Effect of cadmium on the mesophyll parameters, pigments, and lipid profile of the photosynthetic apparatus of *Suaeda salsa* 

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Abstract. Halophytes were found to accumulate HM ions in their tissues; therefore, they are potentially useful for remediation of soil contaminated by salt or HM. In this study, we evaluated the effect of Cd\(^{2+}\) on the characteristics of mesophyll, pigments, and lipid profile of the photosynthetic organelles of the halophyte *Suaeda salsa*. Seeds were sprouted in distilled water for 1–3 days and then sown in containers with sand. Plants were watered with Robinson's nutrient solution. After 3 months, plants were divided into two groups: experimental and control. In the experimental group, soil was treated with 200 µM Cd(NO\(_3\))\(_2\) for 24 hours. Our findings suggest that Cd\(^{2+}\) causes changes in the anatomical structure of the leaf and the ultrastructure of the photosynthetic organelles in *S. salsa* due to alterations in the composition of lipids and fatty acids.

1. Introduction

Global climate change and human activity have caused massive migration of elements that had never been observed earlier. They also increased the concentration of salts in soil, including heavy metal (HM) salts, posing a threat to human health, ecosystems, and national economies [1, 2]. Halophytes were found to accumulate HM ions in their tissues and are potentially useful for remediation soil contaminated by salt or HM [3-5].

*Suaeda salsa* (Chenopodiaceae) is one of potential candidates for this role. This euhalophyte grows on saline soils with a large range of mineralization. Several studies have demonstrated that *S. salsa* can grow on soils containing high concentrations of HM [6, 7]. The results of field studies have shown that this euhalophyte perfectly tolerates highly polluted environment, thereby contributing to gradual restoration of vegetation in HM-contaminated areas [6].

It is well known that plant adaptation to environment is usually accompanied by changes in the structure of photosynthetic tissues [8–10] and the content of the main photosynthetic pigments [11–13]. In leaves, photosynthetic activity is regulated by modulating the number of chloroplasts per unit of leaf area and by altering functional characteristics of chloroplasts [10, 13]. A chloroplast has a double-membrane envelope and internal thylakoid membranes [14]. The structural components of thylakoid membranes include glycolipids, monogalactosyldiacylglycerol (MGDG), digalactosyldiacylglycerol (DGDG), sulfoquinovosyldiacylglycerol (SQDG), and phosphatidylglycerol (PG) [15]. They interact directly with the complexes of the photosystem I and II [16]. The lability and dynamic properties of thylakoid membranes, as well as molecular interactions.
between the complexes of the photosynthetic apparatus, are modulated by fine adjustment of the MGDG/DGDG ratio [17]. However, the role of adaptation of the photosynthetic apparatus to HMs in leaf mesophyll and lipid profile of halophyte plants remains poorly studied.

In this work, in model experiments, we evaluated changes in the structural and functional parameters of the photosynthetic apparatus of S. salsa in response to Cd^{2+}.

2. Materials and Methods

2.1. Plant material

The objects of the research were photosynthetic organs of salt-accumulating halophytes S. salsa. Seeds of the wild plants had been selected at the end of October 2018 in the area of Prieltone (49°07′N, 46°50′E) and stored at a room-temperature for 6 months. Seeds had been sprouted in a distill water in Petri dishes at temperature of 22–24 °C. The seedlings were transferred to containers with sand. Watering had been carried out with Robinson nutrient solution [15]. Plants had grown at an air temperature of 20–22 °C, iluminance of 1200 µmol m^{-2} s^{-1}, photoperiod of 10 h for 3 months (juvenile stage). Afterwards plants were divided into a two group, control (C) and experimental (Cd). The experimental plants during 10 days were treated solution by using Cd^{2+} [as Cd(NO₃)₂] to a final concentration of 200 μM. Three independent biological samples for each analysis type (0.5–2 g) of fresh weight were made from overall leaves.

2.2. Leaf traits

The leaf traits were evaluated using the leaf mesostructure analysis developed by [16], and the parameters of mesophyll cells were estimated by the projection method [10]. The cell and chloroplast number was measured on leaf samples fixed in a 3.5% solution of glutaraldehyde in phosphate buffer (pH = 7.4). The number of cells per unit of leaf area was calculated in a suspension obtained after the maceration of leaf samples. The suspension was prepared by heating leaf discs in 20% KOH to the point of boiling. The number of cells was counted in a Goryaev counting chamber at a magnification of × 200 under a Zeiss Axiosstar light microscope (Carl Zeiss, Germany). The cell size and the number of chloroplasts per cell were estimated in a cell suspension obtained after the maceration of leaf discs in 1 N HCl at 50 °C for 10 minutes. The cell volume was calculated by the projection method, on the basis of average values of the cell projection area and perimeter, as well as coefficients dependent on the shape of cells [17]. The two-dimensional shape factor of cells (K2D) was calculated as a ratio of the squared projection perimeter of a cell to its area [17]. The chloroplast sizes were measured on leaf cross sections using the Zeiss Axiosstar light microscope (Carl Zeiss, Germany) and a Simagis Mesoplant analyzer (SIAMS LLC, Russia). The chloroplast number per unit leaf area was calculated by multiplying the number of chloroplasts per cell by the number of cells per unit of leaf area. Total area of external cell membranes (Ames/A) was calculated by multiplying the number of cells per unit leaf area and average surface area of the palisade and spongy mesophyll cells. Similarly, we calculated total surface of the outer membrane of chloroplasts (AChl/A).

2.3. Pigment analysis

The middle part of fresh leaves (0.5 g) was extracted with ice-cold 80% acetone. Photosynthetic pigment content was measured in 3–4 biological replicates by means of spectrophotometer (UV–1700, Shimadzu, Japan,) in acetone extract at wavelengths of 662 and 644 nm chlorophyll (Chl) and 470 nm carotenoids (Car) with corrections in absorbance maximums. The concentrations of Chl a, b and Car were calculated according to method [18]. The relative contents [%] of Chl of light-harvesting complexes (LHC) were calculated according the formula: (1.2 Chl b + Chl a)/Σ (Chl a +Chl b)×100 (%).
2.4. Lipid extraction and analysis
The lipids were extracted three times by chloroform/methanol mixture (1:2, v/v) with simultaneous mechanical destruction of tissues [19]. The lipids were separated by thin-layer chromatography method as described earlier [23]. Glycolipids (GL) were measured densitometrically (Lenchrom, Russia, Denskan-04). PG value was determined by phosphorus content. The chromatograms were analyzed in the parabolic approximation mode by calibration curves using MGDG (Sigma-Adrich, Germany) as standards.

2.5. Fatty acid profiles
FA were analysed in the form of their methyl esters (FAME). The esters were purified by preparative thin-layer chromatography and analyzed using a Crystal 5000.1 gas–liquid chromatography system (Crystal 5000.1, Chromatek, Russia). Separation of esters was conducted using an isothermal regime, with a RESTEK capillary column. The temperatures of the column and detector were 180 and 260 °C, respectively. The flow rate of the carrier gas (helium) was 2 mL min⁻¹. The FAME were identified by comparing their retention times with FA standards (RESTEK, USA), and quantification was performed using an internal standard of heptadecanoate.

2.6. Statistics
The data obtained are represented as means of three independent experiments with standard errors (SE). The confidence of the difference between two values was estimated by a Student t-test (p < 0.05). The results were statistically processed using Statistica 10 (Statsoft Inc., USA).

3. Results
In our previous studies, we have shown that most of Cd²⁺ is accumulated in the roots of S. salsa [25]. Nevertheless, the incubation of plants with 200 μM Cd²⁺ led to changes in the mesostructure of leaves. Leaf anatomy of S. salsa is characterized by a clear separation of the palisade and spongy mesophyll layers. The total number of photosynthetic cells per unit of leaf area in the experimental plants decreased by half compared to the control (figure 1A).

![Figure 1](image_url)

Figure 1. Effect of Cd²⁺ on integral parameters of mesostructure in S. salsa leaves. C – control group, Cd – experimental group. Ames/A – total surface area of mesophyll cells per unit of leaf area, cm²/cm²; Achl/A – total surface area of chloroplasts in cells of mesophyll per unit of leaf area, cm²/cm²; p. – pieces. Different letters indicate significant differences at p < 0.05.
There was a negative correlation between the number of mesophyll cells and changes in their volume: the larger the cells were, the fewer cells were observed per unit of leaf area (figure 1 B). The studied plants also differed in the number and size of chloroplasts in photosynthetic cells (figure 1D). The change in the size of cells and chloroplasts was reflected in the parameters characterizing the total surface area of mesophyll and chloroplasts. Cd\(^{2+}\) caused a reduction in the total surface area of mesophyll and chloroplasts (figure 1C, F).

One of the main parameters characterizing the process of photosynthesis is the concentration of pigments. In the leaves of control plants, the amount of pigments was 1 mg/g \(-1\) of fresh leaves (table). Exposure to Cd\(^{2+}\) resulted in a decrease in Chl \(a\) and the Chl \(a/b\) ratio, but caused an increase in Chl/Car ratio and LHC.

**Table.** Effect of Cd\(^{2+}\) on the level of photosynthetic pigments in leaves of *S. salsa* (mg/g of fresh leaves)

| Heavy metal | \(\Sigma\text{Chl} a+b\) | Car | Chl \(a/b\) | Chl/Car | LHC, % |
|-------------|--------------------------|-----|-------------|---------|--------|
| C\(^{a}\)    | 0.6 ± 0.1a               | 0.2 ± 0.03a | 3          | 4       | 49     |
| Cd\(^{2+}\) | 0.4 ± 0.02b              | 0.1 ± 0.02b | 2          | 6       | 57     |

\(^a\) C – control group, Cd – experimental group. Different letters indicate significant differences at \(p < 0.05\)

The amount of lipids containing one (MGDG) and two (DGDG) monosaccharide residues was more than 70% of total GL. In the leaves of control plants, the most abundant GL was MGDG, followed by DGDG, PG and SQDG (figure 2A). Cd\(^{2+}\) changed the lipid ratio, as well as the number and size of chloroplasts. Smaller chloroplasts in experimental plants were characterized by high levels of DGDG and SQDG.

**Figure 2.** Effect of Cd\(^{2+}\) on the ratio and content of lipids and FA in leaves of *S. salsa*. C – control group, Cd – experimental group. Different letters indicate significant differences at \(p < 0.05\)

Lipid molecules form a matrix of thylakoid membranes, the dynamic and functional properties of which depend on the FA composition. As we expected, the content of unsaturated FA exceeded 75% (figure 2B). We observed a trend to a decrease in the relative level of saturated FA (SFAs) and monounsaturated FA (MUFA); moreover, there was a decrease in the size of chloroplasts in experimental plants. At the same time, we revealed an increase in the content of polyunsaturated FA, both dienoic (DUFA) and trienoic (TUFA).
4. Discussion
Photosynthesis is usually characterized by an extremely high sensitivity to HM that affect many aspects of this process. They reduce \( \text{CO}_2 \) accumulation and cause structural and functional changes in the chloroplasts [26]. Exposure to HM may lead to changes in the size of cells and chloroplasts. \( \text{Cd}^{2+} \) decreased the total number of mesophyll cells and increased the cell volume due to water flow into the cell to maintain the water potential [27]. Our study showed that exposure of \( S. \ salsa \) to \( \text{Cd}^{2+} \) increased the number of chloroplasts, while their volume decreased.

Analysis of the pigment complex involved in the process of photosynthesis showed that \( \text{Cd}^{2+} \) reduced the level of Chl \( a \) and Car in the leaves of \( S. \ salsa \). Lower Chl \( a/b \) ratio and increased LHC concentration indicate a reorganization in the ultrastructure of the photosynthetic apparatus. An increase in the Chl/Car ratio also suggests an increase in the role of pigments with protective function in plants.

The detected alterations in the level of MGDG and DGDG indicate compensatory changes required to maintain physical properties of the membrane, smoothly correct phase transitions and curves of the membrane to preserve photosynthetic activity [7, 23, 28].

5. Conclusion
In general, our findings demonstrate that exposure of \( S. \ salsa \) to \( \text{Cd}^{2+} \) led to changes in the anatomical structure of the leaf and the ultrastructure of the photosynthetic apparatus due to changes in the composition of lipids and fatty acids.

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