the membranes [25]. A decrease in the ST content is noted only in *A. santonica* under both chloride and soda salinity. The sterol component of *S. perennans* membranes was found to be more resistant to the effects of salts than in *A. santonica*.

The physical properties of lipids in the membrane bilayer are largely determined by the level of their FA unsaturation or the number of FA double bonds. The UI of lipid FAs in the studied plant species remained practically unchanged. That is, the physical properties of lipids also remained unchanged.

We found that chloride- and soda-dominated salinity negatively affects the concentration of WP and MP in the leaves of the studied plant species. An exception was found only in experiments with NaHCO₃ for *A. santonica*. In general, the content of WP decreased by 23–31%, MP – by 27–52%. That is, NaCl and NaHCO₃ generally negatively affect the MP of leaves of both types of halophytes.

### Table 1. Influence of chloride and soda-dominated salinity on physiological and biochemical parameters of halophytes with different strategies of salt metabolism

| Parameters                  | Sp Control | Sp NaCl | Sp NaHCO₃ | As Control | As NaCl | As NaHCO₃ |
|-----------------------------|------------|---------|-----------|------------|---------|-----------|
| Water content, %            | 92,5       | 87,1▼   | 87,2▼     | 72,5       | 68,7▼   | 70,6●     |
| LPO (µM/g DW)               | 0,013      | 0,031▲  | 0,031▲    | 0,295      | 0,260●  | 0,362▲    |
| GL (mg/g DW)                | 10,3       | 9,9●    | 8,0▼      | 18,5       | 17,3●   | 19,0●     |
| PL (mg/g DW)                | 12,5       | 8,2▼    | 11,2●     | 16,9       | 11,8▼  | 11,7▼     |
| ST (mg/g DW)                | 0,5        | 0,5●    | 0,5●      | 1,8        | 1,1▼   | 1,2▼      |
| Unsaturation index          | 1,9        | 1,9●    | 1,9●      | 1,8        | 1,8●   | 1,8●      |
| WP (mg/g DW)                | 21,3       | 15,5▼   | 16,4▼     | 98,2       | 67,4▼  | 93,5●     |
| MP (mg/g DW)                | 16,0       | 11,6▼   | 10,9▼     | 29,1       | 14,1▼  | 21,1▼     |
| Chl a (mg/g DW)             | 3,7        | 3,3▼    | 3,0▼      | 3,8        | 3,8●   | 4,0●      |
| Chl b, mg/g DW.             | 1,3        | 1,2●    | 1,2●      | 1,4        | 1,2●   | 1,4●      |
| Car (mg/g DW)               | 1,1        | 0,9▼    | 0,7▼      | 1,0        | 1,1●   | 1,1●      |

*The RSD is relative and is not more than 10%*

Sp – *S. perennans*, As – *A. santonica*;
▲, ▼, ● – respectively, increase, decrease, stability of the parameter relative to the control
The leaf of a higher plant is a complex optical system with the ability to efficiently use solar energy. Organic compounds (pigments) containing systems of conjugated bonds absorb light quanta in the visible part of the spectrum (400–700 nm) [26]. The chloroplasts of the leaves of higher plants contain two Chl (a and b), which are Mg-containing porphyrins. Accordingly, disturbances in the content and condition of pigments can affect the functioning of the entire photosynthetic apparatus of plants. We found that NaCl and NaHCO₃ negatively affect the content of Chl a and Car in experiments with S. perennans. At the same time, soda-dominated salinity had a greater negative effect on the content of these components than the chloride one. In turn, the content of Chl a, b, and Car in A. santonica remained at the level of control values. It can be concluded that the photosynthetic apparatus of the glycohalophyte is more protected from the toxic effects of salts than that of the euhalophyte.

4. Conclusion
Thus, the particularity of the effect of chloride dominated salinity on S. perennans euhalophyte is a negative effect on PL, and soda-dominated salinity on GL. With regard to A. santonica, it was found that soda-dominated salinity had a less effect on the studied components of leaves, but at the same time caused an increase in LPO level. Chloride-dominated salinity, in comparison with the soda one, had a greater negative effect on the water content of A. santonica leaves and the WP content in them. In general, both types of salinity negatively affected the MP content in both plant species, regardless of their strategy of salt metabolism.

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