Boron removal by the duckweed *Lemna gibba*: A potential method for the remediation of boron-polluted waters

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**Abstract**

Boron (B) is often found in polluted and desalinated waters. Despite its potentially environmental damaging effects, efficient treatments are lacking. The duckweed *Lemna gibba* has been shown to remove toxic elements from water; however, its applicability to B removal is unknown. In this study, *L. gibba* was examined for its tolerance to B in water and its B removal efficiency. Duckweed plants were grown in outdoor 12-day batch experiments in nutrient solution containing 0.3–10 mg B L\(^{-1}\). Plant biomass production was not affected by B over the tested concentrations during the 12-day cultivation period. Boron removal and the bioconcentration factor of B in *L. gibba* were highest at initial B concentrations below 2 mg L\(^{-1}\), and decreased as the initial B concentration increased. Boron content in the plants at the end of the experiment ranged between 930 and 1900 mg kg\(^{-1}\) dry weight, and was comparable to that of wetland plants reported to be good B accumulators. Boron removal by *L. gibba* may therefore be a suitable option for the treatment of water containing B concentrations below 2 mg L\(^{-1}\).

**1. Introduction**

Boron (B), a metalloid essential for plant growth, is often found at elevated concentrations in wastewater as a result of its use for domestic purposes and in industrial production processes. When present in excessive concentrations in the soil solution, B can be toxic to plants (Yermiyahu et al., 1995). Consequently, B removal from wastewater has gained increasing interest, mainly in arid regions where wastewater reuse for irrigation is a favored solution (Campos et al., 2000; Oron et al., 2001). The main processes that have been studied for B removal are precipitation-coagulation, ion exchange, membrane filtration, use of B-selective resins, and adsorption on various compounds or materials (Simonnot et al., 2000; Ferreira et al., 2006; Dionisiou et al., 2006). All of these methods are associated with high operation and maintenance costs, and the use of chemicals.

Phytoremediation, a method developed to remove pollutants from the environment using plants, has been shown to be a promising cost-effective, environmentally friendly technology for the remediation of water polluted by toxic trace elements (Raskyn et al., 1997). However, limited information is available concerning the use of this technology for B removal from wastewater.

Duckweed plants have been widely used in phytoremediation studies as part of constructed wetland systems for...
wastewater treatment (Oron, 1994; Alaerts et al., 1996; Van der Steen et al., 1999; Vaillant et al., 2004). Duckweed-based wetland systems have been reported to remove nutrients, organic matter (Körner and Vermaat, 1998; Körner et al., 1998), suspended solids (Valderrama et al., 2002; El-Shafai et al., 2007), and various trace elements (Cd, Cr, Cu, Ni, Pb, and Zn) (Zayed et al., 1998; Wang et al., 2002; Oporto et al., 2006). Some duckweed species are also known to be good B accumulators (Glandon and McNabb, 1978; Frick, 1985).

Know-how and understanding concerning B removal from water by duckweed species is limited. Davis et al. (2002) found that the species Spirodella polyrrhiza did not remove significant amounts of B from solution when cultivated at concentrations between 0.5 and 37 mg B L$^{-1}$. The effect of B concentration in solution on plant growth and plant B accumulation was also studied. The potential use of this species as a phytoremediation system for B removal from wastewater is discussed.

2. Materials and methods

2.1. Site description

The study was conducted from May to October 2002 at the research facilities of the Blaustein Institutes for Desert Research, Kibbutz Sde Boker, located in the Negev Desert, 50 km south of the City of Beer Sheva in Israel. Climatic conditions in the area during this period were mean daily temperature, 25 ± 6°C; mean daily relative humidity, 65 ± 25%; daily maximum global radiation, between 600 and 1054 W m$^{-2}$; and direct radiation, between 370 and 1012 W m$^{-2}$ (information was provided by the Israeli Meteorological Service).

2.2. Stock cultures

Cultures of L. gibba were obtained from a pilot wastewater treatment system, and grown outdoors in 10-L plastic containers filled with half-strength Hoagland’s nutrient solution (Landolt and Kandeler, 1987). In this study, plastic containers and laboratory ware were employed to prevent B contamination from glassware. The duckweed plants were cultivated under these conditions for 2 weeks prior to the beginning of the experiments. The nutrient solution contained 0.25 mg B L$^{-1}$ with a pH ranging from 6.0 to 7.0 and was replaced weekly.

2.3. Experimental setup

Twelve-day batch experiments were conducted to evaluate B removal by L. gibba. The experiments were performed outdoors in 3-L plastic vessels (surface area: 432 cm$^{2}$). A series of tests were carried out to select the range of B concentrations most suitable for L. gibba cultivation. Preliminary observations showed that B concentrations above 10 mg L$^{-1}$ were severely toxic to the plants (data not shown). Therefore, the B concentrations selected were 0.3, 0.6, 0.9, 1.2, 1.5, 1.8, 2.0, 4.0, 6.0, 8.0, and 10.0 mg L$^{-1}$. For each B concentration, three replicates were inoculated with L. gibba (treatments), and three vessels without L. gibba were set as controls. Each replica was filled with 2.5 L of half-strength Hoagland’s nutrient solution, with B added according to the assigned concentrations (in the form of boric acid). The pH of the solutions was adjusted with NaOH to be between 6.0 and 7.0. Every day during cultivation the evaporated volume was replaced with deionized water.

Twenty-five grams fresh weight (FW) of plants was added to each treatment replicate to have an initial plant density of 580 g FW m$^{-2}$. Treatment and control vessels were randomly arranged. On days 3, 6, 9, and 12 of cultivation, after making up the volume, water and plant samples were collected from each replicate for B analysis and the pH of the water was recorded using pH-indicator strips.

2.4. Biomass production measurements

Plant samples were collected with a sieve (surface area = 20.2 cm$^{2}$). Once inside the sieve, the plants were rinsed thoroughly with deionized water, drained and then blotted on paper towels for 2 min. After that, the plants were weighed on an analytical balance. Plant biomass production Pr (g FW m$^{-2}$ d$^{-1}$) was calculated as follows:

$$\text{Pr} = \frac{(\text{FW}_2 - \text{FW}_1)}{\Delta t},$$

where FW$_1$ and FW$_2$ are plant fresh weight (gm$^{-2}$) at time 1 and time 2, respectively, and $\Delta t$ is the difference between time 1 and time 2 (days).

2.5. Boron analysis of plant and water samples

Water samples were filtered (Whatman No. 42 filter) and analyzed for B content by the azomethine-H method (Gupta and Stewart, 1975). Plant samples, after being weighed, were dried at 70°C for 48 h in an oven. Dry plant biomass was ground to a powder with a pestle and mortar. Samples of 0.4 g of ground material were put into porcelain crucibles, and burned in a furnace at 550°C for 2 h. The crucibles were allowed to cool at room temperature. Five milliliters of HCl (1N) was then added to each crucible, and the mixture was allowed to stand for acidic digestion for 15 min. Finally, digested samples were filtered through a Whatman filter No. 42 and subsequently analyzed for B content by the azomethine-H method (Gupta and Stewart, 1975).

The bioconcentration factor (BCF) of B in L. gibba was calculated by dividing B concentration in plant tissues (mg B kg$^{-1}$ dry weight (DW)) at harvest by the initial B concentration in solution (mg L$^{-1}$) (Zayed et al., 1998).

2.6. Quality control and quality assurance

A standard plant sample was purchased from WEPAL, Wageningen University, The Netherlands (carrot leaf, B concentration: 40.5 ± 4.3 mg B kg$^{-1}$ DW). The standard plant sample allowed assessment of the quality and accuracy of our analysis. One crucible containing 0.4 g of the standard plant and an empty crucible, used as a blank control, were
processed simultaneously with the plant samples as described above. Boron concentration of the standard plant in our analysis was $39.5 \pm 3.2$ mg B kg$^{-1}$ DW ($n = 10$). Boron was not detected in the blanks.

2.7. Statistical analysis

The data were tested for equal variance using Levene’s test and for normality using the Shapiro–Wilk $W$ test with a degree of significance of 0.05 (Sall et al., 2001). One-way analysis of variance (ANOVA) was implemented to identify significant differences in B concentration at different times of cultivation ($p < 0.05$). All pairwise mean comparisons were performed using the Tukey–Kramer honestly significant difference (HSD) test with a degree of significance of 0.05 (Sall et al., 2001). Since unequal variances were detected for the biomass production data among different initial water B concentrations, Welch ANOVA was applied to find significant differences between the means ($p < 0.05$). Non-linear regression analysis was conducted for both B removal from water and BCF in relation to initial B concentration in the solutions. All statistical analyses were performed with the software JMP™ 5.0 (Sall et al., 2001).

3. Results

3.1. Plant biomass production

The results of biomass production are shown in Table 1. Mean biomass production for the 12-day cultivation period varied between 37.7 and 51.7 g FW m$^{-2}$ d$^{-1}$ and there were no significant differences between the B concentrations studied ($p > 0.05$). These results give evidence that B concentration in water up to 10 mg L$^{-1}$ does not have adverse toxic effects on L. gibba growth.

3.2. Boron removal

Boron concentration during the cultivation period in both the treatment and control vessels is shown in Fig. 1. In the treatment vessels (with L. gibba), B concentration decreased throughout the cultivation period. This reduction was more significant at lower initial B concentrations (0.3, 1.2, and 1.8 mg B L$^{-1}$). In the treatments with 4, 6, and 10 mg B L$^{-1}$, B concentration decreased up to the ninth day of cultivation, and a slight increase was subsequently observed. Slight variations were observed in B content in the control vessels. The pH of the solution was between 6.0 and 7.0 throughout the entire cultivation period; within this pH range, B is dissolved in the solution as boric acid and readily available for plant uptake (Blevins and Lukaszewski, 1998).

Percent B removal for the 12-day cultivation period decreased logarithmically proportional to the increase in initial B concentration in the water (Fig. 2). Consequently, B removal was most efficient at concentrations below 2 mg BL$^{-1}$.

3.3. Boron accumulation in duckweed plants

The content of B in the plants increased continuously during the experiments in all the B concentrations tested (Fig. 3). Furthermore, B concentration in the plants was observed to rise along with increased initial B concentration in the water. At the end of the 12-day cultivation period, B content in the plants ranged between 900 and 1900 mg B kg$^{-1}$ DW. BCF is an index indicating the plant’s ability to accumulate trace elements (e.g. B) relative to their concentration in the external nutrient solution (Zayed et al., 1998). The BCF values referring to B content in L. gibba fitted a log-log function, depending on initial B concentration in the solution (Fig. 4). Similar to the results of B removal, the highest BCF values (1100–2400) were obtained when the initial B concentration ranged from 0.3 to 1.0 mg L$^{-1}$. At B concentrations between 1.0 and 2.5 mg L$^{-1}$, the BCF decreased to 500, and at B concentrations above 5 mg L$^{-1}$, the BCF values further decreased to less than 300. Under the conditions of this study, bioaccumulation of B by L. gibba was most efficient when water B concentration was less than 2 mg L$^{-1}$.

4. Discussion

The adaptability of the duckweed L. gibba to grow in B-containing nutrient solutions and its capability for B removal were studied over a concentration range of 0.3–10 mg B L$^{-1}$. It was found that L. gibba tolerated a B concentration of up to 10 mg B L$^{-1}$ throughout the 12 days of cultivation. Other

<table>
<thead>
<tr>
<th>Initial B concentration in solution (mg L$^{-1}$)</th>
<th>0.3</th>
<th>0.6</th>
<th>0.9</th>
<th>1.2</th>
<th>1.5</th>
<th>1.8</th>
<th>2.0</th>
<th>4.0</th>
<th>6.0</th>
<th>8.0</th>
<th>10.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duckweed biomass production (g FW m$^{-2}$ d$^{-1}$)</td>
<td>44.4</td>
<td>43.4</td>
<td>37.7</td>
<td>45.4</td>
<td>38.0</td>
<td>40.3</td>
<td>46.0</td>
<td>47.3</td>
<td>48.8</td>
<td>45.6</td>
<td>51.7</td>
</tr>
<tr>
<td>SE$^a$</td>
<td>0.21</td>
<td>0.33</td>
<td>0.31</td>
<td>0.28</td>
<td>0.06</td>
<td>0.28</td>
<td>0.80</td>
<td>1.42</td>
<td>2.86</td>
<td>1.45</td>
<td>1.93</td>
</tr>
</tbody>
</table>

$^a$ SE = standard error, $n = 3$. 

Table 1 – Mean plant biomass production of L. gibba after 12 days of cultivation with different initial B concentrations in solution.
Duckweed species have also demonstrated tolerance to B. Davis et al. (2002) found unaffected growth rates in the duckweed *S. polyrrhiza*, following a 10-day static renewal test with up to 6.1 mg B L$^{-1}$, where above this concentration toxic adverse effects were encountered. Frick (1985) found that *Lemna minor* was tolerant to concentrations of up to 20 mg B L$^{-1}$ after 6 days of exposure. Aquatic plant species can be selected for specific wastewater nutrient removal based on toxicity characteristics (Davis et al., 2002). The tolerance of *L. gibba* to B indicates that this species might be suitable for B removal from water.

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**Fig. 1** – Boron (B) concentration in treatment (■) and control (□) vessels during the cultivation period. The number in each sub-figure indicates the initial B concentration in solution. Error bars represent standard error ($n = 3$). Different letters indicate significant differences in B concentration within the treatment (capital letters) or control (small letters) vessels according to the Tukey-Kramer HSD test ($\alpha = 0.05$).

**Fig. 2** – Percentage of B removal by *L. gibba* after 12 days of cultivation. Error bars represent standard error ($n = 3$).

**Fig. 3** – Boron (B) concentration in *L. gibba* plants during the cultivation period. The number next to each line indicates the initial B concentration in solution. Error bars represent standard error ($n = 3$).

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*Lemma minor* was tolerant to concentrations of up to 20 mg B L$^{-1}$ after 6 days of exposure. Aquatic plant species can be selected for specific wastewater nutrient removal based on toxicity characteristics (Davis et al., 2002). The tolerance of *L. gibba* to B indicates that this species might be suitable for B removal from water.
Selection of adequate wetland plant species for trace element removal is also based on the known accumulation capacities of the different species (Zayed et al., 1998). Our results provide evidence that L. gibba is a good B accumulator as compared with other wetland plants previously reported. In this study, B concentration in L. gibba at the end of the cultivation period ranged from 900 to 1900 mg B kg\(^{-1}\) DW (Fig. 3). In a study by Qian et al. (1999), Hippuris vulgaris was found to be the best B accumulator among 12 species of wetland plants, with a plant B concentration of 1132 mg kg\(^{-1}\) DW when supplied with 1 mg B L\(^{-1}\) for a period of 10 days. Frick (1985) reported that L. minor accumulated 1168 mg B kg\(^{-1}\) DW after 7 days of cultivation in a solution containing 10 mg B L\(^{-1}\). Qian et al. (1999) found that other commonly used wetland plants, such as Mimulus guttatus (monkey flower) and Marsilea drummondii (fuzzy water clover), were moderate B accumulators (700–1000 mg B kg\(^{-1}\) DW), while species including Spartina alterniflora, Juncus xiphioides, Myriophyllum brasiliense, and Pistia stratiotes had a B uptake of less than 500 mg kg\(^{-1}\) DW.

Percent B removal by L. gibba and the BCF values of B in this plant were highest when B concentration in the water was less than 2 mg B L\(^{-1}\). These findings imply that bioaccumulation of B by L. gibba is efficient as a method for B removal from water that contains low B concentrations. At B concentrations higher than 2 mg B L\(^{-1}\), bioaccumulation and B removal are significantly reduced.

This study is one of a few dealing with the efficiency of B removal by duckweed plants. In the study of Davis et al. (2002), no B removal was found with S. polyrrhiza, even at low B concentrations. The fact that B removal by L. gibba is efficient at low B concentrations suggests that the proposed phytoremediation system would be suitable for municipal effluents after primary or secondary treatment, in which B content is commonly in the range of <0.1–2.5 mg L\(^{-1}\), and where B removal by conventional treatment methods is limited (Feigin et al., 1991). However, this system would not be suitable for industrial effluents, such as electric utility wastewater, in which B concentrations may reach more than 40 mg L\(^{-1}\) (Ye et al., 2003).

Removal efficiency of any given trace element can be assessed by the product of plant density and the rate of element accumulation in the plants (Qian et al., 1999). According to our results, the potential B removal of a L. gibba-based treatment system for effluents that contain 2 mg L\(^{-1}\) is 20.5 g B ha\(^{-1}\) d\(^{-1}\). This estimate is based on duckweed cultivation with enriched nutrient solution. For cultivation with wastewater, other variables should be considered, e.g. hydraulic retention time of the system, chemical species of B in the effluent, concentration of other wastewater constituents such as nutrients, organic matter, and total suspended solids, and their chemical interactions with B.

For continuous long-term operation of a L. gibba-based treatment system, adequate harvesting is required to prevent plant overcrowding and settling (Crites et al., 2006). In our experiments, L. gibba biomass nearly doubled after 12 days of cultivation. Accordingly, plant harvesting should be performed approximately once every 2 weeks.

5. Conclusions

The duckweed L. gibba was found to remove B efficiently from water that contains less than 2 mg B L\(^{-1}\). Boron removal efficiency was drastically reduced along with B concentrations above 2 mg B L\(^{-1}\) in the solution. Plant biomass production, over a 12-day cultivation period, was not affected by B concentration up to a concentration of 10 mg B L\(^{-1}\). In addition, we have shown that L. gibba is a good B accumulator as compared with other wetland plants previously investigated. For long-term operation of a L. gibba-based treatment system for B removal, variables such as hydraulic retention time, chemical composition of the effluent, and plant harvesting should be considered.

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