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Pysiological effects of NaCl on *Ceratophyllum demersum* L., a submerged rootless aquatic macrophyte

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Abstract

In the present study, effects of NaCl concentrations (0, 20, 40 and 80 mM) were investigated on *Ceratophyllum demersum*. Macronutrients N, P and K and micronutrients Zn and Cu were significantly decreased by NaCl salinity. In general, however, Fe and Mn contents of macrophyte tissues increased. As a result, NaCl toxicity disturbed the uptake and translocation of macro and micronutrients in the macrophyte and induced its nutrient imbalance. Although proline, ascorbate and non-protein protein thiols amounts were increased with salinity, contents of photosynthetic pigments, total soluble carbohydrate, reducing sugar and protein were reduced. Observed increases in malondialdehyde contents, one of the most frequently used indicators of lipid peroxidation, showed that oxidative damage occurred in membranes resulting from NaCl. High salinity stress developed some toxicity symptoms. These symptoms probably occurred due to ionic toxicity, imbalance of macro and micronutrients and/or oxidative stress triggered by salinity.

Keywords: *Ceratophyllum demersum*, NaCl, Physiological effects, Morphological effects

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Introduction

Plants are greatly affected by a wide range of environmental stresses. Among these, salinity is one of the major effect on plant growth and development. There is general agreement that there are three ways that salinity can injure plants; salinity can alter water relations, cause ion toxicity, or cause ion imbalance and deficiency. There are environmental variables such as humidity, temperature, soil structure and aeration, light intensity, composition and concentration of ion species in solution, and duration of exposure to salinity. Also, there are genetic and developmental variables which influence the plant's sensitivity to salinity (Cramer, 1997). Salinity stress affects many physiological and biochemical plant processes. Elevated NaCl levels reduce plant nutrient uptake, resulting in ion imbalances (Gabr, 1999; Khan *et al.*, 2000). There are many processes for salt stress responses in plants including various compatible solutes, transport of ion and compartmentalization of toxic ion (Sairam and Tyagi, 2000). In addition, salt stress can cause oxidative stress in plants due to the excessive production of reactive oxygen species (ROS). Consequently, the ROS can damage lipid, carbohydrates, membranes, DNA and proteins (Menezes-Benavente *et al.*, 2004; Hichem *et al.*, 2009).

Due to human activities, aquatic ecosystems are sensitive to the introduction of organisms. Increasing salinity in freshwaters is known to have a negative impact on many aquatic organisms (Thouvenot *et al.*, 2012). At salinities above 1000 mg L^{-1} freshwater macrophytes have reduced growth and development (Nielsen *et al.*, 2003).

According to field study, normally widespread freshwater macrophytes are no longer found at salinities of around 4000 mg L^{-1} (Brock, 1981). Many studies have been carried out to determine the effects of NaCl application on freshwater macrophytes (Rout and Shaw, 1998; Rout and Shaw, 2001; Upadhyay and Panda, 2005).

Ceratophyllum demersum (Ceratophyllaceae) is considered a native in many areas of the world, including Turkey. It is a submerged, rootless and free floating macrophyte. It has a high vegetative propagation capacity. The aim of the present study was to determine the effects of NaCl salinity on some physiological and morphological processes in *C. demersum*.

Materials and methods

Plant exposure

The test macrophyte, *C. demersum* (coontail), is commonly available in Turkey. The macrophytes were collected from the local water bodies. These were acclimatized in 10% Arnon and Hoagland nutrient solution (Arnon and Hoaglan, 1940), at a day temperature of 25-27 °C with a daily photoperiod of 16 h of light (6000 lux) for 14 days. The macropyhte was placed in 1 L glass vessels (14-16 g of healthy macrophyte per vessel), and this trial was repeated three times. Saygideger *et al*. (2004) reported that *C. demersum* was properly growing at near neutral pH levels. According to this, pH of solutions was adjusted to 6.5–6.7. Macrophytes were treated with salinity as NaCl at 20, 40 and 80 mM during 96 h, as supplied with nutrient solution. Macrophyte without added NaCl served as control. The test media were changed after 48 h, and NaCl concentrations were replenished. The macrophyte was harvested after 96 h of exposure.

Biochemical analyses

To determine photosynthetic pigment contents, fresh macrophyte samples were homogenized with 80% acetone. The supernatant was separated and the absorbance was read using a UV/VIS spectrophotometer at 662, 645 and 470 nm. Photosynthetic pigment contents were calculated using the formula by Lichtenthaler and Wellburn (1985). Proline content of macrophyte tissues was determined by Bates *et al*. (1973). Lipid peroxidation was determined by measuring the amount of malondialdehyde (MDA) according to Hodges *et al.* (1999). Total ascorbic acid (AsA) and non-protein protein thiols were determined by Cakmak and Marschner (1992). The amount of total phenolic contents in macrophyte tissues was determined with Folin-Ciocalteu reagent according to the method of Ratkevicius *et al.* (2003). Gallic acid was used as the standard. Protein content was determined according to Lowry *et al.* (1951). Total soluble carbohydrates were determined using the phenol-sulphuric acid method with D(+)-glucose as the standard (Dubois *et al.,* 1956) Reducing sugars were determined using 3,5-dinitrosalicylic with D(+)-glucose as the standard (Miller, 1959).

Determination of element contents

Macrophyte samples were dried in an electric furnace at 80 °C. To determine Na, K, Zn, Fe, Cu and Mn contents, the samples were mineralized in 14 M HNO_3 and residues were dissolved in 1 M HCl. After mineralization, the elements were determined using an atomic absorption spectrometer. Micro-Kjeldahl method was used to determine of total nitrogen in the macrophyte tissues (Kacar, 1972). Phosphorous was determined according to Olsen and Watanabe (1957).

Data analysis

All analyses were carried out with three replicates. The least significant difference (LSD) test was used to compare the parameters by using SPSS 11.0. Pearson's correlation was analyzed between Na concentration in macrophyte tissues and other parameters.

Results

Element contents

At all NaCl concentrations, Na accumulations were significantly incresed in macrophyte tissues (Fig. 1). The rate of Na increases in the tissues for 20, 40 and 80 mM NaCl applications can be calculated as 1.49, 1.73 and 2.31-fold, respectively compared to that found in control tissues. NaCl application disturbed macro and micronutrient uptakes (Fig. 1). Macronutrients N, P and K contents were reduced by NaCl. The maximum reduction rates were estiamated as 32.7%, 23.1% and 14.0% for N, P and K, respectively at 80 mM NaCl concentration. There were negative correlations between Na accumulation and macronutrients contents in macrophyte tissues (r=-0.0877; $p<0.001$ for N, r=-0.728; $p=0.007$ for P and r=-0.649; $p=0.022$ for K). Micronutrients Zn and Cu contents also decreased up to 59.9% and 41.8%, respectively at 80 mM NaCl concentration. There were negative correlations between Na accumulation and micronutrients Zn and Cu contents in macrophyte tissues (r=-0.829; $p<0.001$ for Zn and r=-0.870; $p<0.001$ for Cu). Moreover Fe content was decreaed up to 10.4% at 20 mM NaCl concentration, and then increased up to 16.8% at 40 mM NaCl concentration. No significant correlations were estimated between Na and Fe contents in macrophyte tissues (r=0.299; $p=0.345$). Manganese content increased up to 24.0% at 80 mM NaCl concentration. There is positive and insignificant correlation between Na and Mn contents in macrophyte tissues (r=0.522; $p=0.082$).

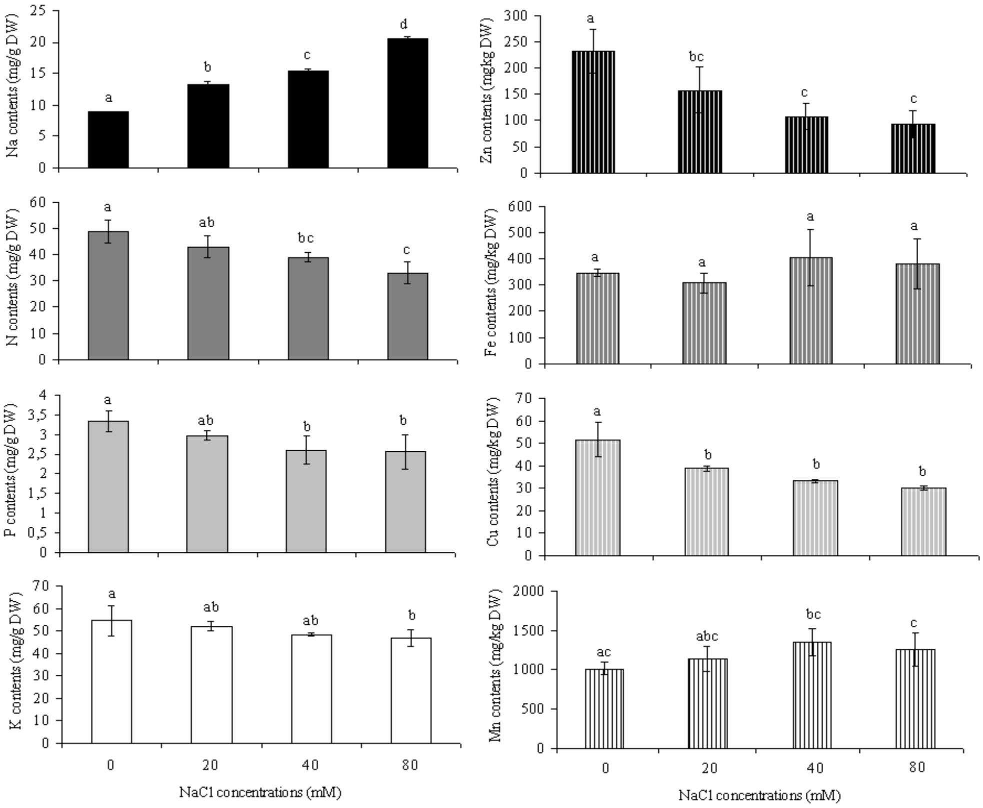

Figure 1: Element contents of *Ceratophyllum demersum* tissues after NaCl apllications. Error bars represent the standard deviation of means. Means with different letters are significantly different from one another according to LSD test ($p<0.05$).

Photosynthetic pigment contents

Chlorophyll a, b and carotenoid contents were reduced in response to NaCl (Fig. 2). The reduction was dose-dependent and the least amounts were measured at 80 mM NaCl-treated macrophytes. Total amounts of all pigments were found to be negatively correlated with Na contents in tissues (r =-0.642; *p*=0.024 for chl-a, r=-0.701; *p*=0.011 for chl-b and r=-0.374; *p*=0.231 for carotenoids).

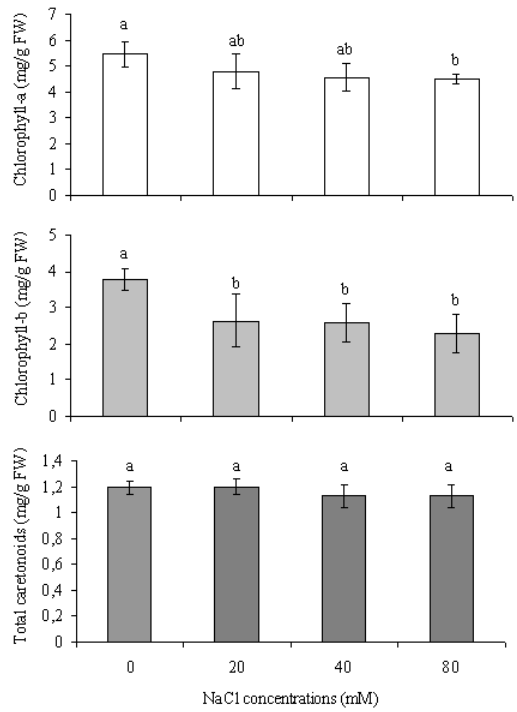

Figure 2: Photosynthetic pigment contents of *Ceratophyllum demersum* after NaCl apllications. Error bars represent the standard deviation of means. Means with different letters are significantly different from one another according to LSD test (*p*<0.05).

Biochemical changes

As shown in the Fig. 3, some biochemical changes were determined under the influence of NaCl in macrophyte tissues. Total soluble carbohydrate, reducing sugar and protein contents showed decreases under NaCl stress. With respect to their control, reduction rates of total soluble carbohydrate were astimated as 10.5%, 9.9% and 23.8%, at 20, 40 and 80 mM NaCl concentrations, respectively. Similarly, reducing sugar and protein contents decreased up to 43.3% and 44.5% at 80 mM NaCl concentration, respectively. There were negative and significant relationships between Na contents and these parameters (r=-0.628; *p*=0.029 for total soluble carbohydrate, r=-0.668; *p*=0.018 for reducing sugars and r=-0.793; *p*=0.002 for protein content). On the other hand, proline, ascorbic acid, non-protein SH groups and MDA contents were increased in response to NaCl. Ascorbic acid (AsA) content was increased up to 52.8% at 40 mM NaCl concentration. The content, however, was decreased at 80 mM NaCl with respect to other applied NaCl concentrations. Non-protein SH groups were increased by 59.8%, 91.2% and 115.4%, at 20, 40, 80 mM NaCl concentrations, respectively, when compared with the control. Similarly, proline and MDA contents also increased up to 68.5% and %58.1, respectively. These parameters were found to be positively correlated with Na contents in tissues (r=0.847; *p*<0.001 for proline, r=0.434; *p*=0.158 for ascorbic acid, r=0.718; *p*=0.009 for non-protein SH groups and r=0.760; *p*=0.004 for MDA). Total phenolic compound content was increased up to 7.3% at 40 mM NaCl, then decreaed up to 21.4% at 80 mM NaCl concentration. There were negative and insignificant relationships between Na contents and phenolic compound contents (r=-0.535; *p*=0.056).

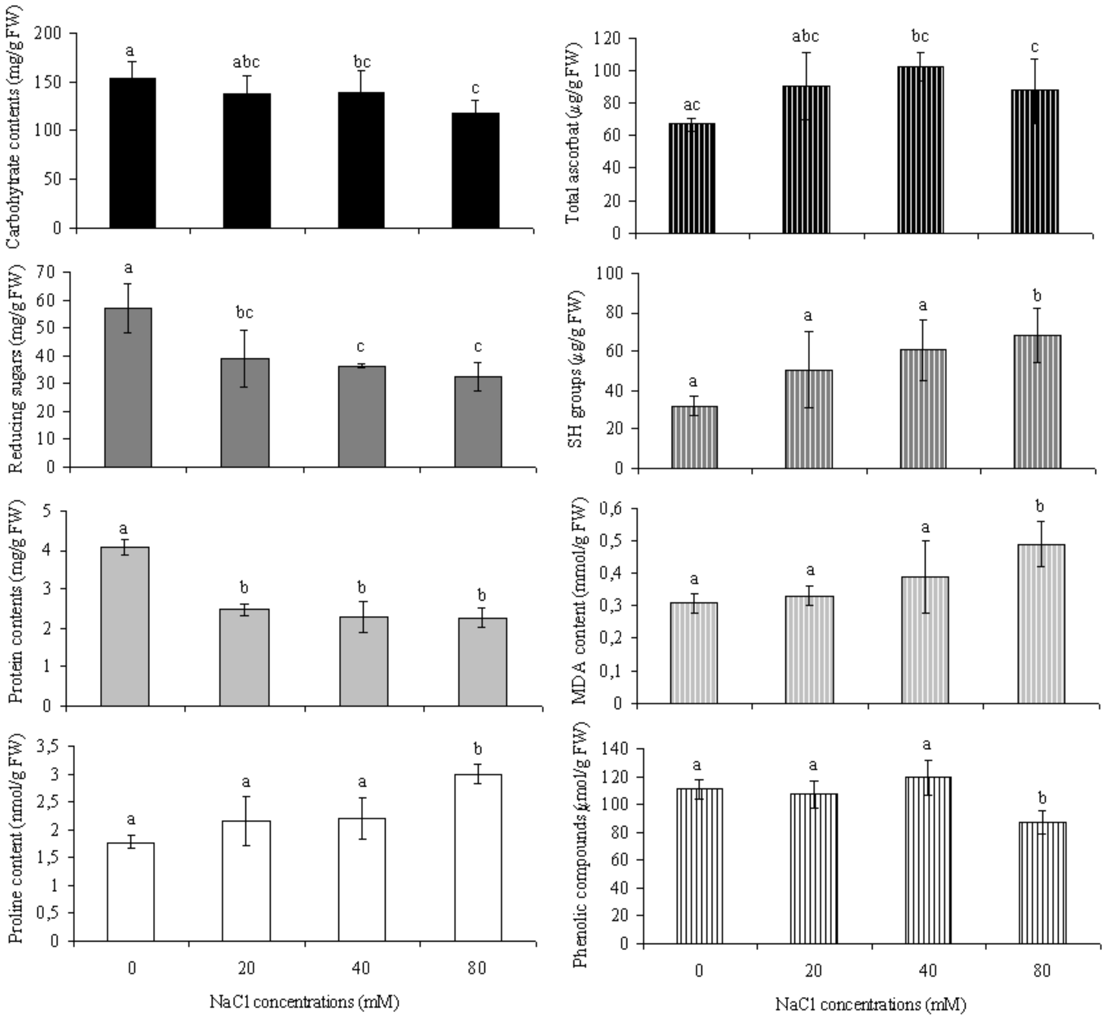

Figure 3: Some biochemical changes of *Ceratophyllum demersum* tissues after NaCl apllications. Error bars represent the standard deviation of means. Means with different letters are significantly different from one another according to LSD test (*p*<0.05).

Discussion

Salinity is one of the abiotic stress factors which restricts growth and development of plants (Lauchli and Epstein, 1990). Plants exposed to salinity affect various physiological and biochemical prosesses (Munns and Tester, 2008). One of the most important process that is affected by salt stress is photosynthesis (Stepien and Klobus, 2006). After NaCl applications, photosynthetic pigments decreased in *C. demersum*. Our findings agree with many reports (Rout *et al.*, 1998; Agastian *et al.,* 2000). According to Yeo and Flowers (1983) chlorophyll content of salinity stressed rice can be described as a function of the leafs sodium content. Our correlation analyzes confirmed this situation. The decrease in chlorophyll contents at high NaCl concentration might possibly be due to changes in the lipid protein ratio of pigment–protein complexes or increased chlorophyllase activity (Iyengar and Reddy, 1996).

Sodium contents of *C. demersum* tissues were increased with increasing NaCl concentrations. Similar findings have been reported in many aquatic macrophytes (Rout and Shaw, 2001; Jampeetong and Brix, 2009). NaCl salinity differentially affected the mineral contents of *C. demersum*. NaCl concentrations increased concentrations of Na but reduced concentrations N, P and K contents in macrophyte tissues. Antagonist effect of Na^+ on $N\text{-}NH_4^+$ and Cl^- on $N\text{-}NO_3^-$ may be responsible for decrease in nitrogen concentration in the macrophyte tissues (Bradley and Morris, 1991; Bar *et al.,* 1997). Phosphorus contents declined with increasing salt concentrations in the macrophyte. Probably Cl^- ion in the simulated salt solutions depressed the uptake of PO_4^{3-}. According to Jacoby (1999) K content response of plants is not uniform in salt conditions. It is reported that in general, many salinity tolerant plants grown in saline conditions maintain K content constant or increase it. However, salt-sensitive plants fail to maintain K content at high salinity conditions. Such decrease of K content may indicate damage (Winter and Kirst, 1991), as determined in our study. As findings in macronutrients N, P and K contents, data showed that salt-dependent reductions were found in micronutrients Zn and Cu contents subjected to different salinity concentrations. Correlation analyzes confirmed this situation as well. In general, however, Mn and Fe contents were increased by NaCl. As a result, NaCl application caused nutrient imbalances, probably due to the competition of Na^+ and Cl^- with macro and micronutrients.

According to a study, salt stress decreased soluble and hydrolyzable sugars in *Vicia faba* (Gadallah, 1999). On the other hand, sugar content increased in some genotypes of rice under salt stress but also decreased in some genotypes (Alamgir and Ali, 1999). In our study, total soluble carbohydrate and reducing sugars in *C. demersum* tissues showed decreases under NaCl stress. Correlation data shown that this reduction is dependent on NaCl stress. Like these parameters, protein content of macrophyte tissues were reduced in response to NaCl. Many authors reported that contents of soluble protein in plant tissues decreased in response to salt stress as well (Agastian *et al.,* 2000; Parida *et al.,* 2002).

Under salt stress plants increase their osmotic potential by accumulation of some solutes like proline. In the present study, NaCl stress increased proline content in *C. demersum* tissues as a compatible compound. Correlation analysis between Na and proline also supports this situation. Accumulation of the solute in the macrophyte can show an indirect protective function due to its antioxidant properties in addition to the direct effect to stabilize subcellular structures such as membranes and proteins (Ashraf and Foolad, 2007).

Salinity stress, like other abiotic stresses, generates reactive oxygen species (ROS). ROS can directly attack membrane lipids and cause lipid peroxidation (Mittler, 2002). MDA content increased with increasing NaCl treatment. Correlation analysis demonstrated a significant positive relationship between Na and MDA contents. This result shows NaCl-induced lipid peroxidation, and therefore, an onset of oxidative stress in *C. demersum*.

In order to cope with ROS, plants possess various cellular enzymatic and nonenzymatic antioxidants, such as ascorbic acid, glutathione and phenolic compounds (Foyer, 1993). The increased AsA contents exhibited that oxidative stress occured in the macrophyte tissues. According to our findings, non-protein -SH groups showed marked increases in the macrophyte with the severity of NaCl toxicity. Namely, NaCl-induced increase in the level of the thiol compounds represented another defensive mechanism against oxidative stress in *C. demersum*. Content of total phenolic compounds, however, negatively correlated with Na. This indicates that phenolic compounds of *C. demersum* may be ineffective in salinity stress.

C. demersum is a submerged rootless aquatic macrophyte, the leaves of which are fragmented and all the surface directly interacts with contaminants. Untreated macrophytes (control) had grown very healthily under the test conditions. Namely, toxicity symptoms (excluding normal detachment of leaves) were not observed in the control. Similar observations were noted at 20 mM NaCl concentration. Detachment of some leaves and chlorosis were especially observed at 80 mM NaCl. According to our findings, these symptoms probably occurred due to Na^{+} and Cl^{-} toxicity, nutrient imbalances and/or oxidative stress induced by NaCl salinity.

In conclusion, our results revelated that NaCl salinity affected various physiological processes. The salinity created an ionic imbalance by the uptake of beneficial elements, decreased contents of photosythetic pigments, total soluble carbohydrate, reducing sugars and total protein. Increases in proline, non-protein thiols and AsA contents may indicate their roles in response to salinity stress. Observed increase in MDA contents showed that oxidative damage occurred in membranes resulting from NaCl toxicity.

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