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Effects of salinity on growth and nickel accumulation capacity of *Lemna gibba* (Lemnaceae)

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Abstract

The effect of salinization of water on the growth rate and nickel accumulation capacity of *Lemna gibba* was studied under laboratory conditions. At high salinity (375 and 500 mol m^{-3} NaCl), we observed that decreasing growth rate with increasing water salt level. Especially, at a salinity of 500 mol m^{-3} NaCl, the net growth rate became negative. Additionally, growth rate of roots was significantly reduced at salinity of 250 mol m^{-3} NaCl and significantly reduced at 500 mol m^{-3} NaCl compared to a freshwater control. Biomass production was inhibited by an increase in salinity. Furthermore, high salinity caused decreasing nickel accumulation by *L. gibba*. In this study, a simple model was created according to data obtained from this study. The model has suggested that salinity is an important factor in limitation of growth of *L. gibba*.

Keywords: *Lemna gibba*; Salt stress; Accumulation; Nickel; Growth rate

1. Introduction

Heavy metal contamination from different kind of sources is an important environmental problem. Many industries, such as automotive, metal finishing, electroplating, battery manufacturing, mining, electric cable manufacturing, tannery, steel and textile industries, release various concentrations of heavy metals like cadmium, nickel and copper in wastewaters. These heavy metals are toxic to aquatic ecosystem and human health and these heavy metals also accumulate by organisms in tolerance levels. Uptake and accumulation of elements by plants may follow two different paths, that is, the root system and the foliar surface [1]. Metal bioaccumulation depends upon numerous biotic and abiotic factors, such as temperature, pH, salinity and dissolved ions in water [2,3]. According to Yang et al. [4], higher nickel concentrations cause weak plant growth, yield depression, disorder in plant metabolism and chlorosis. In many agro climatic areas, the metal co-occurs in combination to other stresses such as salinity, alkalinity, water stress, drought and water logging [5]. Especially, salt stress is an important issue for different ecosystems around the world, so an understanding of responses of plants to salinity is of great practical significance. High concentrations of salt in aquatic environments have harmful effects on plant growth and excessive concentrations kill growing plants [6]. Ramoliya et al. [7] stated that there is retardation of germination and growth of seedling at high salinity. They indicated that salt stress suppresses shoot growth. Furthermore, Brady and Weil [8] indicated that plant species differ in their sensitivity or tolerance to salt stress. Because, there are many different type of salts in the environment and organs, tissues and cells at different developmental stages of plants exhibit varying degrees of tolerance to these kinds of stresses. High salt concentration can cause to decrease in osmotic potential of environment and in consequence the availability of water in plants decreases. Many investigators have studied that the concentrations of heavy metals in aquatic plants. However, the relationship between heavy metal accumulation and salt-stressed in plants remains poorly understood.

Duckweeds have a cosmopolitan distribution in freshwaters in most areas of the world. This plant can be used to remove nutrients from sewage effluents. In this study, nickel and salt stress were exposed to *Lemna gibba* in controlled laboratory tests to determine if growth and metal accumulation capacity were affected. Specifically, to assess the pattern of nickel accumulation within the Lemna gibba in response to salt stress.

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From the results obtained, a simple model was constructed to explore the salinity stress involved in growth and heavy metal accumulation capacity of aquatic macrophytes.

2. Materials and methods

2.1. Crowding experiment

L. gibba (Lemnaceae) are small and green vascular plants rapidly growing under favourable conditions supplied from Kayseri, Turkey. This plant is known to have a very high tolerance capacity to waste-waters in removing pollutants from urban wastewater [9]. The plants used in this experiment were collected the previous autumn. The growth rate and nickel accumulation capacity of biomass of this plant was determined under laboratory conditions (such as: 23 °C and a 14-h photoperiod; 350 μmol m^2 s^{-1}). In this study, nickel nitrate [$Ni(NO_3)_2{\cdot}6H_2O$] and sodium chloride (NaCl) were used without further purification for treatments. The salt and nickel solutions were prepared by mixing the appropriate amount of sodium chloride and nickel nitrate to suitable volume of deionized water in each case. Preliminary tests for nickel concentrations were performed to determine the appropriate sensitivity range for test plant. So, the nickel exposure concentration for *L. gibba* was determined 20 mg Ni L^{-1}.

The plants were grown in a liquid medium according to methods from Smart and Barko [10]. A total of five different salinity concentrations (0, 125, 250, 375 and 500 mol m^{-3} NaCl, which are equivalent to 0, 25, 50, 75 and 100% sea water salinity) were tested [11]. The plants were placed in vertical cylinders (height: 15 cm, diameter: 16.2 cm) filled with the medium [12]. 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. For each treatment, five replicates (both for two groups) were applied. The initial biomass was determined as fresh weight.

After Day 4, the cylinders were replaced with fresh medium. At the start of the test and every day, as well as at test termination, changes in plant development, root length and appearance were recorded under the stereomicroscope. Before weighing, the plants were placed on blotting paper and allowed to drain for 5 min. After Day 10, the experiment was stopped and the fresh weight and dry weight (24 h at 70 °C) of *L. gibba* were measured. The initial dry weight was calculated using the dry weight to fresh weight ratio at the end of the experiment. Each sample was then digested with 10 ml pure HNO_3, using a CEM-MARS 5 (CEM Corporation Mathews, NC, USA) microwave digestion system (digestion conditions are; maximum power: 1200 W, power (%): 100, ramp. (min): 20:00, pressure (psi): 180, temperature (°C): 210 and hold time (min): 10:00). After digestion, the volume of each sample was adjusted to 25 ml using double deionized water. Determinations of the nickel concentrations in all samples were carried out by inductively coupled plasma optical emission spectroscopy (Varian-Liberty II, ICP-OES). The samples were analyzed in triplicate. Statistical Package for the Social Sciences (SPSS) statistical program was used for statistical analysis [13].

2.2. Modelling

Salt concentration at the root–water or root–soil interface dramatically increases, as soil water is lost [14]. Therefore, the effects of increased salinity on water potential in environment may be compounded when accompanied by drying conditions. Salinity has been shown to severely affect the uptake and transport of elements enough that shoots may show signs of element deficiency such as calcium [15]. For these reasons, in this study, a simple model created by Driever et al. [16] was constructed to explain the effect of salinity, temperature and nutrients:

$$\frac{dB}{dt} = \mathrm{Br}\, f(T, B, N, P) - lB$$

The variation of the biomass of *L. gibba* was modeled as the function of the maximum growth rate (r). The gross production was modified by a limitation function (f (T, B, N, P)), which was a function of air temperature (T), biomass (B) and nutrients (N and P). Additionally, the production of plants was corrected for the loss (l), which included mortality [16]. The limitation function [$f(T, B, N, P)$] was defined as:

$$f(T, B, N, P) = [T - T_{\min}/T_{\mathrm{opt}} - T_{\min}][N/N + hN] \times [P/P + h_P][h_B/B + h_B]$$

Temperature (T) limitation was assumed to be linear from the minimum temperature ($T_{\min}$, 5 °C) up to the optimum temperature (T_{opt}, 26 °C) [16,17]. According to the statements of Lüönd [18], nutrient limitation of ammonia and nitrate (N) and phosphate (P) were also modelled as Monod-type functions (with the following half saturation values: $h_N = 0.04$ mg N L^{-1} and $h_P = 0.05$ mg P L^{-1}). The restrictive effect of biomass was simply assumed to be another Monod-type function dependent on biomass B and with half saturation (h_B) determined during this study [16,17].

3. Results and discussion

The growth rates of *L. gibba* plants were significantly inhibited by high salinity (500 mol m^{-3} NaCl; Fig. 1). This may be due to the greater sensitivity of plants to the physiological stress of salinity and nickel compared to the control plants. The studies of plant morphogenesis showed that root length was remarkably reduced by an increase of water salinity. Fig. 2 shows that on average, root extension for control group was 30% taller than those of at the 500 mol m^{-3} NaCl. The results of root length for each treatment also showed a similar pattern, with mean length decreasing significantly ($P < 0.05$) from 30.2 mm in the control down to 11 mm in the 500 mol m^{-3} NaCl and 13.77 mm in the plants exposed to 375 mol m^{-3} NaCl (Fig. 2). Furthermore, roots of affected plants (375 and 500 mol m^{-3} NaCl) were also noticeably thinner than those in control and other treatments. In these treatments especially at 500 mol m^{-3} NaCl total root loss was also more apparent. Compared with control treatment, average

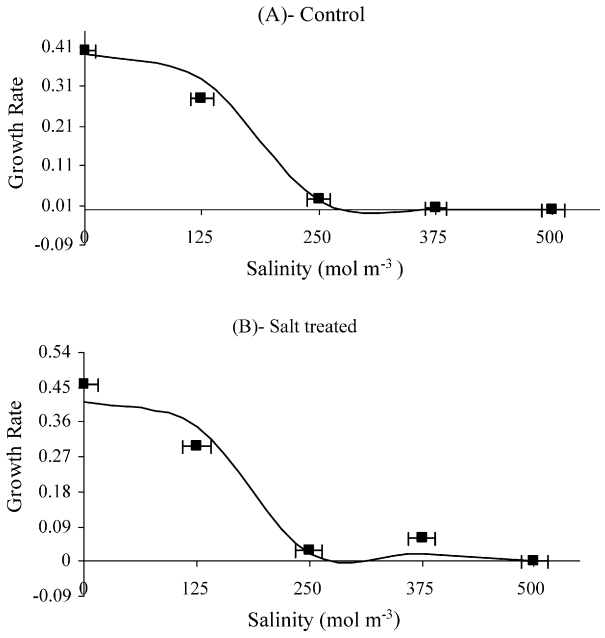

Fig. 1. Growth rate (control and salt-treated) of *Lemna gibba* both laboratory-scale experiment and simple model. Dots represent the measured growth rate and solid line represents the curve described by the model. Error bars show standard deviations.

root extension and nickel accumulation were inhibited by 36.4% and 75%, respectively.

The one way ANOVA indicated that *L. gibba* plants, grown at lower salinity levels, were significantly affected in their nickel accumulation capacity (F: 57.4, $P<0.001$). Nickel accumulation capacity of plants grown at 125 and 250 mol m^{-3} NaCl was significantly higher than those of plants grown at the higher salinity levels (375 and 500 mol m^{-3} NaCl; $F=5.48$ and 14.15, respectively; $P<0.001$ for both; Fig. 3). Although only four treatments were examined, it appeared that there is a negative relationship between tissue nickel content and water salt content especially between water salinity and concentration for nickel in plants ($r=-0.72$, $P<0.05$). Earlier works made by other authors indicated that higher salinity level cause decreasing element accumulation by plants. For example, Ramoliya et al. [7] stated that there was a gradual decline in copper and zinc content in leaves and stems of *Salvadora persica*. Furthermore, they indicated that there was a negative relationship between manganese content in roots and increase in salt concentrations.

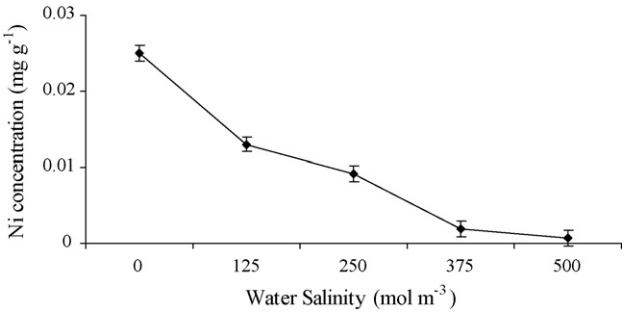

Fig. 3. Effects of water salinity on nickel accumulation capacity of *Lemna gibba* plants at different salinity levels. Error bars show standard deviations.

Fig. 4 shows, dry mass production of *L. gibba* decreased significantly in the higher salinity treatments. The highest growth rate was observed at a salinity of 125 mol m^{-3} NaCl in this study. Extraordinarily, the lowest biomass had a significantly lower growth rate of 375 mol m^{-3} NaCl salinity level ($P<0.001$). The highest salinity of 500 mol m^{-3} NaCl caused a negative net growth rate. Results from present study agree with those of obtained by Kramer [19] who reported that plants subjected to different kind of water stress, such as salt stress, show a general reduction in size and dry matter production. So, dry weight obtained from *L. gibba* significantly decreased ($P<0.05$) for whole plants in response to increasing concentration of salt in water. A negative relationship was observed between dry weight of plants and salt concentrations ($r=-0.593$, $P<0.05$).

Results obtained indicated that salinity leads to a transient decrease of the concentrations of nickel in whole plant. This trend seemed to be related to the growth of the plant. In this regard, these results obtained from *L. gibba* are similar to those of obtained from *Spergularia marina* by Adam [20] and *Schoenoplectus triqueter* by Deegan et al. [21]. They stated that growth levels of these plants were stimulated at moderate salinities, but were severely reduced in growth at higher salinity concentrations.

Results of this study indicated that with an increase in salinity, nickel accumulation capacity of plants was inhibited. Results

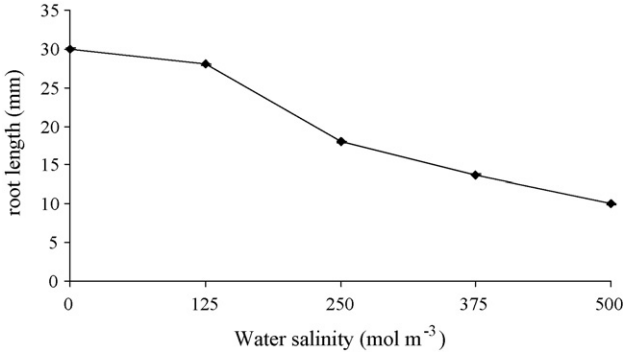

Fig. 2. Effects of water salinity with nickel on root length of *Lemna gibba* plants at different salinity levels. Error bars show standard deviations.

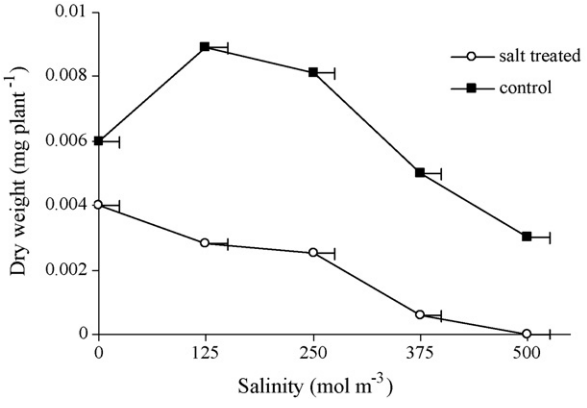

Fig. 4. Effects of water salinity on dry weight (mg $plant^{-1}$) of *Lemna gibba* plants at different salinity levels. Error bars show standard deviations.

obtained by Ushakova et al. [22] agree with our results. They stated that at a NaCl concentration of 2% (150 W/m^2 PAR), the portion of the potassium taken up by the roots reduced twice, P 3.1 times, and Ca 3 times. Data from this study were used to calibrate the model which was created by Driever et al. [16], assuming no nutrient limitation. The maximum growth rate (r) was obtained by extrapolation and was corrected for the optimum temperature of 23 °C. Using a combination of a laboratory-scale experiment and a simple model, we obtained insight in the growth dynamics of *L. gibba* in the field even several processes were neglected or oversimplified in the model. For example, the model assumed that the plants were homogenously distributed over the pot. In fact, this situation is not true in nature because of the wind, animals and birds [23]. The laboratory experiment showed that there is a relationship between salinity and growth rate and between salinity and nickel accumulation for *L. gibba*.

Driever et al. [16] and Landolt [24] stated that in this model the loss (l) was assumed to be constant for all pots and it included respiration, grazing and mortality implicitly. However, it has neglected the fact that respiration is strongly dependent on temperature. In addition, there is also no information about the influence of grazing pressure by birds or other animals, and the influence of temperature, on crowding and decomposition, and on mortality. Therefore, it could not be accounted for, in the mortality rates. Dale and Gillespie [25] stated that temperature can vary widely between water, air and within floating plants. Furthermore, Driever et al. [16] indicated that the maximum air temperature better describes the temperature of the fronds in the upper layers than the water temperature, so we used air temperature for calculations of model in this study.

Despite these difficulties, the negative effect of salinity on growth rate of *L.gibba* was accurately described in the model [25]. As a result, the higher temperature within the mat will cause increasing of the relative growth rate.

4. Conclusions

The growth rate and metal accumulation capacity of *L. gibba* are significantly affected by salinity. Plants grown at the highest salinity had significantly lower dried mass than plants grown at lower salinity. This has to be confirmed by further investigations. Furthermore, results showed that salt become a limiting factor, causing a reduction with increasing in plant biomass. Additionally, the results from modeling and laboratory experiment indicate that salt level of water influences the production and elongation of roots of this plant.

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