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The impact of different levels of sodium chloride on the quantitative changes of chlorophyll and carotenoids in chloroplasts of *Elodea canadensis* (Michx. 1803)

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In this study we used spectrophotometry to investigate the effect of negative concentrations of sodium chloride ions on photosynthetic pigments in *Elodea canadensis* (Michx. 1803). The concentrations of pigments, carotenoids, chlorophyll *a* and chlorophyll *b*, in plant leaves provide information about the physiological state of plants and were determined using a spectrophotometer. Quantity and dynamics analyses of photosynthetic pigments are effective methods which allow determining changes in metabolites of plant cells even at insignificant cellular damage. During this research photosynthetic pigments in leaves were obtained at the different sodium chloride levels: 0.0, 0.025, 0.05, 0.1, 0.5 and 1.0 M. The results of this research indicate that these types of stressors at high concentrations: 0.1, 0.5 and 1.0 M after a prolonged time of impact on plant leaves lead to a decrease of photosynthetic pigments and inhibit growth and development of a plant as a whole.

Key words: *Elodea canadensis*, spectroscopy, photosynthetic pigments, sodium chloride, stress factor

INTRODUCTION

Nowadays salinity is recognized as one of the main environmental factors that limits the productivity and development of crops. In general, investigations of the effect of salinity on agricultural crops are being studied more actively but aquatic plants may also experience negative effects of NaCl in H_2O and may be more sensitive to these types of changes or vice versa have specific mechanisms of adaptability, for example, in plants growing near the ocean or sea.

The quantitative changes of pigments in leaves of *Elodea canadensis* (Michx., 1803) provide important information about the physiological state of plants and resilience to changing environmental conditions (Gang et al., 2010). The research aimed to evaluate the effect of NaCl various levels on quantitative changes of plant photosynthetic pigments in leaves as an indicator to the degree of influence on the general condition of the stress factor to the plant growth.

High salt concentrations reduce the solubility of trace elements in the substrate and thereby reduce their bioavailability for plants. High concentrations of NaCl have the destructive effect

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on normal physiological processes and in the plant cell, and increase production of reactive oxygen species (ROS) in different cellular compartments (Yadav et al., 2011). In the contemporary world the impact of toxic effects of sodium and chloride ions on the intracellular mechanisms of plants regulation processes and linkages with the synthesis of pigments has been insufficiently studied, especially with regard to primary molecular damage caused by this type of stressors (Amirjani, 2011).

Increased salt concentrations in the substrate of water disturb aqueous ion homeostasis of aquatic plants on the cellular level and the whole plant. In turn, this leads to various toxic effects which are expressed in the damage of biopolymers in the cytoplasm. In general, soil salinity inhibits plant growth and often causes the programmed cell death (PCD). The fluorescence of plant pigments is observed during excitation of chlorophyll *a*, chlorophyll *b* and carotenoids. The energy levels of plant chlorophyll *a* and chlorophyll *b* in cells are the lowest compared to the energy levels of other photosynthetic plant pigments, so the transport of energy in chloroplast always directs to chlorophyll *a* (Lichtenthaler et al., 1999).

Salt-tolerant plants have mechanisms that allow them to maintain the normal course of photosynthesis in the presence of high concentrations of salts. High concentrations of NaCl influence the nucleic acids, proteins (in the plasma membrane, nucleus and cytosol), lipids (plasma membrane and membranes of organelles). As a consequence, the influence causes oxidative stress and increases production of ROS. The aggregate of all these factors induces malfunctions and somatic mutations in the plant process of metabolism. Studying the main reaction mechanism of photosynthesis requires deep structure analysis and physical, chemical properties of pigment systems of the photosynthetic apparatus. Pigments are involved in the implementation of the three major stages of photosynthesis (Green, Dunford, 2009; Agastian et al., 2011). Plant pigments are responsible for the absorption of light energy on the photophysical stage and perform energy conversion in photochemical reactions of photosynthesis that are essential components of the electron transport chain.

The aim of the present research was to determine and investigate the dynamics of Canadian waterweed *Elodea canadensis* (Michx., 1803) photosynthetic pigments after NaCl (0.0, 0.025, 0.05, 0.1, 0.5 and 1.0 M) exposure on the plant.

MATERIALS AND METHODS

Aquatic herb *Elodea canadensis* (Michx. 1803) leaves were taken as the object of the research. The plant for the experiment was taken from the natural environment and propagated in the laboratory in an aquarium tank. All samples were transferred into standard Petri dishes Ø 90 mm. For the experiment, we used bi-distilled water with the nutrient solution Sigma-Aldrich Hoagland's NO. 2 basal salt mixture with the addition of various concentrations of NaCl: 0.0, 0.025, 0.05, 0.1, 0.5 and 1.0 M. The plants were grown under the optimal conditions in a climate chamber: Versatile Environmental test chamber with photoperiodicity: 16 hours day and 8 hours night, 26 °C temperature, and relative humidity 70%. The plant pigments were analyzed within three weeks with the initial stage and statistically processed by StatFi 2009 software. The following methods have been used in this paper: extraction of pigments, pigments spectrophotometry and mathematical analysis of the results.

The acetone extracts of photosynthetic pigments (chlorophyll *a*, chlorophyll *b* and carotenoids) were extracted from leaves after NaCl exposure. The total pigment content was investigated in Canadian waterweed under the influence of different NaCl concentrations: 0.0, 0.025, 0.05, 0.1, 0.5 and 1.0 M. We used a spectrophotometric method for determining the content of pigments. The samples were collected in the laboratory, in the daytime, in the period of the maximum photosynthetic activity. The leaf sample (200 mg) was homogenized in 80% acetone (0.4 ml) with the addition of $MgCO_3$ (0.05 g). Absorbance (optical density

(D)) of each acetone extracts of pigments and concentration was determined by the method of a three-wave spectrophotometer (Cary 50 UV/Vis Scan, Varian): wavelengths 663.0, 646.0, 470.0 nm; Ordinate Mode ABS; average scan time of each sample: 0.1000 s; 3 replicates. It corresponds to the maximum absorption of chlorophyll *a*, chlorophyll *b* and carotenoids in acetone extracts. The pigment concentrations were calculated from the absorbance of extraction at 663.0, 646.0, and 470.0 nm using the formulas:

$C_a = 12.21D_{663} - 2.81D_{646}$;

$C_b = 20.13D_{646} - 5.03D_{663}$;

$C_{car} = (1000D_{470} - 3.27C_a - 100C_b)/229$;

C_a, C_b, C_{car} – pigment concentration (mg/l);

D_{663}, D_{646}, D_{470} – optical density.

To determine the reliability of the results of this research we used the mathematical analysis. The results are presented as mean values ± standard error with three replications.

RESULTS AND DISCUSSION

During the experiment, from the obtained data it is evident that the samples in the control group (without the addition of NaCl) have higher concentration of chlorophyll *a* (Fig. 2) and chlorophyll *b* (Fig. 3) compared with other experimental samples. It should be noted that concentration of carotenoids in chloroplasts (Fig. 4) in the samples, that grow in the substrate concentrations of NaCl: 0.025, 0.05 and 0.1 M significantly increased as early as in the first week of experiment.

Carotenoids absorb certain portions of the solar spectrum and transmit energy on

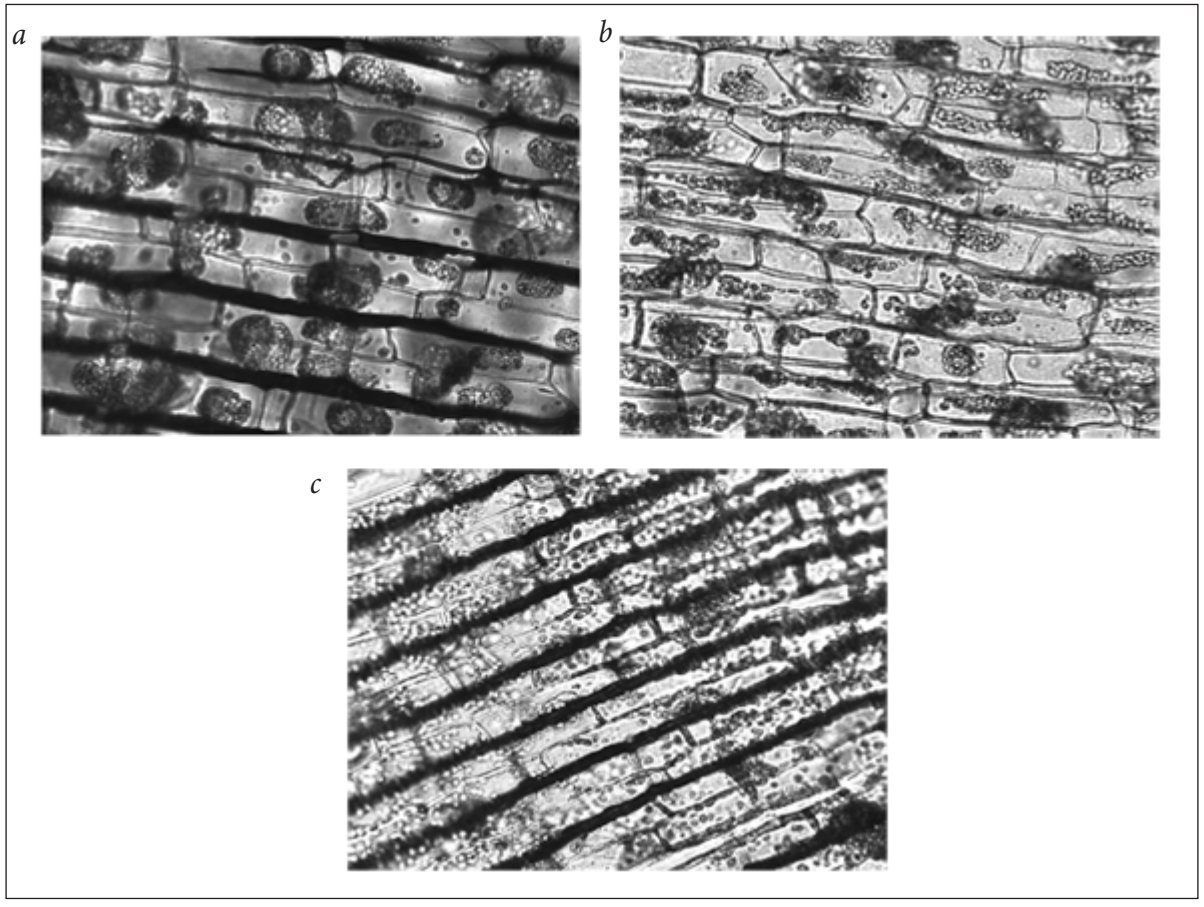

Fig. 1. *Elodea canadensis* (Michx., 1803) Leica microscope HC Plan 10×/20; ×20 (The third week of experiment, concentrations of NaCl in aqua solutions: 1.0 M (*a*), 0.5 M (*b*) and 0.0 M (*c*) control sample

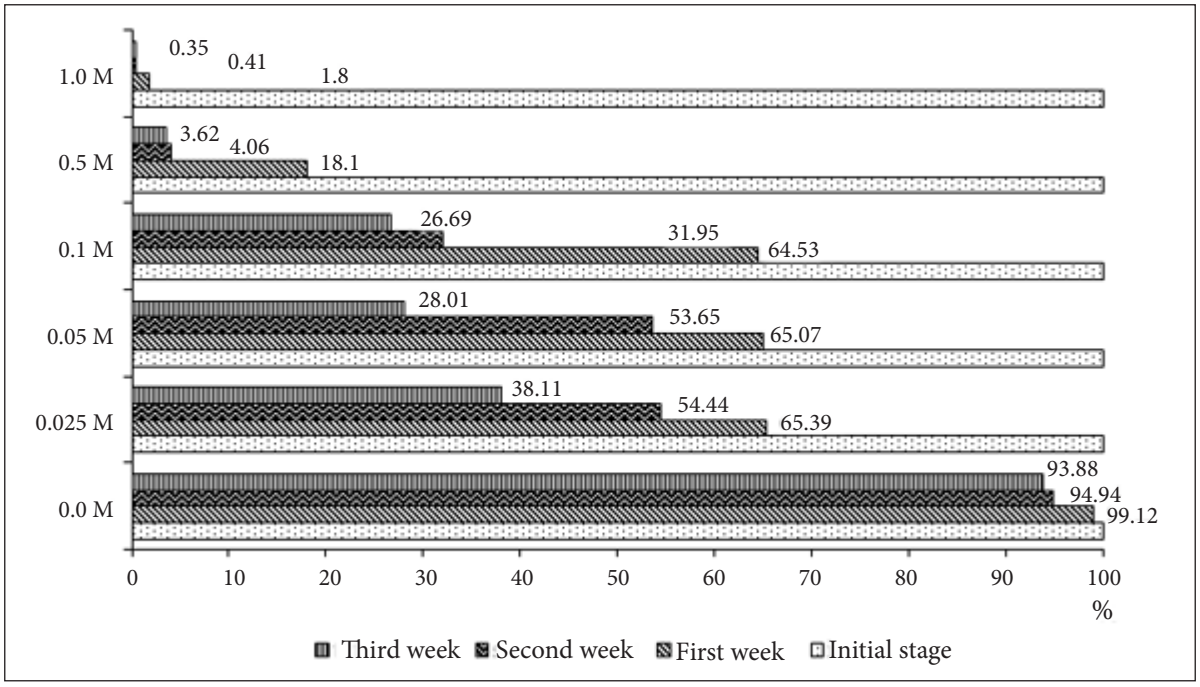

Fig. 2. Chlorophyll *a* (%) dynamics in leaves of *Elodea canadensis* Michx. (1803) (Different concentrations of NaCl solution with Hoagland's NO 2 Basal salt mixture: initial stage, first, second and third weeks)

chlorophyll molecules. There is evidence that carotenoids serve a protective function in chloroplasts and an antioxidative function under the oxidative stress, protecting organic matter (primarily chlorophyll molecule) from the destruction of light in the process of

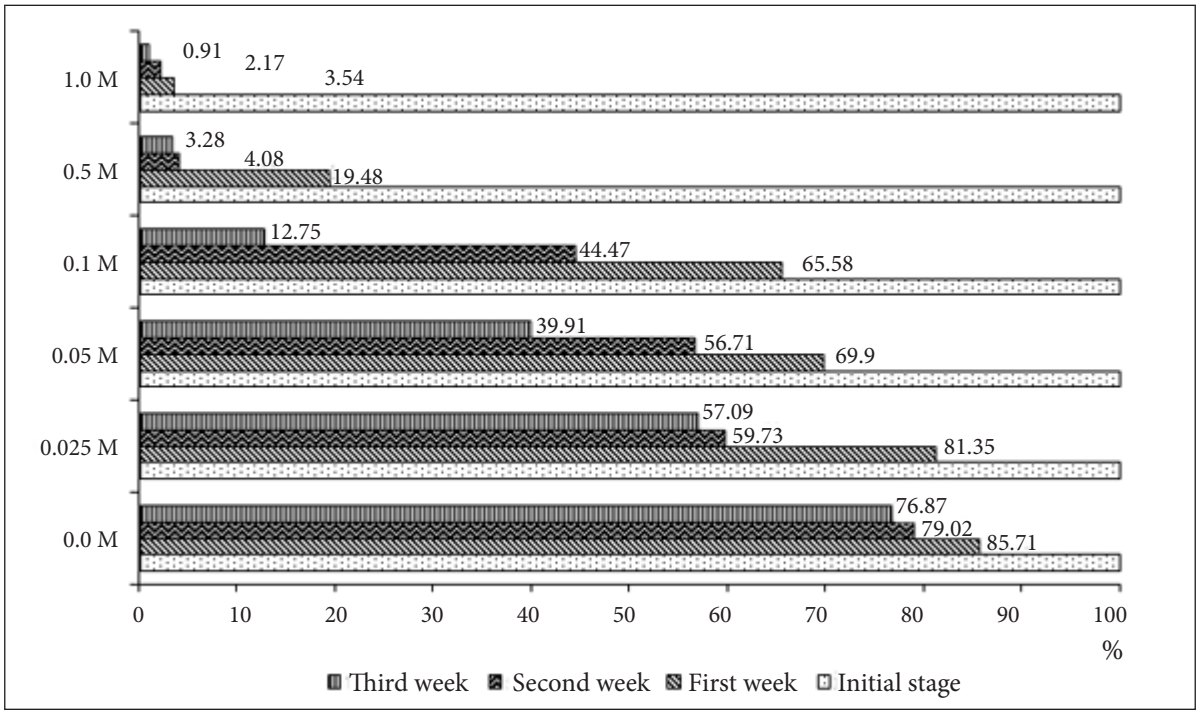

Fig. 3. Chlorophyll *b* (%) dynamics in leaves of *Elodea canadensis* leaves (Different concentrations of NaCl solution with Hoagland's NO 2 Basal salt mixture: initial stage, first, second and third weeks)

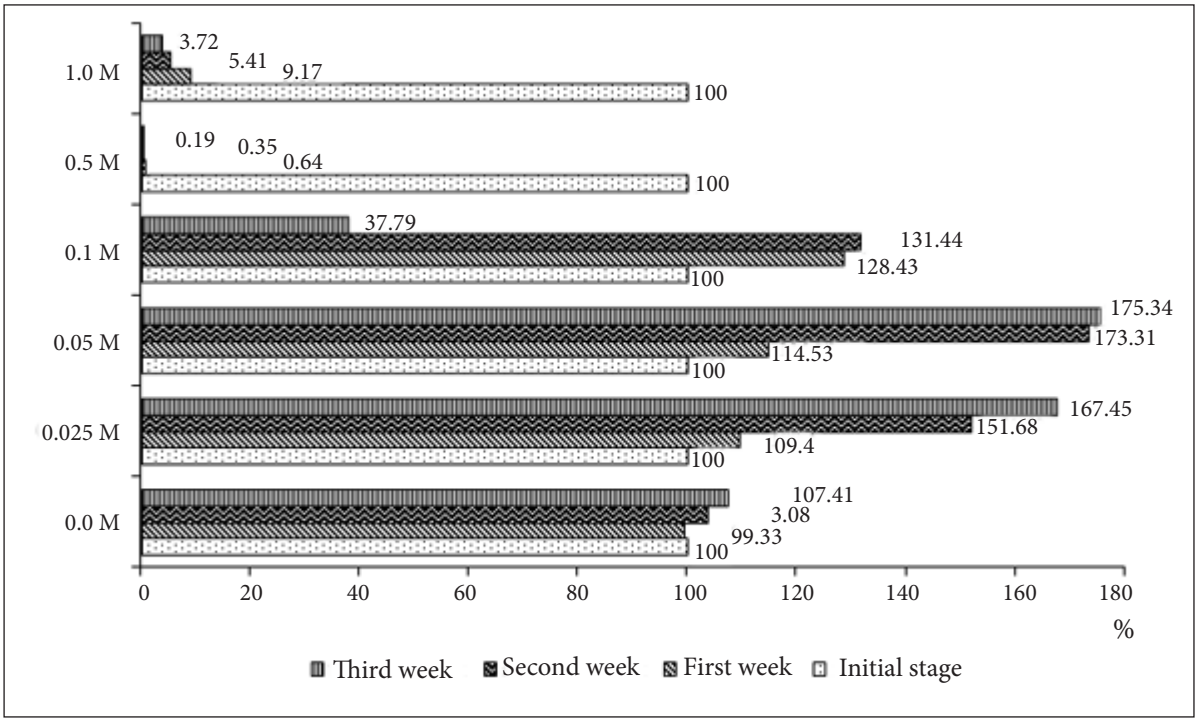

Fig. 4. Carotenoids (%) dynamics in *Elodea canadensis* leaves (Different concentrations of NaCl solution with Hoagland's NO 2 Basal salt mixture: initial stage, first, second and third weeks)

photooxidation and in the stress factors caused by adverse environmental factors, in this case, the simulated salinization (Merzlyak et al., 2008; Green, Dunford, 2009). Carotenoids are present in all photosynthetic organisms and are integral constituents of the thylakoid membrane in chloroplasts (Parida et al., 2008). The tables (Tables 1–3) show the average values of changes in the content of photosynthetic pigments: chlorophyll *a* (Fig. 2), chlorophyll *b* (Fig. 3) and carotenoids (Fig. 4) extracted from leaves of *Elodea canadensis* (Michx., 1803) under the influence of different NaCl levels and depending on the exposure time. It was experimentally determined that the following concentrations of NaCl: 0.1, 0.5 and 1.0 M had a negative effect as early as in the first week of the experiment and only in the third week the level of degradation in chloroplasts increased. Enhanced leaf samples had almost completely lost their photosynthetic pigments and lost the ability to recover the previous condition (Fig. 1).

In samples which were exposed in a substrate with concentration of NaCl 0.5 M, in the third week the amount of chlorophyll *a* decreased by 96% compared to that of control samples. The amount of chlorophyll *b* decreased nearly by 97%, that of carotenoids by almost 94%. For the samples with the concentration of NaCl: 1.0 M in the third week the amount of chlorophyll *a* decreased by almost 98%, that of chlorophyll *b* decreased by 99%, and the amount of carotenoids decreased by 96%, compared to the results of the control samples.

The decrease of chlorophyll content in the chloroplasts under the influence of salinity was also observed, which reduces the absorption of light by chloroplasts. It is a commonly reported phenomenon in other plants and may be due to the membrane deterioration (Mane et al., 2010; Tester et al., 2003; Barhoumi et al., 2007). Usually there is dominance of chlorophyll *a* over chlorophyll *b* in plants, but their values become closer with increasing salinity (Mane et al., 2010). It was experimentally determined that in the samples, which

Table 1. Chlorophyll *a* concentration dynamics under the effect of different NaCl levels in leaves of *Elodea canadensis* Michx. (1803)

Chlorophyll *a*	0.0 M		0.025 M		0.05 M		0.1 M		0.5 M		1.0 M	
	Amount, mg/l	$\bar{x} \pm m_A$	Amount, mg/l	$\bar{x} \pm m_A$	Amount, mg/l	$\bar{x} \pm m_A$	Amount, mg/l	$\bar{x} \pm m_A$	Amount, mg/l	$\bar{x} \pm m_A$	Amount, mg/l	$\bar{x} \pm m_A$
Initial stage	16.02	±0.017	15.98	±0.014	16.03	±0.011	15.96	±0.018	16.02	±0.014	16.03	±0.02
1st week	15.88	±0.012	10.45	±0.018	10.43	±0.018	10.3	±0.014	2.9	±0.013	1.8	±0.018
2nd week	15.21	±0.022	8.7	±0.016	8.6	±0.017	5.1	±0.015	0.65	±0.014	0.41	±0.011
3rd week	15.04	±0.017	6.09	±0.012	4.49	±0.016	4.26	±0.015	0.58	±0.018	0.35	±0.017

Table 2. Dynamics of chlorophyll *b* concentration under the effect of different NaCl levels in leaves of *Elodea canadensis* Michx. (1803)

Chlorophyll *b*	0.0 M		0.025 M		0.05 M		0.1 M		0.5 M		1.0 M	
	Amount, mg/l	$\bar{x} \pm mA$	Amount, mg/l	$\bar{x} \pm mA$	Amount, mg/l	$\bar{x} \pm mA$	Amount, mg/l	$\bar{x} \pm mA$	Amount, mg/l	$\bar{x} \pm mA$	Amount, mg/l	$\bar{x} \pm mA$
Initial stage	8.82	±0.016	8.74	±0.012	8.87	±0.014	8.86	±0.015	8.83	±0.011	8.76	±0.014
1st week	7.56	±0.014	7.11	±0.013	6.2	±0.017	5.81	±0.016	1.72	±0.013	0.31	±0.017
2nd week	6.97	±0.011	5.22	±0.021	5.03	±0.012	3.94	±0.011	0.36	±0.013	0.19	±0.019
3rd week	6.78	±0.008	4.99	±0.017	3.54	±0.014	1.13	±0.012	0.29	±0.017	0.08	±0.015

Table 3. Dynamics of carotenoids concentration under the effect of different NaCl levels in leaves of *Elodea canadensis* Michx. (1803)

Carotenoids	0.0 M		0.025 M		0.05 M		0.1 M		0.5 M		1.0 M	
	Amount, mg/l	$\bar{x} \pm m_A$	Amount, mg/l	$\bar{x} \pm m_A$	Amount, mg/l	$\bar{x} \pm m_A$	Amount, mg/l	$\bar{x} \pm m_A$	Amount, mg/l	$\bar{x} \pm m_A$	Amount, mg/l	$\bar{x} \pm m_A$
Initial stage	2.97	±0.012	2.98	±0.021	2.96	±0.016	2.99	±0.013	3.01	±0.014	2.96	±0.015
1st week	2.95	±0.014	3.26	±0.019	3.39	±0.022	3.84	±0.016	0.64	±0.017	0.27	±0.019
2nd week	3.08	±0.014	4.52	±0.015	5.13	±0.016	3.93	±0.016	0.36	±0.018	0.16	±0.016
3rd week	3.19	±0.011	4.99	±0.017	5.19	±0.015	1.13	±0.016	0.19	±0.013	0.11	±0.02

were exposed in the substrates at a concentration of NaCl 0.025 and 0.05 M, the amount of carotenoids increased with the amount decrease of chlorophyll *a* and chlorophyll *b*. By the end of the third week, in the samples which were exposed by NaCl solution with the concentration 0.025 M there was a decrease of chlorophyll *a* nearly by 62% and chlorophyll *b* by 43%. For the samples that were growing at a concentration of NaCl 0.05 M in the third week there was the reduction of chlorophyll *a* by 72%, chlorophyll *b* by 60% (Tables 1–2). By the third week of the experiment, concentrations of carotenoids on the average increased by almost 68% (NaCl 0.025 M) and by 75% (NaCl 0.05 M) in comparison with the control samples. Our results have shown that the natural senescence of the plants together with the prolonged action of high concentrations of NaCl affected the photosynthetic apparatus of plants. This leads to the initiation of the chloroplast degradation process (Fig. 1) and, as a consequence, leads to the destruction of chlorophyll *a* and chlorophyll *b* while increasing the synthesis of carotenoids. Protection mechanisms occur until the complete destruction of chloroplasts. These processes can be considered as responses to the generation of ROS in the cell under adverse abiotic factors in the first stage included in the mechanism of protection of plant chloroplasts.

The depressive effect of salt stress on chlorophyll biosynthesis may be due to the formation of proteolytic enzymes such as chlorophyllase which is responsible for the chlorophyll degradation and damaging of the photosynthetic apparatus (Tanaka et al., 2008; Lichtenthaler, 1988). Reducing the rate of photosynthesis under high salt concentrations, which are associated with decreased plant stomatal conductance and absorption of carbon dioxide, takes place outside the stoma, and, as a result, the content of carbon dioxide in the intercellular space is reduced (Hasegawa et al., 2000). Also, the reaction rate of photosynthesis in the dark phase is reduced and absorbed light has a damaging effect on the reaction centers of chloroplasts (Munns, 2002).

The impaired growth and development of plants under salt stress is a consequence of physiological response reactions and involves changes in the cellular ionic balance, mineral nutrition, transfer of water through the plants stomata conductance, photosynthetic rate and ultimately in the fixation and utilization of carbon dioxide (Brugnoli, Bjorkman, 1992). The disrupted photosynthetic electron transport chain or the instability of the pigment protein complex with increased activity of chlorophyllase may also be the reason for a decrease in the chlorophyll content under saline conditions (Mane et al., 2010; El-Samad et al., 2011).

CONCLUSIONS

The reduction in chlorophyll content under salinity is due to the suppression of enzymes required for chlorophyll synthesis under higher salinity. Salt stress may cause the destruction of chloroplast and the instability of the pigment protein complex. The results of our study suggest that long-term influence of sodium chloride ions in the substrate in small concentrations activates the *Elodea canadensis* (Michx., 1803) synthesis of carotenoids, as a response of defensive reaction to adverse environmental factors. At the same time, prolonged exposure to high concentrations of sodium chloride disturbed physiological processes in plants and blocked processes of pigment synthesis, apparently through a direct effect on the activity and/or metabolic enzymes synthesis of plant pigments.

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