

## Influence of salinity on the growth and heavy metal accumulation capacity of *Spirodela polyrrhiza* (Lemnaceae)

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**Abstract:** Given that the characteristic of free floating plants to uptake metals can have a positive effect on the efficiency of the remediation process of a wetland, it is important to investigate the factors that may impact the process of uptake of metals by the plants. By conducting this present study over a short duration of time, the effects of salinity on the growth, the content of the photosynthetic pigments (chlorophyll a, b, and carotenoid), and heavy metal uptake by *Spirodela polyrrhiza* were investigated. It was also observed that at a high level of salinity (100 and 200 mM NaCl) the relative growth rate (RGR) of the plant decreased. Especially, at a salinity level of 100 mM NaCl, the RGR values were noted to become negative. The content of photosynthetic pigment was negatively correlated with the salt level. Additionally, a high level of salinity was observed to cause a decrease in the accumulation of the metals cadmium (Cd) and nickel (Ni) by *S. polyrrhiza*.

**Key words:** *Spirodela polyrrhiza*, heavy metal, salt stress, photosynthetic pigment

### *Spirodela polyrrhiza*'da (Lemnaceae) tuzluluğun büyüme ve ağır metal birikim kapasitesine etkisi

**Özet:** Serbest yüzen bitkilerin karakteristik metal alabilme özelliği sulak alanların temizlenmesi üzerine pozitif etkili olabilir ki buda bitkiler tarafından metallerin alınımında etkili olan faktörlerin belirlenmesinde önemlidir. Kısa süreli bu çalışma ile, *Spirodela polyrrhiza*'da tuzluluğun büyüme, fotosentetik pigment içeriğine (Klorofil a,b, ve karoteneid) ve ağır metal alınımı üzerine etkisi incelenmiştir. Yüksek tuzluluk düzeyinde (100 ve 200 mM NaCl) bitkideki doğrusal büyüme oranının (RGR) azaldığı gözlenmiştir. Özellikle, 100 mM NaCl düzeyinde, RGR değerlerinin negatife gittiği not edilmiştir. Fotosentetik pigment içeriği tuz düzeyi ile negatif ilişkilidir. Bununla birlikte *S. polyrrhiza*'da yüksek tuz düzeyinin, kadmiyum ve nikel birikiminin azalmasına neden olduğu gözlenmiştir.

**Anahtar sözcükler:** *Spirodela polyrrhiza*, ağır metal, tuz stresi, fotosentetik pigment

### Introduction

Salinity is the major environmental factor limiting plant growth and productivity. The detrimental effects of high salinity on plants can be observed at the whole-plant level as decreases in productivity and/or the death of plants. Many plants develop mechanisms

either to exclude salt from their cells or to tolerate its presence within the cells. During the onset and development of salt stress within a plant, all major processes such as photosynthesis, protein synthesis, and energy and lipid metabolism are affected (1). The contamination of water with heavy metals is a very

critical issue causing numerous problems all over the world. Several aquatic macrophytes have been used for the removal of heavy metals from waste water. Many researchers have based their investigative works on different aspects of the process of removal of metals from water (2), the degree of toxicity of these metals causing harm to plants (3,4), the use of plants as biofilters for polluted water (5), and the biomonitoring of metals (6). The metal uptake capacity of aquatic plants may be influenced by several factors, including pH, redox potential, and surrounding metal concentrations as well as the temperature and salinity level of the surrounding water (7). The salinity of water may affect the growth rate of the plant and its metal uptake capacity through the toxic effects of both the sodium ( $\text{Na}^+$ ) and the chlorine ( $\text{Cl}^-$ ) ions (8). The  $\text{Na}^+$  ions may release cadmium (Cd) from the sediment to the water, thereby increasing the concentration of Cd in the water (9). Further, Greger et al. (9) also determined that for the plant species *Potamogeton pectinatus* (L.) that grows in highly saline water, a low number of free Cd ions present in the water are noted to correlate with a low Cd uptake capacity of the plant. At a high level of salinity, the increased concentration of  $\text{Na}^+$  reduces both the intracellular and extracellular uptake of Cd in the free-floating plant *Lemna polyrrhiza* (L.) (10). It has been determined not only in aquatic plants (9) and free-floating plants (10), but also in macroalgae, such as the seaweed *Fucus vesiculosus* (L.), that the metal (Zn) uptake capacity of organisms decreases with an increase in the level of salinity (11). Thus, an increase in the level of salinity can be expected to induce a general decrease in the metal uptake capacity of organisms.

In the present study, the plant species *Spirodela polyrrhiza* was exposed to certain amounts of cadmium, nickel, and salt stress during controlled laboratory tests. Further, evaluations were made to determine the impact of the metals on the growth of the plant, the production of photosynthetic pigments in it, and its metal accumulation capacity. This study was specifically conducted to assess the procedure that is undertaken for the accumulation of cadmium and nickel within the plant species *Spirodela polyrrhiza* in response to being subjected to salt stress. The information obtained from this experiment is

relevant for the purpose of aiding phytoremediation technology and can possibly be a progressive step in supplementing the efforts made to widen the sphere of knowledge of this area and, ultimately, to fulfill the objective of optimizing the treatment of polluted areas.

## Materials and methods

### Plant material and treatment conditions

The aquatic macrophyte species *Spirodela polyrrhiza* was collected from the Soysalli Barrage of Kayseri, Turkey. Prior to the experiment, plants were washed in distilled water and acclimatized for 3 days in a climate chamber with a water temperature of 23 °C, a relative humidity of 70%, and light/dark conditions of 16 h light/8 h dark cycle. In this study, cadmium chloride ( $\text{CdCl}_2$ ), nickel nitrate [ $\text{Ni}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ ], and sodium chloride (NaCl) were used without further purification for experimental treatments. Preliminary tests for nickel concentrations were performed to determine the appropriate sensitivity range for the test plants. Nickel and cadmium exposure concentration for *S. polyrrhiza* were determined to be 20 mg Ni L<sup>-1</sup> (12) and 2 mg Cd L<sup>-1</sup>. Plants were treated with different concentrations of sodium chloride (0, 25, 50, 100, and 200 mM NaCl) (4). The plants were grown in Hoagland medium according to methods from Smart and Barko (13). The plants were placed in vertical cylinders (height: 15 cm, diameter: 16.2 cm) filled with the medium. Distilled water was added daily to each cylinder to replace water lost because of evaporation and transpiration. The pH (mean pH value: 7) of the samples was measured daily during the experiments (12). After day 4, the cylinders were replaced with fresh medium. After day 10, plants were washed with double distilled water. Plants were placed on blotting paper and allowed to drain for 3 min before weighing. For each treatment, 3 replicates (for each group) were applied. The initial biomass was determined as fresh weight.

For *S. polyrrhiza*, relative growth rates (RGRs) were calculated in each group according to Hunt's equation:

$$R = \frac{\ln W_2 - \ln W_1}{T_2 - T_1}$$

where  $R$  is the relative growth rate,  $W_1$  and  $W_2$  are the initial and final dry weights, respectively, and  $(T_2 - T_1)$  is the experimental period (14). Dry weight was estimated by drying the plants at 80 °C until constant weight was reached (15).

### Nickel and cadmium quantification

Harvested plants were washed thoroughly with double deionized water, blotted, and oven dried at 80 °C. Each sample was then digested with 10 mL pure  $\text{HNO}_3$ , using a CEM-MARS 5 (CEM Corporation Mathews, NC, USA) microwave digestion system (maximum power: 1200 W, power: 100%, ramp: 20:00 min, pressure: 180 psi, temperature: 210 °C and hold time: 10:00 min). After digestion, the volume of each sample was adjusted to 25 mL using double deionized water. Determinations of cadmium and nickel concentrations in all samples were carried out by inductively coupled plasma optical emission spectroscopy (Varian-Liberty II, ICP-OES) (12). Peach leaves (NIST, SRM-1547) and CRM 039-050 were used as reference material, and all analytical procedures were also performed on reference materials. Samples were analyzed in triplicate.

### Photosynthetic pigments concentrations

Plant biomass was measured on the basis of fresh weight. Photosynthetic pigments of treated and untreated plants (100 mg) were extracted in 80% chilled acetone in the dark. After centrifugation at  $10,000 \times g$  for 10 min, absorbance of the supernatant was measured at 450, 645, and 663 nm. The content of chlorophylls and carotenoids were estimated as previously described (16).

### Statistical analysis

Two-way analysis (ANOVA) was done with all the data to confirm the variability of data and validity of results and Duncan's multiple range test (DMRT) was performed to determine the significant difference between treatments. For each treatment, 3 replicates were applied. Statistical Package for the Social Sciences (SPSS) was used for statistical analysis.

### Results and discussion

Salt stress is considered to have 3 effects: it reduces water potential, causes ion imbalance or disturbances

in ion homeostasis, and is toxic. This alters the status of the water significantly, thus leading to a reduction in the initial growth of the plant and, consequently, limits its productivity. Since salt stress entails both osmotic as well as ionic stress (17,18), the suppression of growth is directly related to the total concentration of soluble salts (19,20).

The RGR value of *S. polyrrhiza* was noted to be significantly inhibited due to the increase in the level of salinity (100 mM NaCl; Figure 1). This may be attributed to the high degree of sensitivity of plants that are subjected to the physiological stress caused by salinity and the presence of heavy metals in comparison with the control plants. In earlier studies, it was determined that salt stress also causes a considerable decrease in the fresh and dry weights of the leaves, stems, and roots of plants (21-23). Additionally, Demirezen (12) indicated that the highest growth rate was observed at a salinity of 125  $\text{mol m}^{-3}$  NaCl. Extraordinarily, the lowest biomass had a significantly lower growth rate of 375  $\text{mol m}^{-3}$  NaCl salinity level ( $P < 0.001$ ) and the highest salinity of 500  $\text{mol m}^{-3}$  NaCl caused a negative net growth rate; hence, this experimental work is in agreement with the results obtained in the present study.

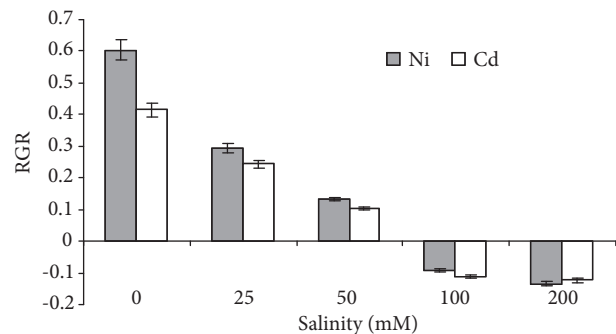


Figure 1. Relative growth rates of salt-treated plants of *Spirodela polyrrhiza*. Error bars show standard deviations.

For *S. polyrrhiza*, the bioaccumulation levels of the metals Cd and Ni were measured at the given contact times for 4 different experimental values of salinity (0, 25, 50, 100, 200 mM) (Figures 2 and 3). The analysis conducted through the one way ANOVA method indicated that the *S. polyrrhiza* plants that were grown under lower levels of salinity had a higher

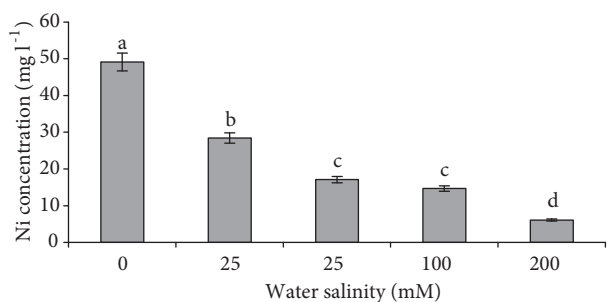


Figure 2. Effects of water salinity on the nickel accumulation capacity of the *Spirodela polyrrhiza* plant at different salinity levels. Error bars show standard deviations. All the values are means of triplicates  $\pm$  SD. ANOVA significance was set at  $P \leq 0.01$ . Different letters indicate significantly different values at a particular point in time (DMRT,  $P \leq 0.05$ ).

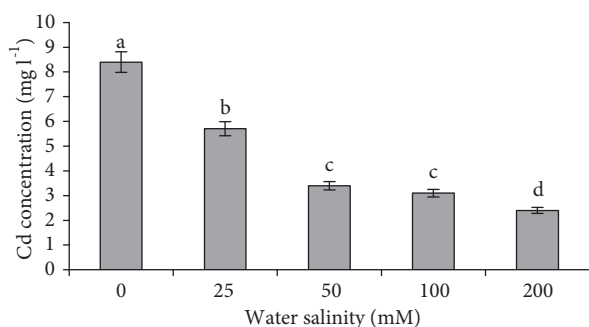


Figure 3. Effects of water salinity on the cadmium accumulation capacity of the *Spirodela polyrrhiza* plant at different salinity levels. Error bars show standard deviations. All the values are means of triplicates  $\pm$  SD. ANOVA significance was set at  $P \leq 0.01$ . Different letters indicate significantly different values at a particular point in time (DMRT,  $P \leq 0.05$ ).

accumulation capacity for nickel ( $P < 0.001$ ). Similarly, the accumulation capacity for cadmium of plants grown at 25 and 50 mM NaCl;  $P < 0.001$  was noted to be significantly higher than those of the plants grown under the higher levels of salinity (100 and 200 mM NaCl;  $P < 0.001$ ). Capacity of *S. polyrrhiza* to accumulate metals was noted to decrease with an increase in the level of salinity in most cases (Figures 2 and 3). Earlier works of other authors indicated that a higher level of salinity causes a decrease in the accumulation of elements by plants. For example, Fritioff et al. (24) stated that an increased

level of salinity causes a reduction in the accumulation of metals in both *Elodea canadensis* and *Potamogeton natans*. This result is quite similar to the one determined in the present study. In this context, the results obtained for *S. polyrrhiza* are found to be similar to those obtained for *Spergularia marina* by Adam (25) and *Schoenoplectus triqueter* by Deegan et al. (26). They stated that the growth levels of these plants were stimulated at moderate salinities, but were severely reduced at higher concentrations of salinity. However, the results of the present study indicate that with an increase in the salinity level, the capacity of plants to accumulate the metals of nickel and cadmium was also inhibited.

In *S. polyrrhiza*, the concentration of chlorophyll was negatively correlated with the addition of salt and heavy metals to its solution (Figures 4 and 5). In addition, the sodium chloride (NaCl) ions could damage the chloroplast envelope and thylakoid through an increased production of free radicals (21). The chlorophyll and carotenoid contents of the leaves develop chlorosis and fall due to being subjected to a prolonged period of salt stress (21,27,28). Additionally, in agreement with the results of the present study, Hou et al. (29) observed in their experimental work that in *Lemna minor* the contents of photosynthetic pigments significantly decreased upon being subjected to high-level metal stress.

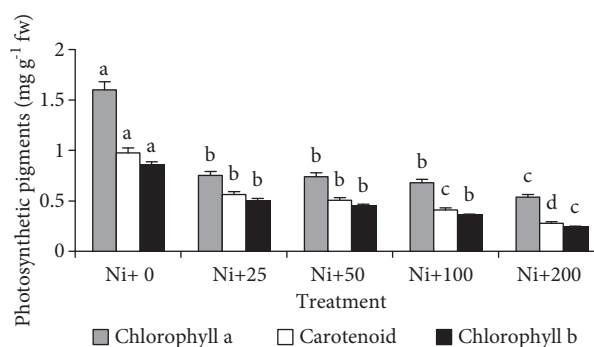


Figure 4. Effects of water salinity and the presence of nickel on the photosynthetic pigments (chlorophyll a and b, and carotenoid) of the *Spirodela polyrrhiza* plant at different salinity levels. All the values are means of triplicates  $\pm$  SD. ANOVA significance was set at  $P \leq 0.01$ . Different letters indicate significantly different values at a particular point in time (DMRT,  $P \leq 0.05$ ).

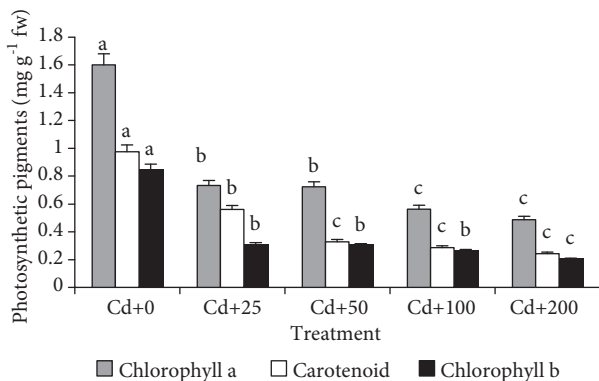


Figure 5. Effects of water salinity and the presence of cadmium on the photosynthetic pigment contents (chlorophyll a and b, and carotenoid) of the *Spirodela polyrrhiza* plant at different salinity levels. All the values are means of triplicates  $\pm$  SD. ANOVA significance was set at  $P \leq 0.01$ . Different letters indicate significantly different values at a particular point in time (DMRT,  $P \leq 0.05$ ).

## Conclusions

The present paper ascertained through investigations that in *S. polyrrhiza* the rate of growth of the plant, its capacity to accumulate metals, and its photosynthetic pigment content were significantly affected by an increase in the level of salinity. *S. polyrrhiza* is an aquatic plant that grows in a freshwater environment. It was noted that increasing the level of salinity of the water may also decrease its

phytoaccumulation capacity. The phytoremediation processes hold enormous potential as a means to clean polluted water and soils. The bioconcentration of heavy metals by plants is a fascinating area of research that should be able to provide answers for some of the fundamental questions that remain unanswered in the fields of plant biochemistry, nutrition, and stress physiology. In order to achieve adequate results using phytoremediation techniques it is essential to choose only plant materials that are tolerant to the specific metals as well as to specific conditions of the environment, such as high levels of salinity, lack of nutrients, or drought conditions. The information generated by the present study may be useful in applying the plant *S. polyrrhiza* as a bioaccumulator. However, this species is not considered appropriate for application in the remediation process of saline water.

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