Excess boron (B) is toxic to aquatic organisms and humans. Boron is often present in water with high salinity. To evaluate the potential of duckweed (*Lemna minor* L.) for removing B from water under salt stress, we cultured duckweed in water with 2 mg/L of B and sodium chloride (NaCl) concentrations ranging from 0 to 200 mM for 4 days. The results show that with increasing salinity, the capacity of *L. minor* to accumulate B initially decreased and then increased. *L. minor* used different mechanisms to accumulate boron at lower and higher levels of salt stress. The growth and chlorophyll synthesis of *L. minor* were significantly inhibited when the concentration of NaCl reached 100 mM. Our results suggest that *L. minor* is suitable for the accumulation of B when NaCl salinity is below 100 mM.

Boron (B), an essential element for plant growth, is often present in excessive concentrations in industrial wastewater, mine effluent, and irrigation water. High concentrations of B in water may exert a negative impact on aquatic organisms and can pose a potential health hazard to humans and animals. B removal efficiency is poor in conventional water treatment systems, leading to the development of various specific B removal techniques, including precipitation–coagulation, ion exchange, solvent extraction, ultrafiltration, and adsorption with B-selective resins. Unfortunately, most of these methods are associated with high operation and maintenance costs, as well as the overuse of chemicals. Thus, it is necessary to explore simple, inexpensive, and environmentally friendly technologies for removing B from water.

Duckweed is a small, free-floating aquatic angiosperm that grows throughout much of the world, and it has been considered as a potential candidate for B removal. Previous studies have shown the ability of duckweed to tolerate and remove B under different conditions. For example, *Lemna minor*, a widespread species of duckweed, was shown to tolerate and accumulate B differently under various conditions. Spirodela polyrhiza, another species of duckweed (also called greater duckweed), reportedly had different growth responses to B toxicity at different initial B concentrations. *Lemna gibba*, also a widespread variety of duckweed, was observed to efficiently remove B at concentrations below 2 mg/L. In another study, however, *L. gibba* was found to be suitable for remediating B-contaminated water at B concentrations of 10 and 25 mg/L.

Boron is often found at high concentrations in association with other salts in saline irrigation water. Excess salt in water can decrease the osmotic potential of water, resulting in oxidative stress on plants. Sodium chloride (NaCl) is recognized as the most common salt. Sodium is not essential for plants but is toxic and often induces cellular damage that inhibits plant growth and development. Growth inhibition from salt stress has been reported for several species of duckweeds including *Spirodela polyrhiza*, *L. minor*, and *L. gibba*. The removal of pollutants (e.g., technetium, nickel, and cadmium) by duckweed was observed to decrease under salt stress. However, the influence of salt stress on the ability of duckweed to remove B is still unknown.

The purpose of the present work is to evaluate the performance of the duckweed species *L. minor* in B removal under salt stress. To this end, we cultivated *L. minor* in water with 2 mg/L of B and NaCl ranging from 0 to 200 mM. We tested the changes in B concentration in the water, the accumulation of B, Na, and K in plant tissue, and the growth and chlorophyll synthesis of *L. minor*. On the basis of this research, we evaluated the potential of duckweed as a candidate for removing B from water under salt stress.

**Materials and Methods**

**Plant cultivation.** *L. minor* colonies were isolated from a lake in Xiqing District of Tianjin, China and were cultured aseptically in half-Hoagland’s solution. The plant was acclimated for one week prior to the formal experiment.
Batch experiment. The acclimated *L. minor* colonies were transferred to a polypropylene container (15 × 10.5 × 7.5 cm³) filled with 750 ml of half-Hoagland's solution. Two grams (fresh weight) of duckweed was cultivated in each container. The boron concentration was set at 2 mg/L by adding boric acid (H₃BO₃) with a concentration of 0.25 mg/L as B into half-Hoagland's solution. NaCl was added to the B-laden solution to generate five concentrations: 0, 50, 100, 150, and 200 mM. One container was filled with 750 ml of half-Hoagland's solution (with 2 mg/L of B and without NaCl) and left unplanted to serve as a control. Each treatment was replicated four times. The plant was cultivated in a culture room with a photoperiod of 16:8 and light intensity of 72 µmol/m²/s. During the experiment, the air temperature ranged from 19 to 26 °C. The experiment lasted for four days. Water loss by evapotranspiration was monitored and corrected by weighing the containers each day and adding deionized water.

Water and plant sampling and analysis. Five millilitres of water was collected from each container at time periods of 1, 2, and 4 days for measurement of B. The water samples for B determination were filtered through a 0.45-µm membrane and then diluted to 25 ml with deionized water. Boron concentrations of the water samples were determined using a spectrophotometer (T6, Persee General, Beijing, China), using the method described by Huang et al. Chlorophyll was calculated using the following equations:

\[ C_a = 12.72A_{663} - 2.69A_{645} \]  
\[ C_b = 22.90A_{645} - 4.68A_{663} \]  
\[ C_{chl} = C_a + C_b \]

where \( C_a \), \( C_b \), and \( C_{chl} \) represent the contents of chlorophyll \( a \), chlorophyll \( b \), and total chlorophyll, respectively; \( A_{663} \) and \( A_{645} \) are the absorbances at 663 and 645 nm, respectively.

Each dried sample was separately ground into powder using a mortar and pestle. The ground duckweed samples were digested using a graphite digester (SH220N, Hanon Instruments, Jinan, China) according to Kaur et al. with minor modifications. Aliquots (0.100 g) of ground samples were digested with 5 ml of nitric acid (HNO₃) and 1 ml of 30% hydrogen peroxide (H₂O₂) at 90 °C for 4 h. The digested solution was filtered through a 0.45-µm membrane and then diluted to 25 ml with deionized water. Boron concentrations of the digested solutions and the water samples were determined using inductively coupled plasma-optical emission spectrometry (ICP-OES) (PS-I, Teledyne Leeman Labs, Hudson, NH, USA).

The B mass balance analysis was performed for the treatment system as follows:

\[ B_t = B_i + B_p + B_o \]  
\[ B_i = C_i \times Q_i \]  
\[ B_p = C_p \times W_p \]  
\[ B_s = C_s \times Q_s \]  
\[ B_f = B_i - B_s - B_p \]

where \( B_t \) is the total B (mg) in the cultivation system; \( B_i \) is the water-soluble B (mg); \( B_p \) is the plant-accumulated B (mg); and \( B_o \) is other forms of B (mg). \( B_i \) is the initial B concentration (mg/L) of the water and \( Q_i \) is the initial water volume (L).

\( B_p \) is the B concentration (mg/g) of the plant tissue and \( W_p \) is the dry weight (g) of duckweed.

\( B_s \) is the final B concentration (mg/L) of the water and \( Q_s \) is the final water volume (L). Thus,

Statistics. Four independent replications were used for each treatment, and the error bars are presented as the mean ± standard deviation. Data were analysed using a one-way ANOVA followed by Duncan's multiple range test (p < 0.05).

Results and Discussion

Growth of duckweed. The growth of *L. minor* was inhibited by NaCl, and visible damage appeared in the fronds at high NaCl concentrations. At the end of the experiment, a normal green colour was observed on the fronds of *L. minor* grown at NaCl concentrations between 0 and 50 mM (Fig. 1). Four fronds were observed on most duckweed plants grown at NaCl concentrations between 0 and 50 mM. In the 100 mM NaCl treatment, only two fronds with slight chlorosis were observed on most plants. In the 150 mM NaCl experiment, two fronds and...
a green-yellow color were recorded for most plants. In the 200 mM NaCl treatment, most plants had only one frond and were severely chlorotic and even bleached. In the 100, 150, and 200 mM NaCl treatment, the duckweed roots became very fragile and easily dropped from the fronds. These results are consistent with the previous studies on duckweed under salt stress\(^{16,25}\). The chlorosis is mainly attributed to the salt-induced oxidative damage, which degrades chloroplasts in the duckweed fronds\(^{26}\). The plasma membrane of duckweed can be damaged by severe salt stress, resulting in fragile stipes and fallen fronds\(^{27}\).

The dry weight of duckweed plants decreased gradually with increasing NaCl concentrations (Fig. 2(A)). Compared with the control, however, no significant decrease in the dry weight of duckweed was observed in the plants grown in 50 and 100 mM NaCl solutions. Significant decreases in dry weight were found in the plants grown in 150 and 200 mM NaCl. These results indicate that in terms of biomass accumulation, duckweed is able to tolerate 100 mM NaCl. A previous study reported that *L. minor* grew well in a 62.5 mM NaCl solution, but growth was inhibited in solutions with NaCl concentrations of 125 mM and higher\(^{28}\). We recently observed that the biomass of *L. minor* decreased significantly in solutions with NaCl concentrations of 50 mM and higher\(^{27}\). The higher tolerance of *L. minor* to NaCl can possibly be attributed to the presence of B, which is able to inhibit the uptake and accumulation of Cl\(^{-}\), a major toxic ion in plants\(^{14}\).

To further understand the effect of NaCl stress on the growth of *L. minor*, the content of chlorophyll was determined at the end of the cultivation. As shown in Fig. 2(B), there was no significant difference in the chlorophyll content of the control and the plant grown in a 50 mM NaCl solution. Compared to the control, chlorophyll contents of the plants grown in the 100, 150, and 200 mM NaCl solutions decreased significantly. Excess NaCl can damage the chloroplast envelope and thylakoid through increased production of free radicals, resulting in the decrease of chlorophyll a and b production\(^{29}\). Chlorophyll in *L. minor* reportedly decreases gradually with increasing NaCl and significantly decreases when NaCl reaches 8 g/L (136.9 mM)\(^{25}\). In the present work, plants grown in solutions with NaCl concentrations of 100 mM and higher also showed a significant decrease in chlorophyll content, suggesting that the chloroplasts of *L. minor* were damaged by solutions with 100 mM NaCl concentrations.

**Boron accumulation in duckweed.** Boron concentrations in duckweed tissue were determined after 4 days of cultivation (Table 1). Boron concentrations in duckweed tissue progressively decreased with increasing NaCl concentrations in the 0 to 100 mM concentration range. However, when NaCl concentrations were increased to 150 and 200 mM, B concentrations in *L. minor* increased to the levels measured in the control. B uptake is a passive process via mass flow within the transpiration stream for most higher plants with adequate and excessive B supply\(^{30,31}\). Excess salt lowers the osmotic potential of water and inhibits transpiration, which reduces the absorption of B by the plant\(^{14}\). This mechanism explains the decrease in B uptake by *L. minor* in solutions with NaCl concentrations of 50 and 100 mM. At higher NaCl concentrations, the increase in B accumulation is attributed to damage of the cell membrane of *L. minor* induced by salt stress. The integrity of plant cell membranes is important for the plant to restrict the uptake of excess B\(^{32}\). Salt-induced loss of membrane integrity and an
The growth and B accumulation of *L. minor* were significantly affected by salt stress. Salt stress inhibited the growth and chlorophyll synthesis of *L. minor*, especially in solutions with NaCl concentrations higher than 100 mM. At lower salinities, *L. minor* accumulated B mainly by absorption, which was inhibited by salt stress. At higher salinities, *L. minor* accumulated B mainly by passive diffusion. Our findings suggest that *L. minor* is suitable for removing B from water with low salinity.
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Author Contributions
C.L. proposed the idea designed the experiment and revised the manuscript. W.G., H.J. and Q.Z. carried out the experiments analysed the data, and wrote the draft. Z.D. contributed to drawing Figure 1 analysing the data and polishing the draft. J.L. contributed to the proof-reading of the manuscript.

Additional Information
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