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Research article

Potential of duckweed (*Lemna minor*) for removal of nitrogen and phosphorus from water under salt stress

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ABSTRACT

Duckweed plays a major role in the removal of nitrogen (N) and phosphorus (P) from water. To determine the effect of salt stress on the removal of N and P by duckweed, we cultured *Lemna minor*, a common species of duckweed, in N and P-rich water with NaCl concentrations ranging from 0 to 100 mM for 24 h and 72 h, respectively. The results show that the removal capacity of duckweed for N and P was reduced by salt stress. Higher salt stress with longer cultivation period exerts more injury to duckweed and greater inhibition of N and P removal. Severe salt stress (100 mM NaCl) induced duckweed to release N and P and even resulted in negative removal efficiencies. The results indicate that *L. minor* should be used to remove N and P from water with salinities below 75 mM NaCl, or equivalent salt stress.

1. Introduction

Excess nutrient, primarily anthropogenic nitrogen (N) and phosphorus (P), is one of the major causes of eutrophication in waterbodies. The need to reduce anthropogenic nutrients into aquatic ecosystems to prevent water eutrophication has been widely recognized (Conley et al., 2009). As an eco-friendly method, cultivation of aquatic macrophytes is attractive for nutrient removal and the restoration of eutrophic waterbodies (Dhote and Dixit, 2009).

Compared to other aquatic plants, duckweed proliferates rapidly and has an excellent capacity for nutrient uptake (Xu and Shen, 2011). Additionally, duckweed is able to grow under a variety of climatic conditions and is easier to harvest than other macrophytes (Zirschky and Reed, 1988). Containing high content of protein, fat, amino acid, and starch, the harvested duckweed can be used for the production of animal feeds, fertilizer, and bioenergy products (Mbagwu and Adeniji, 1988; Soda et al., 2015). Due to these advantages, duckweed is considered to be a promising candidate for the remediation of eutrophic water.

In addition to N and P, salt (mainly sodium chloride) is often brought into waterbodies by anthropogenic activities such as agricultural run-off and industrial and domestic discharge (Wang et al., 2008; Kaushal, 2016). It has been reported that salt stress induced oxidative damages and inhibition of photosynthesis in duckweed (Oukarroum et al., 2015). Salt stress was also observed to influence the removal of contaminants by duckweed. For example, the effects of salt stress on the removal of technetium (Hattink et al., 2001), chromium (Boonyapookana et al., 2002), nickel (Yilmaz, 2007), and cadmium (Leblebici et al., 2011) by duckweed have already been demonstrated.

According to our investigation, in some rivers and lakes of coastal areas of China, salinities of water often exceed 3000 mg/L (unpublished data). Wendeou et al. (2013) studied the influence of salinity (up to 2276 mg/L) on the removal of N and P. Unfortunately, few studies have been conducted on the removal of N and P by duckweed under higher salinities. Furthermore, the responses of N and P removal and the growth of duckweed to the durations of salt stress have not been recorded. The purpose of this study was to evaluate the potential of duckweed (*Lemna minor*) in removing N and P from water under different levels and durations of salt stress using a laboratory-scale batch experiment.

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2. Materials and methods

2.1. Plant cultivation

Duckweed (*L. minor*) colonies were collected from Xiqing Lake in Tianjin, China, and were cultured in sterile 1/4 strength Hoagland's solution in a culture room at 25 ± 2 °C with an irradiance of 72 μmol/m^2/s supplied with a 16-h photoperiod.

2.2. Experimental design

Approximately 0.5 g of duckweed colonies were transferred to 150 mL flask containing 100 mL of modified 1/4 Hoagland's solution. The modified solution contained 5 mg N/L [supplied with $Ca(NO_3)_2$ and KNO_3] and 0.5 mg P/L (supplied with KH_2PO_4). NaCl was added to the solution to obtain the following concentrations: 0, 25, 50, 75, and 100 mM. Two batches of cultivation were conducted in different durations: 24 h and 72 h. Each treatment was replicated four times. At the end of the cultivation, duckweed was harvested to obtain fresh weight (FW). Part of the fresh samples was used to determine chlorophyll and permeability of plasma membrane. Water samples were collected for N and P determination. Part of duckweed samples were dried and then ground into powder for total Kjeldahl nitrogen (TKN) and total phosphorus (TP) determination.

2.3. Analytical methods

Water samples were digested with alkaline potassium persulfate in an autoclave at 108 kPa, followed by spectrophotometric determination for total nitrogen (TN) and total phosphorus (TP). Water samples were filtered through 0.45 μm membrane and then determined ammonia nitrogen (NH_4^+), nitrate nitrogen (NO_3^-), and dissolved orthophosphate (PO_4^{3-}) using colorimetric method. Chemical oxygen demand (COD) of water was determined using the Hach test kits with a UV–vis spectrophotometer (DR 1010, Hach Company, Loveland, CO, USA). These water quality parameters were determined following the Standard Methods (APHA et al., 2007). Total Kjeldahl nitrogen (TKN) of duckweed tissue was determined using a Kjeldahl nitrogen analyzer (Kjeltec 8400, Foss Analyzer, Höganäs, Sweden). Total phosphorus (TP) of duckweed samples were determined by molybdovanadate procedure after digesting with concentrated H_2SO_4 and 30% H_2O_2 (Thomas et al., 1967).

Total chlorophyll was determined according to Huang et al. (2007). Fresh duckweed (0.2 g) was soaked in 10 mL of 95% (v/v) alcohol for 3 days at room temperature in dark. The samples were centrifuged at 2790 × *g* for 10 min and the absorbance of the supernatant was determined at 663 and 645 nm. The concentrations of chlorophyll were calculated according to:

$$C_a = 12.72\,A_{663} - 2.69\,A_{645} \quad (1)$$

$$C_b = 22.90\,A_{645} - 4.68\,A_{663} \quad (2)$$

$$C_{chl} = C_a + C_b \quad (3)$$

where C_a, C_b, and C_{chl} represent the content of chlorophyll *a*, chlorophyll *b*, and total chlorophyll, respectively; A_{663} and A_{645} are the absorbance at 663 and 645 nm, respectively.

Plasma membrane permeability of duckweed was indicated by the rate of electrolyte leakage, which was determined by measuring electrical conductivity (EC) of incubating medium according to Yan et al. (1996). Fresh duckweed (0.2 g) was soaked in deionized water at 30 °C for 3 h, and then the EC of water was measured. After the samples were boiled for 2 min and then cooled to 30 °C, the ECs were measured again. The percentage of electrolyte leakage was calculated using the following equation:

$$EC\ (\%) = (C_1/C_2) \times 100 \quad (4)$$

where C_1 and C_2 are the electrolyte conductivities measured before and after boiling, respectively.

The bioconcentration factor (BCF) of N and P was calculated as

$$BCF = C_p/C_w \quad (5)$$

where C_p and C_w are the nutrient concentrations in plant tissue and water, respectively. A larger BCF value implies greater phytoaccumulation capability.

2.4. Statistics

All data were performed by analysis of variance (ANOVA) and the differences were compared by employing the Duncan's test with a significance of $P < 0.05$ using SPSS 20.0 (IBM Corp., Armonk, NY, USA).

3. Results and discussion

3.1. Removal of nitrogen

Duckweed was able to remove NO_3^- and the removal was inhibited by high levels of salt stress. At lower salt stress, duckweed with longer cultivation removed more NO_3^-. At NaCl concentrations ranging from 0 to 75 mM, NO_3^- concentrations decreased markedly compared to the initial concentration (5 mg/L) (Fig. 1A). As expected, after 72 h of cultivation, NO_3^- concentrations were much lower than those after 24 h of cultivation. Despite cultivation time, NO_3^- concentrations increased progressively with increasing NaCl concentrations. At 100 mM NaCl, after 24 h and 72 h of cultivation, NO_3^- increased to 4.83 and 5.87 mg/L, respectively, indicating little to no NO_3^- removal by duckweed.

Nitrate nitrogen uptake may be reduced by the inhibition of duckweed growth and accompanied NO_3^- demand under salt stress, which is to be discussed in the later section. High concentrations of chloride (Cl^-) may compete with NO_3^- directly for the binding sites of the transporter (Cerezo et al., 1997), which is a possible reason for the inhibition. Moreover, the inhibition of nitrate reductase induced by salt stress may cause an over accumulation of NO_3^- and subsequently an increase in efflux, which even results in the increase in NO_3^- concentrations (Ingemarsson et al., 1987). The increase in NO_3^- under severe salt stress (100 mM NaCl) is mainly due to the decomposition of duckweed, which in turn resulted in nitrification, adding NO_3^- to the medium (Suppadit, 2011).

Although NH_4^+ was not supplied, it was still present in the culture water, indicating the generation of NH_4^+ by duckweed. Higher salt stress and longer exposure induced more NH_4^+ generation (Fig. 1B). At lower NaCl concentrations, the generation of NH_4^+ was mainly due to the degradation of nitrogenous organic matters which were exuded from the duckweed roots. Although duckweed had a higher affinity for NH_4^+ than for NO_3^- (Cedergreen and Madsen, 2002), at lower NaCl concentrations (0 and 25 mM), duckweed grew well and took up more NO_3^- because of the high external NO_3^- concentrations (Ingemarsson et al., 1987). After 72 h of cultivation, NH_4^+ concentrations at 75 and 100 mM NaCl increased significantly ($P < 0.05$) and reached 1.71 and 7.54 mg/L, respectively. This was mainly attributed to the breakdown of duckweed tissue, which was degraded by bacteria and exoenzymes, and eventually released NH_4^+.

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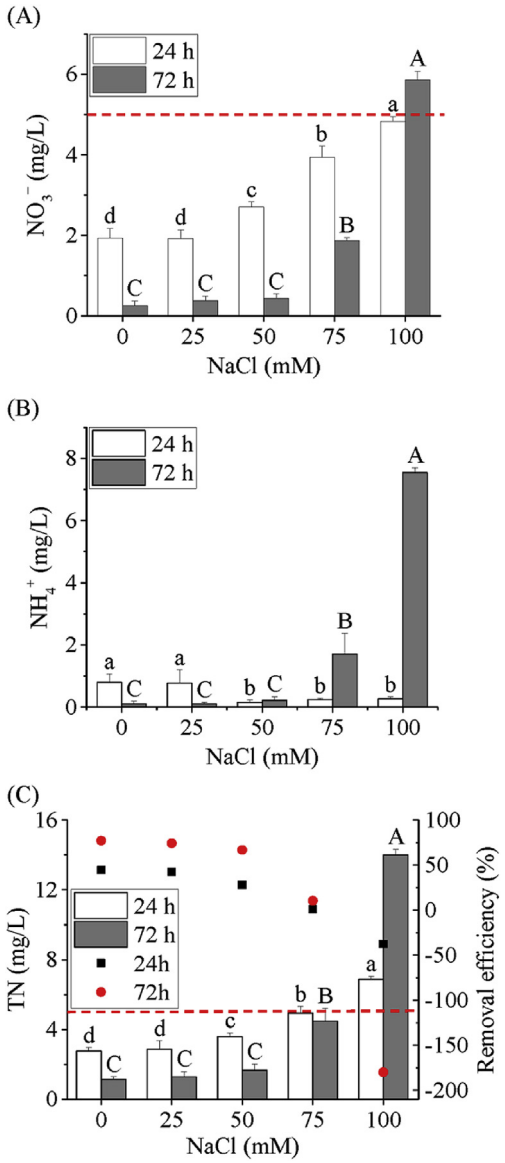

Fig. 1. Effect of NaCl on the removal of N from water by duckweed: (A) Nitrate nitrogen concentration; (B) Ammonia nitrogen concentration; (C) Total nitrogen concentration and removal efficiency of TN. Dash line indicates the initial concentration of N. Values represent average of 4 replicates and error bars represent standard deviations. Means with different letters are significantly different ($P < 0.05$). Lowercase and uppercase letters are for the data of 24 h and 72 h of cultivation, respectively.

Total nitrogen mainly includes NO_3^-, NH_4^+, and organic-N, and thus, the decline of TN means substantial removal of N from water. Similarly to NO_3^- and NH_4^+, the removal of TN was inhibited by salt stress. At 0, 25, and 50 mM NaCl, TN decreased substantially compared to the initial concentration (5 mg/L in the form of NO_3^-), regardless of cultivation duration (Fig. 1C). As expected, after 72 h of cultivation, TN showed more decrease than that after 24 h of cultivation. The concentrations of TN increased with increasing NaCl concentrations eventually exceeded the initial concentration (5 mg/L). At 100 mM NaCl, after 72 h of cultivation, TN was much higher than that after 24 h of cultivation. According to plant element determination, the initial colonies of duckweed contained 50.7 mg TKN/g DW, indicating that duckweed colonies had accumulated excessive N before transferring. According to Bonomo et al. (1997), duckweed was able to excessively accumulate N to the range of 20–60 mg/g DW under N-rich conditions. Mass balance calculations of N suggested that more than 11.3% and 54.5% of N were released from duckweeds which were cultured at 100 mM NaCl for 24 h and 72 h, respectively. This results suggest that the dramatic increase in TN at 100 mM NaCl may have been due to the release of over-accumulated N in duckweed.

To directly evaluate the potential for duckweed in the removal of N under salt stress, the removal efficiencies of TN were calculated. With increasing NaCl concentrations, TN removal decreased progressively (Fig. 1C). At NaCl concentrations ranging from 0 to 75 mM, TN removal efficiencies after 72 h of cultivation were higher than those after 24 h of cultivation. At 100 mM NaCl, regardless of 24 h or 72 h of cultivation, TN removal efficiencies decreased to negative values. The removal efficiencies of TN decreased to −37.5% and −180% after 24 h and 72 h cultivation, respectively. These results suggest that *L. minor* should be used to remove N under salt stress lower than 75 mM NaCl or equivalent salinities.

Bioconcentration factor (BCF) indicates the capacity of a plant to accumulate a substance in the tissue from the medium (generally water) which it is exposed. To evaluate the capacity of duckweed for N accumulation, BCF values were calculated (Table 1). The BCF values of N decreased gradually with increasing NaCl concentrations. The BCF values of 72 h cultivation were greater than those of 24 h cultivation, except when NaCl reached 100 mM. These results demonstrate that the accumulation of N for duckweed is inhibited by salt stress.

3.2. Removal of phosphorus

Duckweed reduced P concentrations, but the removal was inhibited under higher salt stress. Additionally, longer cultivation showed a much better performance in P removal. Orthophosphate and TP showed similar values and tendencies, indicating PO_4^{3-} was the main component of TP (Fig. 2A and B). At NaCl concentrations ranging from 0 to 50 mM, compared with the initial P concentration (0.5 mg/L), the concentrations of PO_4^{3-} and TP both decreased. The decrease after 72 h of cultivation was much more than that after 24 h of cultivation. At 75 mM NaCl, both PO_4^{3-} and TP concentrations after 24 h of cultivation increased significantly ($P < 0.05$) and exceeded the initial concentration. At 100 mM NaCl, PO_4^{3-} and TP increased to much higher levels, and their concentrations after 72 h of cultivation even exceeded those after 24 h of cultivation. Plant element determination showed the initial P of duckweed was 21.3 mg/g DW, which was in the range of P contents previously recorded as 2–29 mg/g DW in P-rich water (Bonomo et al., 1997). According to mass balance calculation of P, more than 18.2% and 45.7% of P were released from duckweed cultured in 100 mM for 24 h and 72 h, respectively. These results suggest that considerable amounts of phosphorus stored in duckweed will be released under severe salt stress.

Table 1
Bioconcentration factor (BCF) of duckweed for N and P.

NaCl (mM)	BCF_N ($\times 10^4$)		BCF_P ($\times 10^4$)	
	24 h	72 h	24 h	72 h
0	1.84 ± 0.13 a	4.48 ± 0.59 a	6.73 ± 0.56 b	88.28 ± 45.19 b
25	1.80 ± 0.28 a	4.07 ± 0.86 ab	8.06 ± 1.62 a	151.69 ± 25.69 a
50	1.41 ± 0.08 b	3.16 ± 0.47 b	7.71 ± 0.63 ab	83.51 ± 42.46 b
75	1.03 ± 0.08 c	1.15 ± 0.20 c	2.79 ± 0.39 c	11.03 ± 1.96 c
100	0.74 ± 0.02 d	0.36 ± 0.01 c	1.21 ± 0.05 d	0.58 ± 0.04 c

BCF_N and BCF_P represent the BCF of duckweed for N and P, respectively. The values represent mean of 4 replicates ± standard deviation. Means followed by the same letter in the same column are not different significantly according to Duncan's multiple comparison test at a level of $P < 0.05$.

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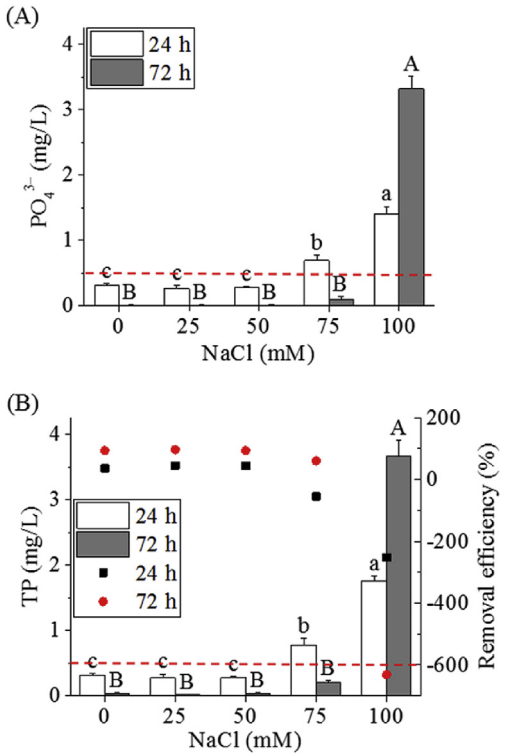

Fig. 2. Effect of NaCl on the removal of P from water by duckweed: (A) Orthophosphate concentration; (B) Total phosphorus concentration and removal efficiency of TP. Dash line indicates the initial concentration of P. Means with different letters are significantly different ($P < 0.05$). Lowercase and uppercase letters are for the data of 24 h and 72 h of cultivation, respectively.

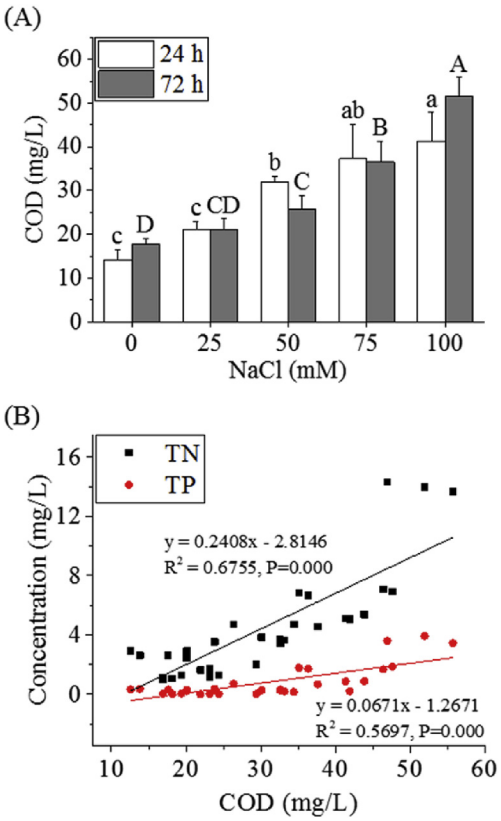

Fig. 3. Effect of NaCl on COD generation (A) and the relationship between concentration of COD and TP and TN, respectively (B). Means with different letters are significantly different ($P < 0.05$). Lowercase and uppercase letters are for the data of 24 h and 72 h of cultivation, respectively.

Removal efficiencies of TP were calculated and shown in Fig. 2B. With NaCl concentrations varying from 0 to 50 mM, removal efficiencies of TP after 72 h of cultivation were much higher than those after 24 h of cultivation. At 75 mM NaCl, TP removal efficiencies after both terms of cultivation decreased markedly, and those after 24 h of cultivation even decreased to negative level (−54.9%). At 100 mM NaCl, TP removal efficiencies after 24 h of cultivation decreased to −251%, and those after 72 h of cultivation even sharply decreased to −631.6%. These results suggest that long-term cultivation at extremely high concentrations of NaCl (e.g., 100 mM) caused dramatic reduction in P removal efficiencies even to negative values.

It has been observed that P uptake by freshwater aquatic plant was inhibited by salt stress in previous studies. For example, P uptake has been recorded to decrease significantly in *Hydrilla verticillata*, *Myriophyllum spicatum*, and *Vallisneria americana* under salinity of 6‰ (~103 mM NaCl), 12‰ (~205 mM NaCl), and 12‰, respectively (Twilley and Barko, 1990). Even for salt-tolerant marine algae (e.g., *Ulva pertusa*), P uptake has also been observed to decrease when salinity exceeds 25 psu (~428 mM NaCl) (Choi et al., 2010). An explanation for the inhibition in P uptake is the decrease in plant growth and P demand that was induced by salt stress. Another possible explanation is the competition between chloride (Cl^-) and PO_4^{3-} for the transport site of cell-surface (Navarro et al., 2001). Besides plant uptake, P was also removed by adsorption onto the biofilms attached to duckweed and the container walls (Körner et al., 2003). The adsorption may also be influenced by water salinity, but the related mechanisms remain unclear.

The BCF of P for duckweed was calculated and shown in Table 1. In both 24 h and 72 h cultivation, the BCF values of P increased at 25 mM NaCl. The increase in P accumulation can be explained by the increase in internal P requirement (Awad et al., 1990). The BCF values of P began to decrease at 50 mM NaCl, and decreased to an extreme low level at 75 and 100 mM NaCl. These results demonstrate that P accumulation by duckweed is facilitated by slight salt stress but inhibited by severe salt stress.

3.3. Organic compounds generation

To investigate the content of organic compounds in water generated by duckweed, COD of water samples was determined. The data showed that COD was generated regardless of salt stress and cultivation time (Fig. 3A). The rapid generation of COD in water is mainly attributed to the release of dissolved organic matters of low molecular weights (<1000 Da) (Baker and Farr, 1987). The major components of the dissolved organic matters may be extracellular polymers, which are easily induced by environmental stresses (Babel et al., 2002). Therefore, COD increased progressively with increasing NaCl concentrations. Through the decomposition of organic compounds, nutrients (mainly N and P) release into the water (Szabó et al., 2000), resulting in the increase in N and P. When the concentrations of TN and TP were plotted against COD, respectively, positive linear correlations were observed (Fig. 3B). Moreover, there is a high degree of correlation between TN and COD ($R^2 = 0.6755$, $P = 0.000$), as well as TP and COD ($R^2 = 0.5697$, $P = 0.000$). These results suggest that the organic N and P induced by salt stress made a significant contribution to the increase in TN and TP.

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3.4. Duckweed growth

With increasing NaCl concentrations, the numbers of fronds of individual duckweed decreased, regardless of cultivation time (Fig. 4). In general, a healthy duckweed colony has 3 or 4 fronds. Previous studies have reported that salt stress can reduce the total number of duckweed frond (Chang et al., 2012; Wendeou et al., 2013). The decrease of frond number of individual duckweed observed in the present work was due to the fragile stipe caused by salt damage. Regardless of NaCl concentrations, after 24 h of cultivation, most fronds looked green and vigorous. In contrast, after 72 h of cultivation, most fronds at 50 mM and higher NaCl concentrations exhibited partially transparent and chlorotic effect. At 100 mM NaCl, the fronds were almost bleached after 72 h of cultivation. The signs of chlorosis were attributed to the loss of photosynthetic pigments, and these results were in agreement with the observations in previous studies (Chang et al., 2012; Cheng et al., 2013).

Chlorophyll content of duckweed decreased with increasing NaCl concentrations (Fig. 5A). This is in corresponding with the variation of frond color presented in Fig. 4. After 72 h of cultivation, chlorophyll contents of duckweed were much lower than those after 24 h of cultivation, which was due to the higher accumulative rates of frond biomass than those of chlorophyll contents. At 50 mM NaCl and higher concentrations, chlorophyll contents decreased significantly ($P < 0.05$), and the chlorophyll contents after 72 h cultivation decreased much more than those after 24 h of cultivation. The loss in pigment contents could be due to the direct oxidative breakdown of pigments and/or the destruction of thylakoid pigment-protein complexes in photosynthetic reaction centers (Chang et al., 2012). Obviously, higher NaCl concentrations and longer exposure time exerted more damage to the synthesis of chlorophyll.

Fresh weight of duckweed decreased with increasing NaCl concentrations (Fig. 5B). Regardless of cultivation time, no inhibitive effect was observed on the growth of duckweed at 25 mM NaCl. At 50 mM and higher concentrations of NaCl, duckweed FW decreased significantly ($P < 0.05$). The reduction of duckweed FW under high salinities is mainly attributed to: (1) the inhibition in biomass accumulation, including the restriction of root expansion and the reduction in frond number (Chang et al., 2012); (2) the breakdown of fronds and roots; and (3) the leakage of electrolyte.

As compared with the initial FW (0.5 g for each flask), duckweed FW showed almost no increase or negative increase at 50 mM NaCl and higher concentrations. This result suggests that duckweed has a lower tolerance to NaCl than that reported by Sikorski et al. (2013). In their study, duckweed (*L. minor*) showed a negative growth rate when NaCl exceeded 250 mM. The growth of other species of duckweed have also been reported to be inhibited by salt stress. For example, *Spirodela polyrhiza* showed negative relative growth rate at 100 mM and higher salinities (Leblebici et al., 2011). Chang et al. (2012) reported that the growth of *S. polyrhiza* completely ceased at 200 mM NaCl. *Lemna gibba* showed negative growth when salinities exceeded 250 mM (Yilmaz, 2007).

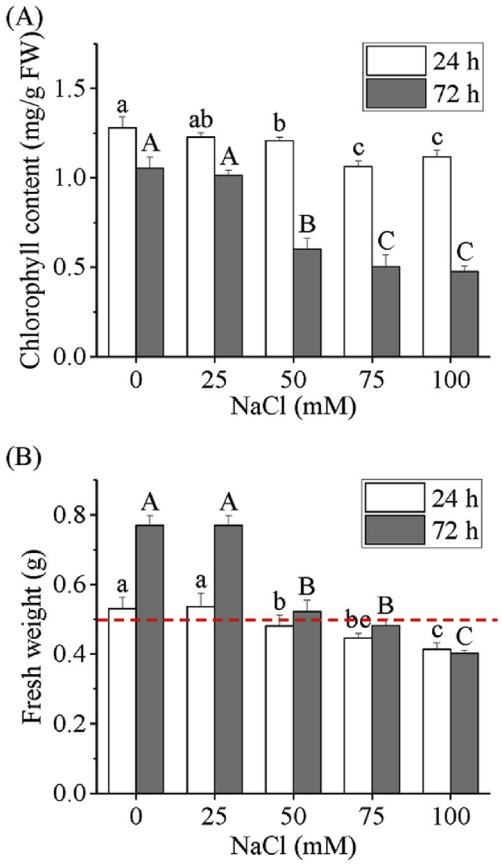

Fig. 5. Effect of NaCl on chlorophyll synthesis and biomass accumulation of duckweed: (A) Chlorophyll content; (B) Fresh weight of duckweed (dash line indicates the initial FW of duckweed). Means with different letters are significantly different ($P < 0.05$). Lowercase and uppercase letters are for the data of 24 h and 72 h of cultivation, respectively.

3.5. Membrane permeability

To investigate the damage of NaCl stress to the plasma membrane of duckweed, electrical conductivity (EC) of incubating medium of duckweed samples was determined (Fig. 6A). Electrical conductivity of incubating medium has been used as an index of

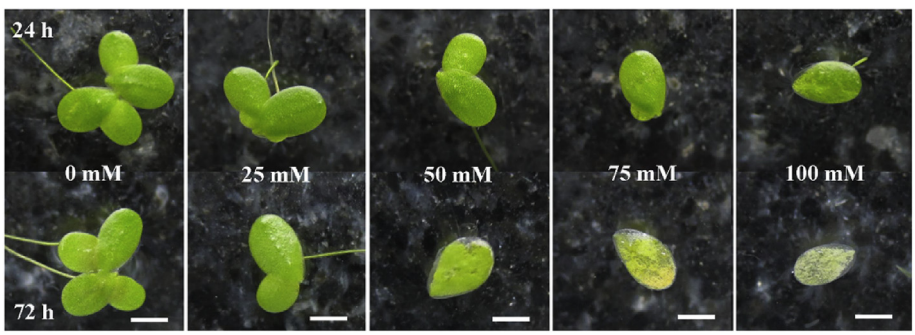

Fig. 4. Effect of NaCl on fronds of duckweed (Bar = 2 mm).

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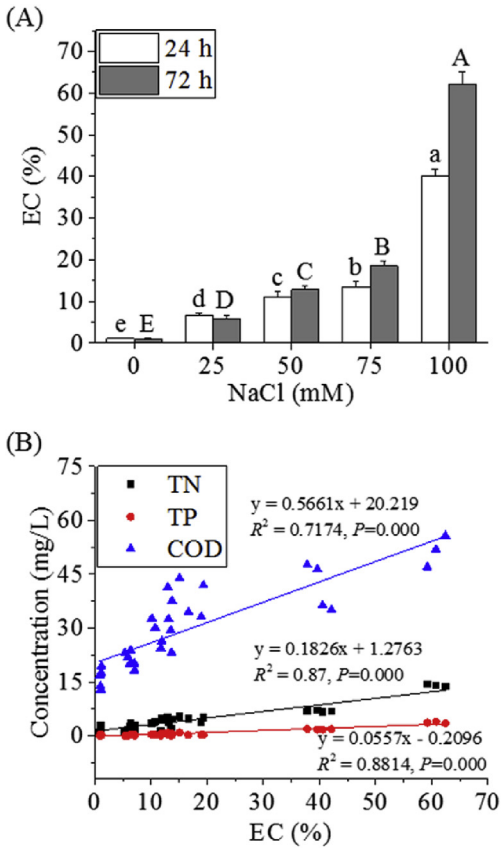

Fig. 6. Effect of NaCl on membrane permeability (A) and the relationship between membrane permeability and TP, TN, and COD, respectively (B). Means with different letters are significantly different ($P < 0.05$). Lowercase and uppercase letters are for the data of 24 h and 72 h of cultivation, respectively.

membrane permeability of damaged plant tissue, which leaks electrolyte with charged inorganic or organic molecules (Bajji et al., 2002). The data show that EC increased progressively with increasing NaCl concentrations, indicating the membrane permeability of duckweed was dependent on salt stress. At 0 and 25 mM NaCl, EC was present at the same level. At 50 mM and higher concentrations of NaCl, EC increased dramatically, especially for that after 72 h of cultivation. This result suggests that under higher salt stress, the damage to plasma membrane of duckweed was dependent on exposure time.

Plasma membrane may be the primary site of salt injury. Thus, it is important for plasma membrane to be less susceptible and maintain its low permeability under high-salt conditions (Mansour, 2013). Previous studies have shown that high salinity increased plant plasma membrane permeability. For example, Ismail (2003) found that 50 mM salinity significantly increased the membrane permeability of maize and sorghum. Panda and Upadhyay (2004) observed the lipid peroxidation and the loss of membrane integrity of duckweed (*L. minor*) under the stress of NaCl ranging from 50 to 200 mM. However, few studies on membrane permeability of duckweed that influenced by salt stress have been conducted. In the present work, membrane permeability was estimated indirectly with electrolyte leakage, which consists of K^+ and so-called counterions (Cl^-, HPO_4^{2-}, NO_3^-, citrate^{3-}, and malate^{2-}) (Demidchik et al., 2014). Some of these ions contributed to TN, TP, and COD in water. When the concentration of TN, TP, and COD (regardless of treatment) was plotted against EC, significant correlations between TN ($R^2 = 0.8700$, $P = 0.000$), TP ($R^2 = 0.8814$, $P = 0.000$), and COD ($R^2 = 0.7174$, $P = 0.000$) and EC were observed respectively (Fig. 6B). Total nitrogen, TP, and COD all increased linearly with increasing EC. Throughout the range of EC, COD showed greater variation than TN and TP. This result suggests that the injury of plasma membrane caused by salt stress may have more contribution to the generation of organic compounds (COD) than TN and TP.

3.6. Implications for application in saline water

According to our work, duckweed should be used carefully for purifying water with high salinities. The tolerable salinities and suitable cultivation time (i.e., harvest frequency) need to be taken into consideration. Besides N and P, the influence of salinity on the removal of other pollutants especially for organic compounds needs to be investigated. Recently, duckweed also showed the capacity of desalination of water by the uptake of dissolved salt (Balla et al., 2014), which confirmed duckweed as a promising candidate for saline water purification.

In addition to *Lemna*, duckweed family consists of several other genera including *Spirodela*, *Landoltia*, *Wolffiella*, and *Wolffia* (Crawford et al., 2006). According to previous studies, the tolerance of different species of duckweed to salt stress are quite different. Consequently, the performance of duckweed for N and P removal under salt stress may be different. Salt tolerance of duckweed has been improved by overexpression of the *Arabidopsis* photorespiratory pathway gene (AtAGT1) (Yang et al., 2013). These transgenic duckweed colonies are likely to be used to remove N and P under severe salt stress.

4. Conclusions

Our research showed that salt stress inhibited N and P removal by *L. minor*. Higher salt stress exerted more inhibitive effects on N and P removal. High-salt stress severely injured duckweed and reduced removal efficiencies of N and P even to negative levels. Longer cultivation time helped duckweed remove more N and P under low-salt stress and, conversely, induced more reduction in removal efficiencies under high-salt stress. Our results suggest that *L. minor* should be used to remove N and P from water with salinities below 75 mM NaCl or equivalent salt stress.

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