Desalination and Water Treatment
www.deswater.com
doi: 10.1080/19443994.2015.1048736

57 (2016) 12037–12046
June

Taylor & Francis
Taylor & Francis Group

Experimental comparisons of three submerged plants for reclaimed water purification through nutrient removal

Xiaoqin Zhou, Zifu Li*, Ruixue Zhao, Ruiling Gao, Yupan Yun, Mayiani Saino, Xuemei Wang

Beijing Key Laboratory of Resource-oriented Treatment of Industrial Pollutants, School of Civil and Environmental Engineering, University of Science and Technology Beijing, Beijing 100083, P.R. China, Tel./Fax: +86 10 62334378; emails: zhouxiaoqin025@163.com (X. Zhou), zifulee@aliyun.com (Z. Li), 642823393@qq.com (R. Zhao), gaoruiling1988@126.com (R. Gao), yun_19850601@163.com (Y. Yun), enolesaino@gmail.com (M. Saino), wangxuemei0000@126.com (X. Wang)

Received 15 September 2014; Accepted 26 April 2015

ABSTRACT

Submerged aquatic plants have attracted increasing attention as an advanced component for wastewater treatment. This study performed a laboratory-scale investigation using three submerged plants, namely *Myriophyllum verticillatum*, *Potamogeton perfoliatus*, and *Najas minor* to determine the response of submerged plants to seasonal changes and intermittent reclaimed water amendment. The three plants were pre-selected from seven commonly used submerged plants. Reclaimed water from a wastewater reclamation plant was used as raw water. The total phosphorus (TP), total nitrogen (TN), ammonium, chemical oxygen demand, and dissolved oxygen were monitored during the experiments to investigate their resistance to changes in water conditions. With seasonal changes from April to June, the water temperature ranged between 19 and 23˚C, and the TN and TP removal efficiencies were negatively affected. The highest TN and TP concentrations in the tanks with *M. verticillatum* and *P. perfoliatus* were reached on day 14. However, *N. minor* exhibited sustained growth, and the water quality remained in good condition. The TN and TP removal efficiencies were 81 and 62%, respectively. In addition, the intermittent reclaimed water amendment tests on the three plants yielded similar results. The TN and TP removal efficiencies of *N. minor* were 55 and 93%, respectively. *N. minor* proved to be a promising plant for water purification.

Keywords: Landscape ponds; Purification efficiency; Reclaimed water; Submerged plants

1. Introduction

Reclaimed water as supplement water or full water resource for urban lakes and rivers is a good remedy to the water crisis being faced by many water-scarce regions. However, maintaining the self-purification capacity of a water ecosystem is crucial when water is reused as scenic water in case of water eutrophication and secondary pollution. A promising technology is the planting of macrophytes, which convert sunlight energy and chemical elements for living plants and replenish the aquatic environment with dissolved oxygen (DO). This process is an effective, low-cost, and environmentally friendly way of keeping a water body healthy.

*Corresponding author.

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Macrophytes play important roles in a water system. On the one hand, they regulate water ecological circulation by absorbing nutrients from both sediment and water to maintain a balance between the output and input of nutrients, such as nitrogen and phosphorus. On the other hand, macrophytes increase the diversity of aquatic organisms. Macrophytes have been used for water purification in nitrogen removal and phytoremediation of heavy metals for many years [1,2]. The three macrophyte species that serve diverse functions in water purification systems are as follows: free-floating macrophytes (e.g. *Eichhornia crassipes*), entire plant body, except the roots, is above water; submerged macrophytes (e.g. *Hydrilla* sp.), whole plant body remains submerged in water; and emergent macrophytes (e.g. *Typha* sp.), plants are rooted in soil but emerge to significant heights above the water [3]. Water hyacinth is a commonly used floating macrophyte that can remove more than 91% of Kjeldahl nitrogen, 99% of ammonium (NH_4^+–N), and more than 98% of total phosphorus (TP) [4]. Emergent macrophytes are widely used in free water surface constructed wetlands, sub-flow constructed wetlands, and aerobic ponds. In these systems, the plants reduce wind speed and thus support sedimentation and prevent re-suspension. They also provide substrate for periphyton and bacteria, as well as absorb nutrients and provide carbon for denitrification during biomass decomposition in carbon-limited systems [5]. Reed has strong roots, is easy to manage, and is thus commonly used as reed bed for domestic wastewater treatment; reed bed is effective in the removal of biological oxygen demand, suspended solids, and pathogenic organisms [6]. *Myriophyllum verticillatum*, *Potamogeton perfoliatus*, *Najas minor*, and *Hydrilla* are frequently used submerged macrophytes in ecological engineering [7]. Submerged macrophytes can absorb nitrogen and phosphorus dissolved in sediment and water through their roots and bodies; thus, submerged macrophytes have larger nitrite removal capacities than emergent macrophytes [8]. In addition, submerged macrophytes assume their photosynthetic function below the surface and accumulate DO in water because these plants are partly suspended in water and partly rooted in bottom sediment; thus, submerged macrophytes provide a conducive reactivation condition for nitrification [9,10]. Submerged macrophytes can obviously reduce the turbidity and prevent re-suspension of sediment [11,12]. Moreover, submerged macrophytes evidently inhibit algal growth because of the competition between these organisms as a result of their similar ecological living conditions.

The growth of macrophytes mainly depends on nutrient supply and temperature, and different species respond differently to environmental changes [13–15]. The effects of surrounding environmental changes on macrophytes and water quality warrant evaluation. Alkaline phosphorus activity in sediment and soluble reactive phosphorus concentration of overlying water are significantly affected by the addition of organic matter and submerged plants, which play important roles in phosphorus release and transportation [16]. In submerged plant treatment systems, nitric oxide enhances NH_4^+–N-induced toxicity and improves the antioxidant capacity of *Hydrilla verticillata* [17]. Five submerged macrophytes showed different capacities to remove phosphorus from the eutrophic lake Donghu in spring and autumn; the removal performance observed in spring was arranged as *Ceratophyllum demersum* > *Elodea Canadensis* > *Potamogeton crispus*, whereas that in autumn was arranged as *C. demersum* > *Vallisneria spiralis* > *Myriophyllum spicatum* [18]. In this study, *C. demersum* was the best functioning plant throughout the year. Senesced submerged plant community was suspected to contribute 40% of nitrogen export with the release of organic and inorganic nitrogen. Therefore, optimal management of macrophytes is important to retain its function in the water body [19]. Harvesting may be an effective way to remove nutrients from the water body [20]. However, macrophytes could also die when they cannot adapt to the surrounding environment. Hence, improper management of submerged macrophytes and decomposition of these plants could cause nitrogen release and secondary pollution. Therefore, proper control and management of submerged macrophytes is critical, especially during seasonal changes.

The Beijing Olympic Park is the heartland for the 29th Olympic Games. A landscape river named "dragon-shaped landscaping water system" runs through the whole park, which starts at the Olympic Forest Park and ends around the National Stadium (The Bird Nest). The water system consists of nine ponds that are connected by underground culvert. The nine ponds are simply named from W1 to W9 in accordance with the flow direction, and W1 has the influent pipe for reclaimed water. This water system is one of the largest artificial waterscapes that use reclaimed water from a wastewater reclamation plant as the sole water resource. Thus, the daily maintenance and control of eutrophication and bloom in this system are challenging, as evidenced in the past years. Macrophytes such as *Vallisneria natans*, *M. verticillatum*, and *Typha orientalis* were planted in this water body.

However, the proper management of such an ecological engineering project is essential to maintain good water quality. Therefore, identifying the roles of plants in the system is important for appropriate management. Accordingly, the present study conducted a laboratory-scale experiment to investigate the purification rules of the plants. Macrophytes were planted and investigated individually. Three submerged plants, namely *M. verticillatum*, *P. perfoliatus*, and *N. minor*, were selected by pre-investigation. The three species were expected to respond differently to changes in temperature and nutrient loading. This study aims to assess the response of submerged macrophytes to temperature increase caused by seasonal change and intermittent reclaimed water amendment with special attention to total nitrogen (TN) and TP removal. The results of this study can serve as a basis for landscape water management on submerged plants in the dragon-shaped landscaping water system and can be replicated to other projects.

2. Materials and methods

2.1. Aquaculture system

The reclaimed water purification experiment was launched using culture tanks (0.6 m × 0.6 m × 0.6 m). The tank contained a 20-cm layer of local soil at the bottom and a depth of around 0.5 m above the soil layer. The impact of the soil was ignored because the local soil was collected from the same sampling point of the dragon-shaped landscaping water system bank for all tanks.

Seven submerged plants (*V. natans*, *M. verticillatum*, *P. perfoliatus*, *Potamogeton malaianus*, *Elodea nuttallii*, *P. crispus*, and *N. minor*) were planted in separate culture tanks. The initial water quality of the water sample is presented in Table 1 (KH_2PO_4 was added into the water to maintain TP concentration at a normal level of approximately 1 mg/L). All plants were obtained from the macrophyte system of the dragon-shaped landscaping water system. The plants were mature in size and good condition because they had been domesticated for 60 d before being used in the experiment.

M. verticillatum, *P. perfoliatus*, and *N. minor* were selected from the seven submerged plants and classified into two groups. For group one, reclaimed water was fed on 1 April and then operated for two months to evaluate its quality variation with temperature increase. For group two, 120 L of reclaimed water was added to the water tank in the beginning. To balance the evaporation loss of the experimental tanks, the reclaimed water was used as supplement water in the following experimental period, such that the water quality was similar to that of the initial reclaimed water. This experiment was evaluated for a month. The initial water quality for the reclaimed water samples is presented in Table 1.

2.2. Sampling and analysis

Water samples were collected approximately once a week from the culture tanks, and sampling was usually conducted at around 11:00 am on each sampling day. The samples were analyzed for TN, NH_4^+–N, TP, and chemical oxygen demand (COD_{cr}). All analytical measurements were conducted in accordance with the Inspective and Analytical Methods of Water and Wastewater (fourth edition). The temperature, pH, and DO of water in the sampling locations were detected using automatic machines HI9125 and Oxi330i/SET, respectively.

All experiments were performed in triplicates and data were calculated based on the mean value of three replicates. Test results were drawn by origin for each set of data; mean values of removal rates were compared between different treatments using one-way analysis of variance. Significance was accepted when $P \le 0.05$.

Table 1
Initial values of the main parameters of reclaimed water

Parameters	Test A	Test B	Test C
TN (mg/L)	13.10 ± 0.50	5.02 ± 0.50	9.18 ± 0.15
TP (mg/L)	1.00 ± 0.03	0.072 ± 0.0003	0.095 ± 0.0005
NH_4^+–N (mg/L)	–	0.77 ± 0.13	1.21 ± 0.20
CODcr (mg/L)	50.64 ± 8.98	38.15 ± 2.50	33.30 ± 3.89
DO (mg/L)	7.50 ± 0.05	7.01 ± 0.50	6.93 ± 0.40
pH	8.20 ± 0.10	8.93 ± 0.06	7.80 ± 0.05

Notes: A refers to the water samples in the seven culture tanks; B refers to the water samples used in three culture tanks, group one; and C refers to the water samples used in three culture tanks, group two.

3. Results

3.1. Performance of seven submerged plants

Fig. 1 shows the TN removal efficiencies of the seven submerged macrophytes. All of the macrophytes exhibited significant TN removal efficiencies within two months. *N. minor* and *P. crispus* achieved TN removal efficiencies of over 90%. After two months, the TN concentrations in the seven culture tanks were arranged as *N. minor* (0.83 mg/L) < *P. crispus* (1.02 mg/L) < *E. nuttallii* (1.80 mg/L) < *P. Malaianus* (2.90 mg/L) < *M. verticillatum* (2.92 mg/L) < *P. perfoliatus* (2.98 mg/L) < *V. natans* (5.42 mg/L), with removal efficiencies of 91, 90, 82, 71.8, 71.6, 71, and 47%, respectively. In addition, the TN content in the tanks with *N. minor* satisfied the third-class standard of the Environmental Quality Standards for Surface Water (GB3838-2002) (TN ≤ 1.0 mg/L).

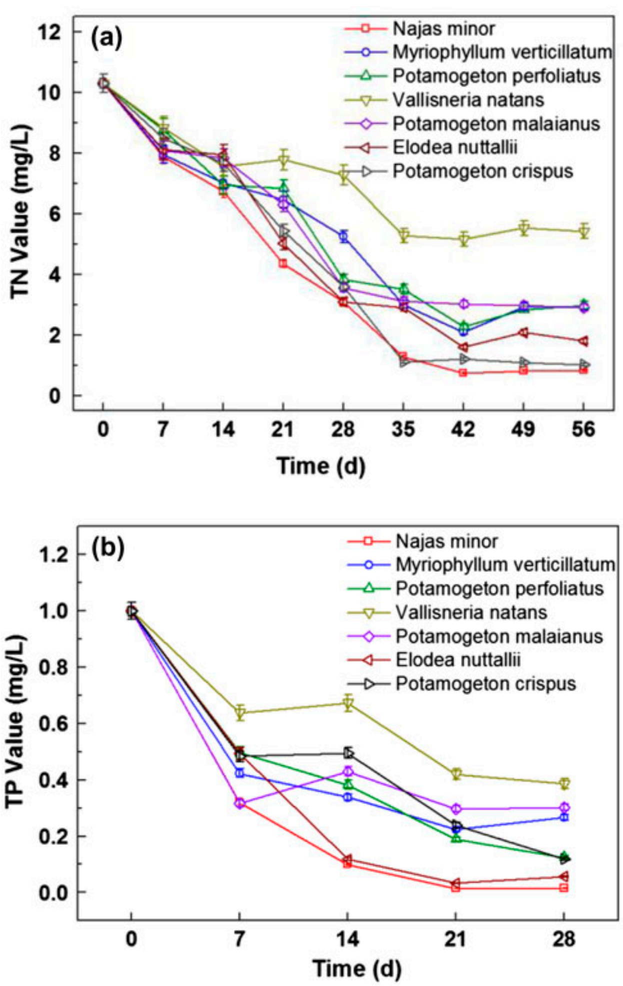

Fig. 1. (a) TN and (b) TP concentrations in seven tanks planted with individual submerged macrophytes.

For TP removal, a remarkable decline in TP concentration within 30 d was observed, which indicated the efficiency of the submerged macrophytes. The final TP concentrations in the seven tanks were arranged as *N. minor* (0.015 mg/L) < *E. nuttallii* (0.056 mg/L) < *P. crispus* (0.119 mg/L) < *P. perfoliatus* (0.125 mg/L) < *M. verticillatum* (0.267 mg/L) < *P. malaianus* (0.302 mg/L) < *V. natans* (0.386 mg/L), with removal rates of 98, 94, 88, 87, 73, 69, and 61%, respectively. Moreover, the TP concentration in the tanks with *N. minor* satisfied the second-class standard of the Environmental Quality Standards for Surface Water (GB3838-2002) (TP ≤ 0.025 mg/L).

On the basis of both nutrient removal efficiency and plant growth condition, *M. verticillatum*, *P. perfoliatus*, and *N. minor* were selected as test plants in the following investigation on seasonal change and nutrient variation.

3.2. Nutrient removal in group one

During the study period, the temperature ranged between 19 and 23˚C, and the pH varied between 6 and 9. Variations in the levels of TN, NH_4^+–N, and TP in the three tanks are presented in Fig. 2. *N. minor* was the most active plant among the three plants because it achieved more than 80% TN removal. This result indicated that a suitable living environment was formed during seasonal changes and had sustainable purification ability to the water. The TN concentrations in *M. verticillatum* and *P. perfoliatus* increased. The highest TN concentrations of 10.12 and 12.57 mg/L were recorded on day 14. Then, this parameter began to slowly decrease from day 15 and then stabilized. This phenomenon indicated that the nitrogen released by plant decomposition was less than the consumption by absorption and sediment. Thus, harvest is important during secondary pollution [16]. The final TN value for *N. minor* was 0.95 g/L, which satisfied the third-class standard of the Environmental Quality Standards for Surface Water (TN ≤ 1.0 mg/L).

Plants are influenced by seasonal change. To obtain detailed insights, NH_4^+–N was measured and presented. This parameter reflects the decomposition of organic nitrogen by micro-organisms in water [21]. For *N. minor*, NH_4^+–N concentration was approximately 0.55 mg/L, indicating stable micro-organism activities in the system. By contrast, the corresponding values for *M. verticillatum* and *P. perfoliatus* increased and then decreased toward the 50-d operation. NH_4^+–N is an inorganic nitrogen form that can be directly absorbed by plants. Variations in this parameter reflects plant growth inhibition and decreases TN removal efficiency. Meanwhile, no significant variation

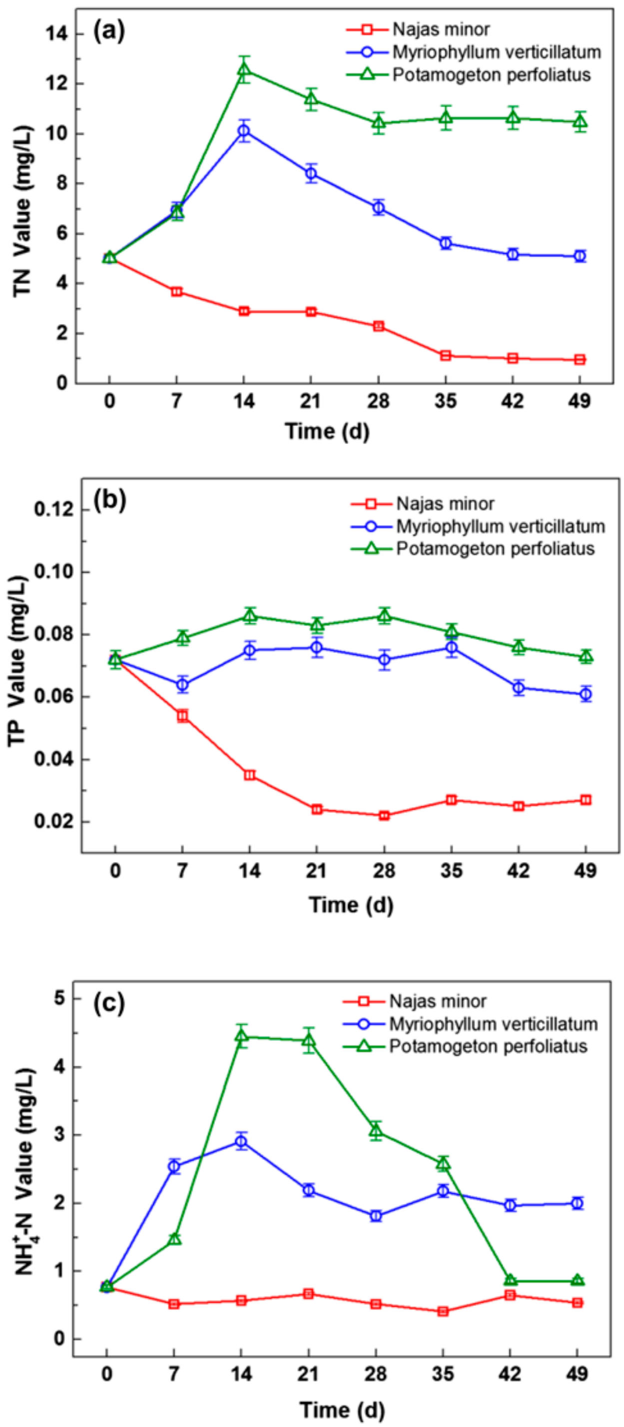

Fig. 2. (a) TN, (b) TP, and (c) NH_4^+–N concentrations of the three culture tanks in group one.

in TP was detected in the culture tanks planted with *M. verticillatum* and *P. perfoliatus* (Fig. 2). However, *N. minor* significantly reduced TP by more than 62%, i.e. from 0.072 to 0.027 mg/L, which satisfied the second-class standard of the Environmental Quality Standards for Surface Water (TP ≤ 0.1 mg/L). Similar to TN concentration, TP concentration increased on day 14 and then decreased from day 30. The initial TP concentration was relatively low, so the increasing concentration almost equaled the amount released by plants themselves. Thus, phosphorus was deposited in the sediments, which decreased the TP concentration in water [22]. These results clearly indicated that *N. minor* was the most resistant plant to seasonal changes in group one.

3.3. Nutrient removal in group two

The three submerged plants in group two differed in removal performance. All parameters decreased during monitoring, which indicated that all of the three submerged plants affected water purification under the experimental conditions (Fig. 3). The TN removal rates of *M. verticillatum*, *P. perfoliatus*, and *N. minor* were 37, 46, and 55%, respectively. *N. minor* removed nitrogen more effectively than the other two plants. Removal results for NH_4^+–N varied for *M. verticillatum* and *P. perfoliatus* had similar tendencies to decrease NH_4^+–N concentration, but *N. minor* showed a more stable removal efficiency during the first two weeks before it subsequently decreased NH_4^+–N concentration from 1.21 to 0.59 mg/L. Organic nitrogen must be converted into inorganic nitrogen so that it can be directly absorbed by plants. The tank with *N. minor* was rich in micro-organisms and had active nitrogen circulation. Thus, it exhibited better removal efficiency than the tanks containing other macrophytes. Besides, photocatalysis of macrophytes participated in the nutrient removal in the water system. Similar results were found in TP removal, with an outstanding efficiency of 93% for the tank with *N. minor* compared with the two other tanks.

3.4. COD and DO removal

The COD and DO values were also tested in the two experimental groups of the three submerged plants. The data are presented in Figs. 4 and 5. The submerged plants showed almost no purification capacity for COD. The COD concentration increased in group one, except for the tank with *N. minor*, which slightly reduced this parameter from 38.15 to 26.15 mg/L. The peak values reached 64.36 and 62.01 mg/L, which subsequently decreased because algae used COD as nutrient for growth. The COD concentration for *M. verticillatum* and *P. perfoliatus* rapidly increased during the first two weeks. In general, all of

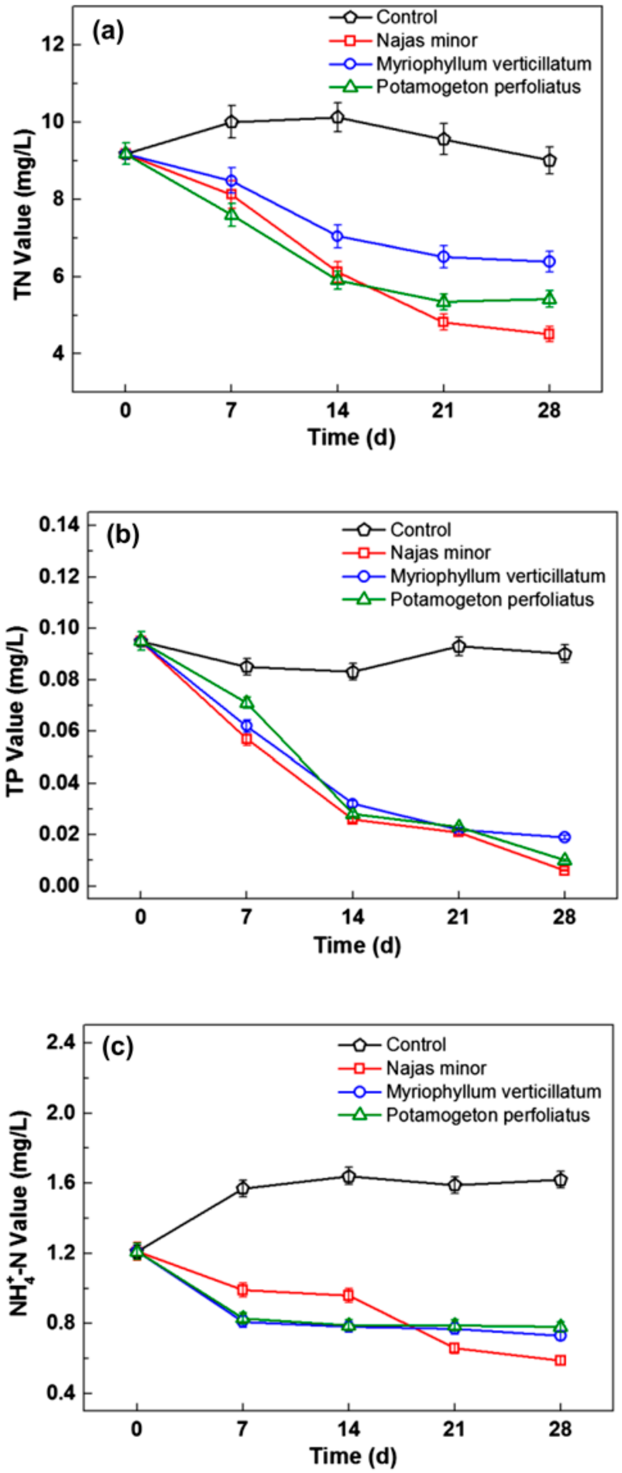

Fig. 3. (a) TN, (b) TP, and (c) NH_4^+–N concentrations of the three culture tanks in group two.

the three plants showed negligible capacities to remove COD. In group one, the DO level increased in the tank with *N. minor*, which indicated good plant

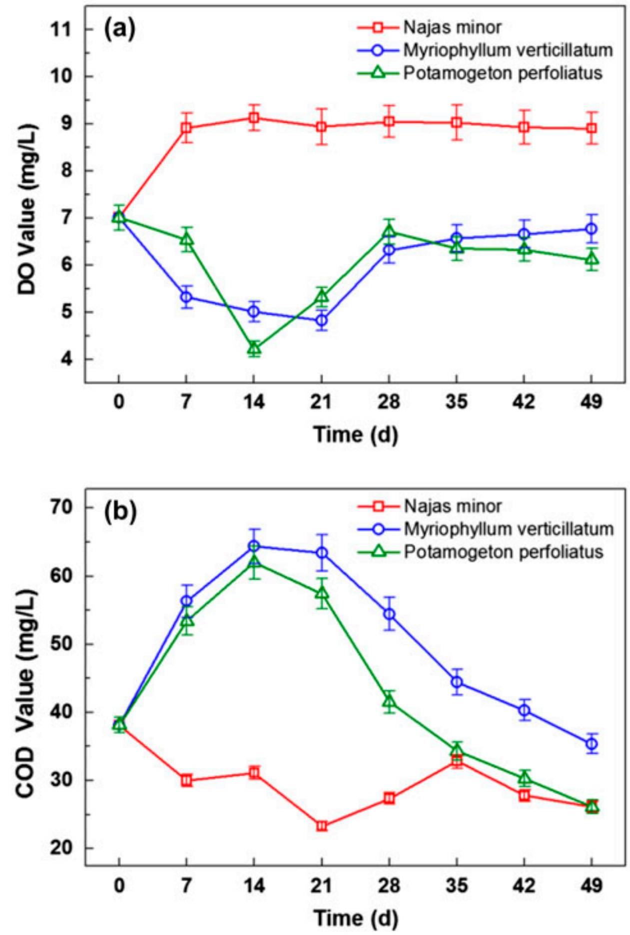

Fig. 4. (a) DO and (b) COD variations in group one.

growth and photosynthesis. This result was contrary to the decreased DO level in the tanks planted with *M. verticillatum* and *P. perfoliatus*. This phenomenon may have resulted from the decay of the plant itself and decomposition of residues that consumed oxygen; it may have also resulted from the consumption of oxygen by nitrification caused by the active aerobic micro-organisms with the increasing nutrient concentration and temperature [23]. In the *M. verticillatum*-cultured tank, the TN value significantly negatively correlated with DO ($R = -0.894$, $p < 0.01$). This finding is possibly due to the decomposition of the submerged plants that released nitrogen to the water body, which consumed a large amount of oxygen. Hence, water quality was influenced, and water turned yellow and smelly in spring [24].

In group two, the DO consumed by the three plants could be arranged as *N. minor* > *P. perfoliatus* > *M. verticillatum*. Compared with the control tank, in which DO decreased from 6.93 to 4.51 mg/L, the submerged plants improved water quality and

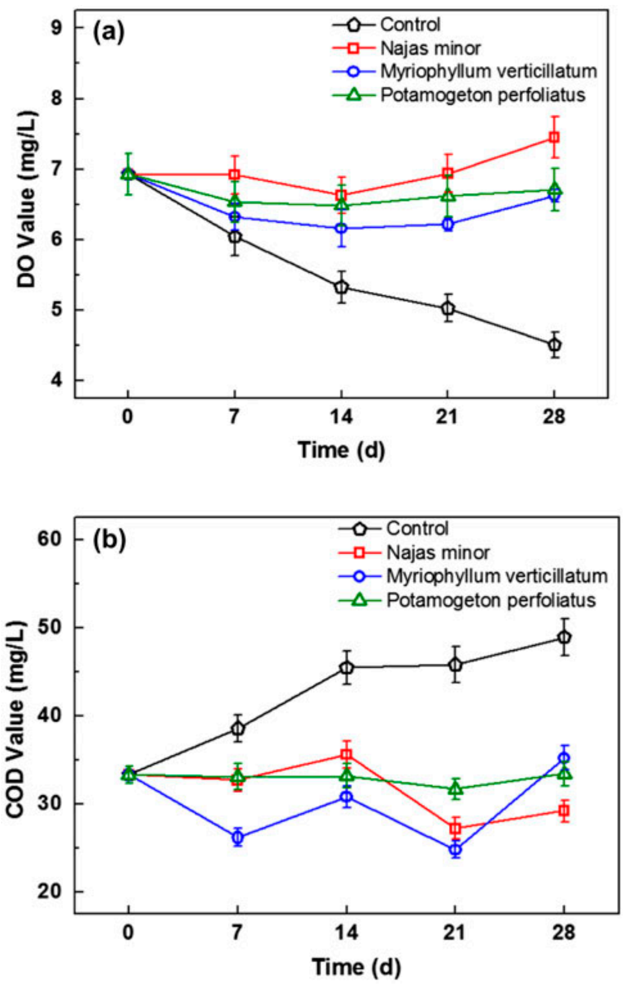

Fig. 5. (a) DO and (b) DO variations in group two.

increased DO concentration by photosynthesis of the macrophytes that consumed carbon dioxide and released oxygen. Similarly, no significant change in COD was found.

All of the three selected submerged plants exhibited good activities in the reclaimed water body. However, the harvest time for *M. verticillatum* and *P. perfoliatus* during seasonal changes should be optimized in practical applications. With the highest nutrient removal efficiency among all the plants, *N. minor* was apparently the most active in both systems. Therefore, *N. minor* is a promising plant for ecological engineering projects.

3.5. Mathematical analysis

The two groups of experiments were mathematically analyzed to further elucidate the behavior of the plants. For group one, the TN and TP concentrations in the culture tank planted with *N. minor* fitted exponential distribution with time. The corresponding fitting equations could be calculated as $C_{(TN)} = 4.941e^{-0.033t}$ ($R^2 = 0.950$, $R = -0.952$, $p < 0.01$) and $C_{(TP)} = 0.065e^{-0.029t}$ ($R^2 = 0.757$, $R = -0.803$, $p < 0.05$). The equations demonstrated that the TN and TP values in this tank significantly negatively correlated with time as the seasons changed. However, the influences of pH, temperature, and plant growth were not considered in this analysis.

For group two, the kinetic and correlation analyses between nutrients (TN and TP) and time (t) are presented in Tables 2 and 3. The numbers associated with correlation were significant at the 0.01 or 0.05 level (two-tailed) for all the three plants. This is simple mathematics that only accounted for TN or TP concentration and time. In a natural environment, factors such as nutrient loads, temperature, intensity of illumination, and pH should be considered, and the kinetic model should be revised when used for engineering application. On the basis of exponential results, the purification capabilities of the three plants were arranged as *N. minor* > *P. perfoliatus* > *M. verticillatum*. The formula could be utilized for two possible purposes. First, in the formula $Y = A * e^{-Bt}$, parameter B reflects the removal rate, which could benefit the proper selection of plants. Second, the retention time t could be calculated and used in ecological project design.

Table 2
Kinetic and correlation analyses between TN and time (t) in group two

Plants	Fitting equation	R^2	Coefficient
Myriophyllum verticillatum	$C = 9.640e^{-0.014t}$	0.929	−0.959*
Potamogeton perfoliatus	$C = 8.707e^{-0.020t}$	0.882	−0.929*
Najas minor	$C = 9.265e^{-0.028t}$	0.966	−0.977**

*$p < 0.05$.
**$p < 0.01$.

Table 3
Kinetic and correlation analyses between TP and time (t) in group two

Plants	Fitting equation	R^2	Coefficient
Myriophyllum verticillatum	$C = 0.087e^{-0.059t}$	0.964	−0.949*
Potamogeton perfoliatus	$C = 0.106e^{-0.081t}$	0.967	−0.959**
Najas minor	$C = 0.107e^{-0.094t}$	0.953	−0.951*

*$p < 0.05$.
**$p < 0.01$.

4. Discussion

Aquatic plant harvesting is very important in macrophyte purification system because it can uptake nutrients from the water body. Thus, regular management of submerged plants is essential. Otherwise, submerged macrophyte decomposition would release nitrogen into the water body and reduce nitrogen removal efficiency [25–27]. The present study demonstrated that reclaimed water, given its low-level pollution load, can be purified in terms of N and P by planting submerged macrophytes. These plants substantially reduced water pollutants, as shown in Figs. 1 and 3. The growth of submerged plants is influenced by periodic seasonal changes, especially after winter. Most macrophytes die and decompose with increasing temperature (Fig. 2) and then release nitrogen into water and sediment. Therefore, regular and timely plant harvest is highly important in water purification systems that use macrophytes [16,18]. In the present study, TN and TP concentrations increased in the group one tanks with *P. perfoliatus* and *M. verticillatum*. This result indicated that purification was blocked by the abnormal growth of submerged plants and the subsequent release of nitrogen into the water. In Ref. [28], a nitrogen and phosphorus transfer model was established in *P. malaianus*, and three processes were used to modify the decomposition. These processes included the dissolution of inorganic nitrogen and phosphorus in *P. malaianus* Miq., the degradation of its organic nitrogen and phosphorus, and the boundary adsorption of nitrogen and phosphorus in water. The plants rapidly released nutrients within the initial one week, and these nutrients were absorbed by sediment. These previous findings coincided with the present results. The TN and TP levels both peaked on day 14 and then decreased with time. Thus, suppressing weeds and replanting suitable plants are important measures to form a sustainable purification ability.

After monitoring the dragon-shaped landscaping water system from 2013 to 2014, the first two of the nine ecological ponds, along with water flow, were chosen for analysis because of their high pollutant content. The results are illustrated in Fig. 6. The TN concentrations in the ponds fluctuated and even reached relatively high levels. However, no eutrophication or algal bloom was observed, and the water remained transparent with good landscape effects.

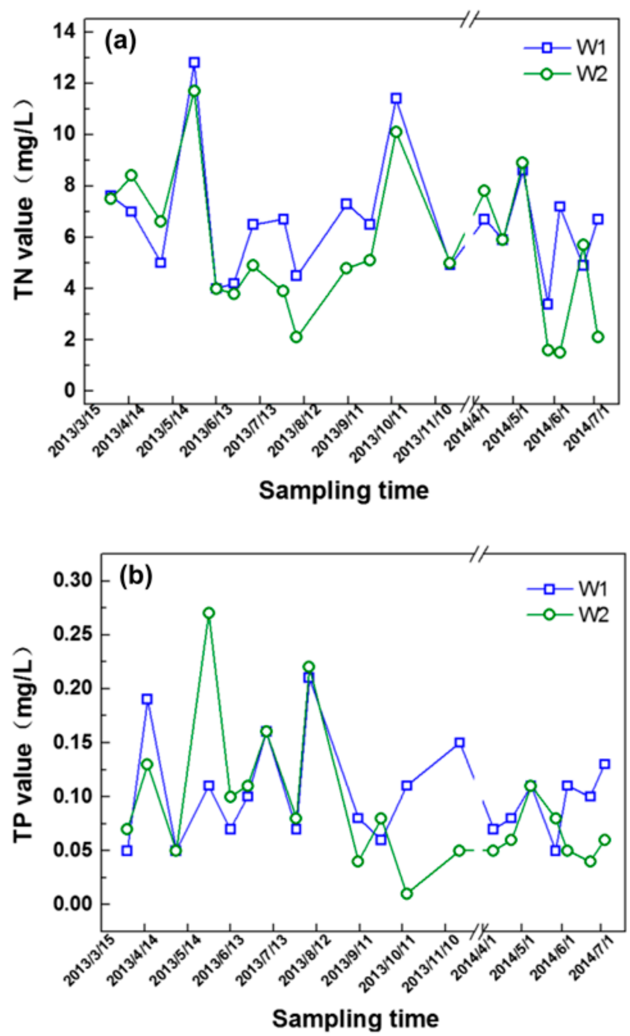

Fig. 6. Variations in (a) TN and (b) TP levels in the W1 and W2 areas of the dragon-shaped landscaping water system.

Therefore, a reasonable construction of an ecological system using submerged plants can guarantee a healthy landscape water environment.

Assimilation, rhizosphere effect, and adsorption are the main processes for water purification by submerged macrophytes [29,30]. Submerged plants purify water through photosynthesis using nitrogen and phosphorus in water as nutrient resources for self-growth. In the present study, the submerged macrophytes had significant removal efficiencies for TN and TP. However, the COD removal efficiencies of the macrophytes were not satisfactory. In both systems, the COD value did not significantly decrease and even increased at some sampling points. COD is an indicator of organic material in wastewater, which can be remediated by the biofilm formation of micro-organisms. Therefore, a subsurface constructed wetland is suggested, followed by macrophyte ponds to improve surface water quantity standard. With the life activity of micro-organisms in the biofilm, organic materials can be decomposed and removed by aerobic or anaerobic degradation [31–33].

5. Conclusions

Under correct management, submerged macrophytes can effectively remove nitrogen and phosphorus from reclaimed water of culture tanks and provide high water quality and good culture environment.

In the two test groups, *N. minor* showed positive activities in the culture tanks, which achieved removal rates of 81 and 62% for TN and TP, respectively, in group one. In group two, 55 and 93% reductions for TN and TP were observed. *P. perfoliatus* and *M. verticillatum* increased TN concentration in water to the highest value of 12.25 mg/L. Hence, environmental change and contamination load negatively influenced water purification, but proper management can improve the situation. In general, the purification capabilities of the three submerged macrophytes could be arranged as *N. minor* > *P. perfoliatus* > *M. verticillatum.*

To reduce COD concentration, a subflow constructed wetland is recommended with particular application of biofilm. Constructed wetland combined with macrophyte pond can be an effective method to maintain a clean and safe landscape water. Further research is required to determine the performance of submerged macrophytes in practical engineering and the enhancement of constructed wetland with macrophyte pond. Future studies should also elucidate the mechanism underlying nitrogen removal during purification using both recirculating system model and onsite monitoring to improve management practices of ecological water systems. For a holistic solution, harvested aquatic plants should also be treated and disposed properly.

Acknowledgment

The authors would like to thank the Beijing Iearth Environmental Engineering for their support in this study and their assistance in the management of the culture tanks.

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