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Site test of phytoremediation of an open pond contaminated with domestic sewage using water hyacinth and water lettuce

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A B S T R A C T

This study was undertaken *in situ* to explore the potential of the alien plants water hyacinth (*Eichornia crassipes*) and water lettuce (*Pistia stratiotes* L.) as phytoremediation aquatic macrophytes for nutrients (nitrogen and phosphorus) removal and algal interception from domestic sewage contaminated pond (approximately 10500 m^2 in area, average 2.5 m in depth) by using self-designed experimental devices from July 7 to August 8 in 2015. The physicochemical properties of water and plant samples as well as N and P mass balance in the phytoremediation system were investigated. The range of physicochemical parameters of influent were shown as follows: water temperature (WT: 24.5 °C –31.0 °C), pH (6.94–8.25), DO (4.58 mg L^{-1}–15.73 mg L^{-1}), COD_{Mn} (5.00 mg L^{-1}–13.15 mg L^{-1}), TN (1.60 mg L^{-1}–5.60 mg L^{-1}) and TP (0.16 mg L^{-1}–0.73 mg L^{-1}). Water hyacinth, which exhibited hyperactive accumulating capacity for nitrogen (58.64% of total reductions), was more suitable than water lettuce for the intensive purification of domestic sewage with high nitrogen concentrations. This result may be attributed to the larger total root surface area (0.97 m^2 g^{-1}–1.10 m^2 g^{-1} fresh weight), active absorption area (0.31 m^2 g^{-1}–0.36 m^2 g^{-1} fresh weight), and leaf area and higher root activity (71.79 μg g^{-1} h^{-1}–98.34 μg g^{-1} h^{-1}), root biomass (kg m^{-2}), and net photosynthetic rate (20.28 μmol CO_2 m^{-2} S^{-1}) of water hyacinth than those of water lettuce regardless of cultivation in oligotrophic water with total nitrogen contents lower than 1.0 mg L^{-1}. Water lettuce exhibited a higher total phosphorus removal efficiency, which benefitted higher P accumulation, adsorption, and precipitation because of its longer roots (approximately 49.0 cm) with higher rhizofiltration capacity. As such, water lettuce achieved higher algal (96.36%) and chlorophyll *a* (96.65%) removal efficiencies. A combined pattern using both macrophytes was recommended for the phytoremediation of most domestic sewages containing dual contaminants (N and P) in the future.

1. Introduction

In recent years, human-related eutrophication has been considered a common environmental issue and has received worldwide attention (Conley et al., 2009), especially for many developing countries. Among the various measures for eutrophication control, phytoremediation, a solar-driven biological method performed directly *in situ* (Salt et al., 1998), has received considerable attention because of its low cost and environmental friendliness (Batty and Dolan, 2013). Phytoremediation, in which plants are used to remediate a contaminated medium, is a well-established environmental protection technique that has received increasing attention since the term has been coined two decades ago (Vamerali et al., 2010; Priya and Selvan, 2014). Plants used for phytoremediation must meet requirements, such as high biomass, rapid growth, and high nutrient accumulation; in addition, the application of this method is limited by its clean-up depth (purification depth from water surface), which is strictly determined by plant root length (Putra et al., 2015).

Water hyacinth (*Eichhornia crassipes* Mart. Solms.), a member of the monocotyledonous family Pontederiaceae (Patel, 2012), is a free-floating perennial aquatic macrophyte native to tropical South America (Tipping et al., 2011). The plant is notorious as a highly noxious alien weed because of its tremendously vigorous growth rate (Mishra and Tripathi, 2009). However, given its dense hairy root system, peculiar physiological characteristics and nutrient absorption efficiency (Kim and Kim, 2000; Paganetto et al., 2001), and wide tolerance to environmental conditions (Rommens et al., 2003), water hyacinth has been widely utilized and has gained increasing

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attention in recent years for the phytoremediation of many types of wastewater (Chavan and Dhulap, 2012).

The monocotyledonous freshwater floating macrophyte water lettuce (*Pistia stratiotes* L.) belongs to the Araceae family (Walsh and Maestro, 2014); the plant spreads predominantly by vegetative propagation (Hussnera et al., 2014) and is native to South America (Hill, 2003). Despite its notorious reputation as an alien plants, water lettuce has also been widely applied in wastewater phytoremediation in tropical areas (Putra et al., 2015) because of its prolific growth characteristics (Chen et al., 2015), great potential in nitrogen and phosphorous removal (Lu et al., 2010), significant absorption, and enrichment in several heavy metals (Lu et al., 2011).

Many studies reported on the use of water hyacinth and water lettuce in phytoremediation to remove different contaminants (Hadad et al., 2011; Lu et al., 2011; Anuradha et al., 2015; Lin and Li, 2016). However, only few comparative studies have explored the purification efficiency of these two macrophytes. In addition, previous studies were mostly conducted under static water conditions; hence, the phytoremediation effects of water hyacinth and water lettuce in sewage treatment cannot be accurately determined under actual liquidity conditions (Sooknah and Wilkie, 2004; Wen et al., 2015). Prior studies reported that removing pollutants from wastewater is inadequate if only one macrophyte is applied in ecological management and suggested plant addition for heavily polluted urban rivers (Hu et al., 2007).

Therefore, the present study aims to determine whether water hyacinth and water lettuce benefit urban sewage phytoremediation in a flowing water system *in situ*, which embodies the fate of nitrogen and phosphorus, particulate matter especially for algal interception, and physicochemical properties of macrophytes. A free-floating, sealed, flow-controlled stainless steel sink was designed as the experimental device for phytoremediation, in which water hyacinth and water lettuce were selected as the test plants. This study may serve as a practical and theoretical reference for contaminant removal from domestic sewage through ecological engineering with water hyacinth or water lettuce.

2. Materials and methods

2.1. Study site

The present study was conducted in a pond (31°17′28.0″N, 119°02′29.3″E; approximately 10500 m^2 in area, average 2.5 m in depth) located inside the campus of the Jiangsu Academy of Agricultural Sciences, Nanjing, China. The image in the figure was captured using Google Earth on July 13, 2015 (Fig. 1a). The pond water was mainly replenished by domestic sewage and rainwater, and maintained in a eutrophic status with an algal bloom that occurs from May to October every year. Changes of the general physical and chemical parameters of water collected from pond during the experiment were shown as the follows: water temperature (24.9 °C–32.2 °C), pH (6.98–8.73), DO (4.55 mg L^{-1}–15.73 mg L^{-1}), algae density (3.88×10^7 cells mL^{-1}–15.13 cells mL^{-1}), and chlorophyll *a* concentration (31.81 μg L^{-1}–520.46 μg L^{-1}).

2.2. Experimental device

The cuboid sink was welded with stainless steel (10.0 m × 1.0 m × 0.5 m) and without the top cover, and the sink was maintained in a floating state with bubble float being fixed on both sides of the sink (Fig. 1b, c). Six sinks were placed in experimental area (approximately 200 m^2) in this study (Fig. 1d). Water inlet (5 cm in diameter) at one end of the sink was located at 40 cm from the bottom of the sink, and the water outlet at the other end of the sink was controlled by a metering pump (5 m^3 day^{-1}). The metering pump was fixed on the sink and remained at a distance of 20 cm above the water surface. The pumps were semi-enclosed with stainless steel plate to avoid hazards from the rain or burning sun. The metering pump can be running with uniform speed for several months and the pump flow was calibrated at a 24 h interval.

2.3. Experimental plants

The free-floating macrophytes water hyacinth and water lettuce were selected as the test plants. Before the experiment, both plants were cultivated in the "propagation area" fenced with a steel pipe next to the experimental device in the same pond (Fig. 1a). Healthy, similarly sized samples of water hyacinth and water lettuce were collected from the "propagation area" and then respectively placed in two sets of three floating sinks (Nos. 1, 3, and 5 for water hyacinth and Nos. 2, 4, and 6 for water lettuce) with fresh plants of equal weight (7.30 kg m^{-2}) (Fig. 1d).

2.4. Water sample collection and analysis

Water samples were obtained from the influent and effluent of all the experimental sinks at a 24 h interval at about 9:00 AM (30 days, from July 7 to August 8 in 2015) for physiochemical parameter analysis. The following parameters were measured *in situ*: water temperature (WT), dissolved oxygen in water (DO), and pH (using a multi-parameter water analyzer, HQ40D, Hach, USA). For water samples used for laboratory analyses, the influent samples (approximately 1 L) were collected from the surface layer (0–0.5 m) at all influent sampling sites, and the effluent samples were obtained from the outlet of the metering pumps (water collected for 1 min, approximately 3.75 L) using 5 L plastic barrels. The physiochemical parameters of the effluent samples were analyzed after being weighed to maintain a constant flow rate daily. Chemical oxygen demand (COD_{Mn}), total phosphorus (TP), orthophosphate (PO_4^{3-}), total nitrogen (TN), nitrate (NO_3^-), ammonium (NH_4^+), and chlorophyll *a* (Chl *a*) were determined in accordance with standard methods (APHA, 1998). Conversions between CODcr and COD_{Mn} were as the equation listed below. $CODcr = 6.815 \times COD_{Mn} - 1.092$ ($R^2 = 0.901$), COD_{Mn} (1 mg L^{-1}–10 mg L^{-1}).

For algal species, 0.5 L sub-samples were harvested from 5 L plastic barrels which were collected as described above, and were fixed with 1% acidified Lugol's iodine solution then concentrated to 50 mL by siphon pipe after 48-h sedimentation (Li and Li, 2012). The dominant species was identified with a light microscope at 4000 magnification (CX41, Olympus, Tokyo, Japan). The algal density was determined by using the flow cytometry (FACSJazz, BD company). TN and TP reduce efficiencies of water hyacinth and water lettuce were calculated as the following equation: Reduce efficiency (%) = 100% × (influent concentration − effluent concentration)/influent concentration.

2.5. Plant sample collection and analysis

The biomasses of the water lettuce and water hyacinth were determined and expressed as fresh weight per unit area (kg m^{-2}) at a 5 day interval, respectively. Accurate 1 m^2 plants were harvested form the influent, middle, and effluent sink sites for getting fresh weight with a portable electronic scale. After being weighted, the plants were put back into the sinks neatly (Fig. 1d). In addition, three plants at each site were harvested for laboratory analysis. The shoot and root lengths were measured using a scale ruler before the plants were separated into shoot (stalk and leaf) and root for nutrient analysis.

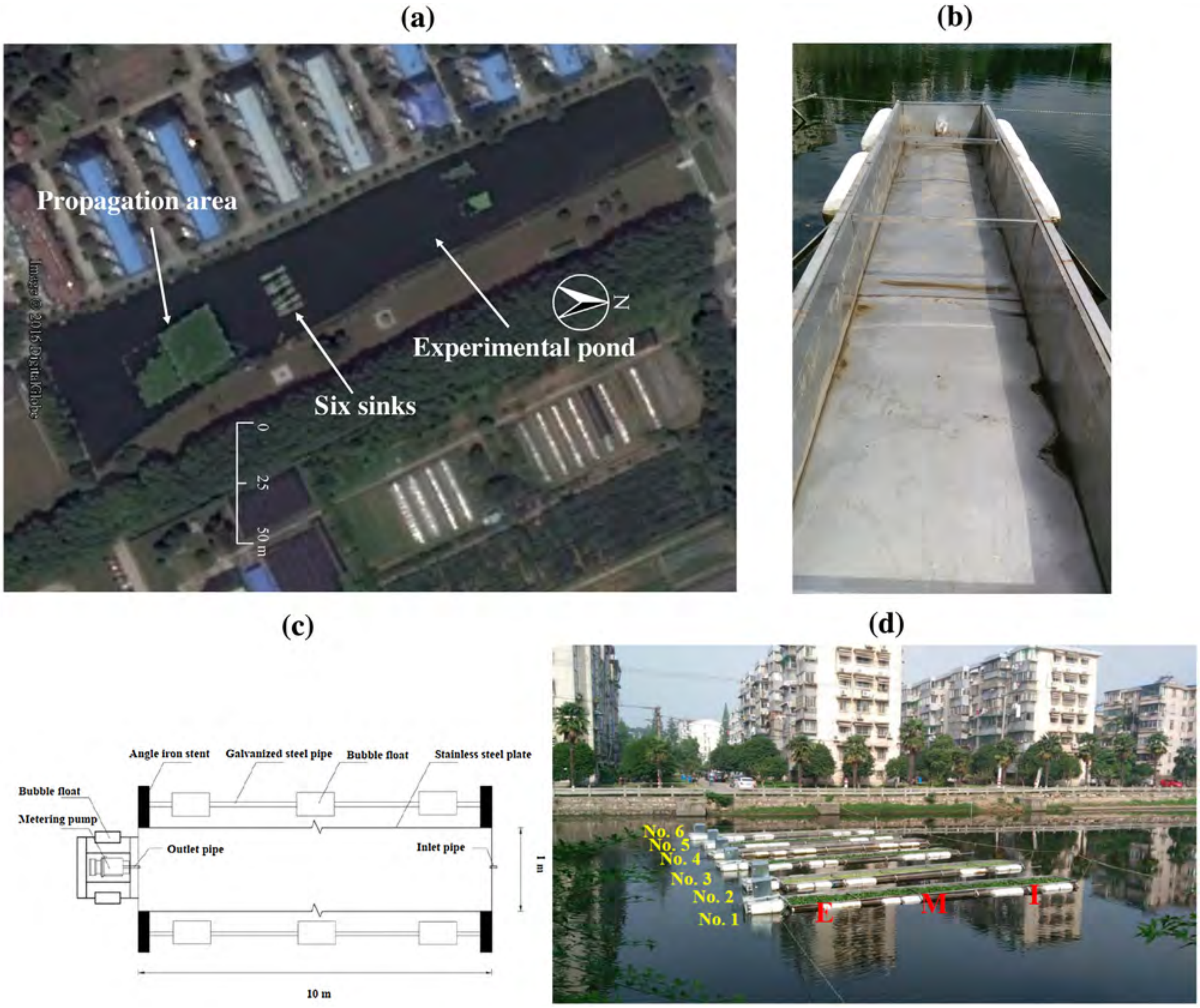

Fig. 1. The study site (a), actual picture (b), structural diagram (c), and actual scene (d) of the sink. The letters I, M, E indicated the plant sampling sites (influent, middle, and effluent in order). Nos. 1, 3, and 5 for water hyacinth and Nos. 2, 4, and 6 for water lettuce.

Both shoot and root parts were washed thoroughly with running tap water to eliminate the adhering particle matters and then maintained on the filter paper to remove surface water. The washed shoots and roots were weighed (fresh weight W_f) and then dried in an oven at 65 °C for at least 48 h to a constant weight (dry weight W_d) after heat treatment under 105 °C for 30 min. Plant water content (M) was calculated using the following equation: $M = 100 \times (W_f - W_d)/W_f$.

The dried samples were ground to fine powder with a high-speed universal disintegrator (FW100, Tianjin Taisite Instrument Co., Ltd., Tianjin, China) and then sieved using an 80-mesh screen before digestion for TN and TP content analyses as described by Bao (2000). TN and TP contents were expressed as percentage (%) based on dry weight. The fate of N or P in the system mainly included plant absorption, root adsorption, N and P in sediments and duckweed, and the mass balance of N or P was based on the four parts.

Root areas, including the root total surface area (TSA) and active adsorption area (AAA), were determined through the methylene blue dyeing method as described by Zhang et al. (1994) after fresh roots were washed thoroughly with tap water. The washed roots were cut into small pieces for root activity assay using triphenyl tetrazolium chloride as described by Li et al. (2000).

The software autoCAD was employed to determine the leaf area on the basis of leaf photograph and the related scale. Photosynthetic parameters, including net photosynthetic rate (Pn), stomatal conductance (Cond), intercellular CO_2 concentration (Ci), and transpiration rate (Tr), were measured with a portable photosynthesis system (LI-6400XT, USA) in an open area near the study sites from 9:00 AM to 10:00 AM on a sunny day as the method described by Li and Cong (2011). The parameters were measured under a photosynthetic photon flux density of 1600 μmol photons $m^{-2}\,s^{-1}$ and a flow rate of 500 μmol photons $m^{-2}\,s^{-1}$ using red and blue light.

A filter screen was used to collect duckweeds growing in water lettuce sinks. The duckweeds were washed with running tap water, and then their fresh weight, dry weight, and TN and TP contents were determined as described above for plant samples.

2.6. Statistical analysis

The test was run in triplicate for 30 days under the conditions described above. Significant differences between the water lettuce and water hyacinth sinks were analyzed through one-way ANOVA followed by the least significant difference post-hoc test (SPSS, Chicago, IL, USA) at the 95% confidence level. All data are presented as mean ± standard deviation (SD).

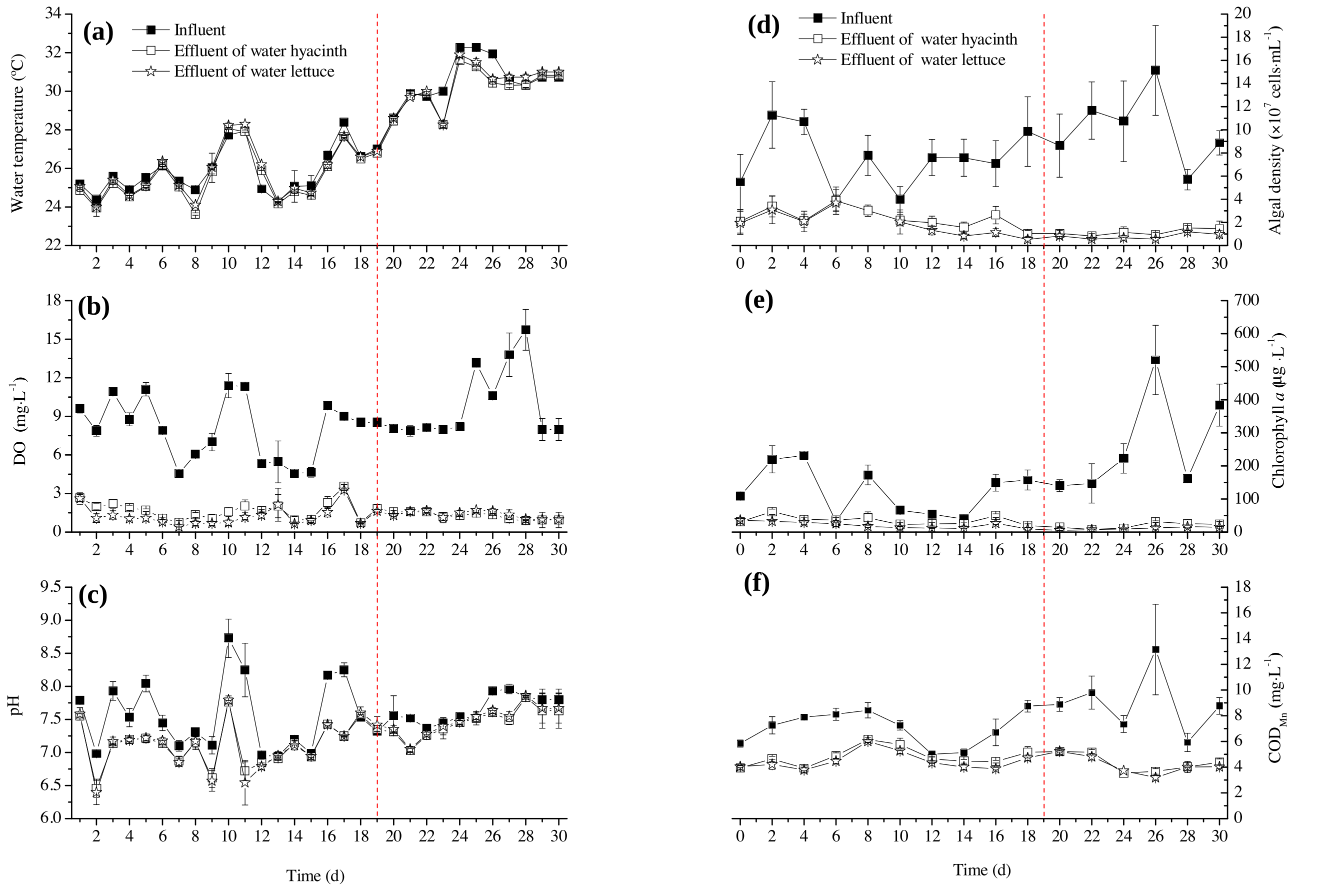

Fig. 2. Physicochemical properties ((a) water temperature WT; (b) DO; (c) pH; (d) algal density; (e) chlorophyll *a* concentration; (f) COD_{Mn}) of water samples. The dotted line in figures indicated different weather on the left (rainy days) and on the right site (sunny days) of the line (the same for the following figures).

3. Results

3.1. Physicochemical properties of water samples

The experiment lasted for 30 days (from July 7 to August 8 in 2015). The physicochemical properties of water at the influent and effluent of the sinks are presented in Fig. 2. The early stage of the experiment (0–19 days) comprised rainy days, whereas the late stage of the experiment (20–30 days) involved sunny days. WT ranged between 24.5 °C and 31.0 °C from the initial stage to the end of the experiment, and no significant difference between the influent WT and effluent WT of the water lettuce and water hyacinth groups was observed ($P > 0.05$) (Fig. 2a).

The DO levels of the effluent in the water lettuce group (0.42–3.29 mg L^{-1}) were slightly lower than those of the effluent in the water hyacinth group (0.73–3.56 mg L^{-1}) in the first 19 days, whereas the opposite phenomenon was observed in the subsequent 11 days ($P > 0.05$). However, both were significantly lower than the data at the influent sites (Fig. 2b) ($P < 0.05$). Changes in pH are shown in Fig. 2c. Overall, the values of the effluent water in both water lettuce and water hyacinth sinks were slightly lower than those in the influents.

Algal density and Chl *a* concentration showed similar changes; a significant decreasing trend was observed at the effluent sites of the water hyacinth and water lettuce sinks compared with the values at their influent sites. In particular, the algal density and Chl *a* concentration at the effluent sites were not more than 6.15% and 6.06% of the water hyacinth group and 3.63% and 2.34% of the water lettuce group compared with their influent sites with maximum values (15.13×10^7 cells mL^{-1} and 520.46 $\mu g\,L^{-1}$) when the experiments were carried out for 26 days (Fig. 2d and e). The effluent COD_{Mn} concentrations were significantly lower than their influent counterparts, especially for the later sampling periods (Fig. 2f). A significant positive correlation was shown between COD_{Mn} and DO. The regression equation was got as $COD_{Mn} = 0.5119 \times DO + 3.8884$ ($R^2 = 0.7005$, n = 45).

The contents of the major nutrients (N and P) in the different water samples are presented in Fig. 3. Compared with those in the effluent of the water hyacinth sinks, the TN and $NO_3{}^-$-N concentrations in the effluent of the water lettuce sinks were slightly lower ($P > 0.05$) in the first 19 days but significantly higher during the subsequent 11 sunny days ($P < 0.05$) (Fig. 3a and b). The influent concentrations of TN (1.60 mg L^{-1}–5.60 mg L^{-1}) and $NO_3{}^-$-N (1.20 mg L^{-1} –2.96 mg L^{-1}) were significantly higher than their effluent concentrations in both water lettuce (TN: 0.51 mg L^{-1}-3.72 mg L^{-1} and $NO_3{}^-$-N: 0.65 mg L^{-1} –2.24 mg L^{-1}) and water hyacinth (TN: 0.25 mg L^{-1} –4.34 mg L^{-1}, $NO_3{}^-$-N: 0.08 mg L^{-1} –2.47 mg L^{-1}) sinks during the entire the experimental period ($P < 0.05$).

No significant difference in $NH_4{}^+$-N level was found among the influent (0.41–0.55 mg L^{-1}) and effluent concentrations of the water lettuce sinks (0.43–0.51 mg L^{-1}) and the effluent concentrations of the water hyacinth sinks (0.42–0.53 mg L^{-1}) over the study periods ($P > 0.05$). However, a rapid increasing trend was shown in all of the study sites after 22 days (Fig. 3c).

Changes in TP and $PO_4{}^{3-}$ levels in different study sites are shown in Figs. 3d and 4e. The change trends of TP concentrations were similar to those of TN concentrations; the effluent concentrations of all the six sinks were significantly lower than their influent concentrations, especially for the later 11 days

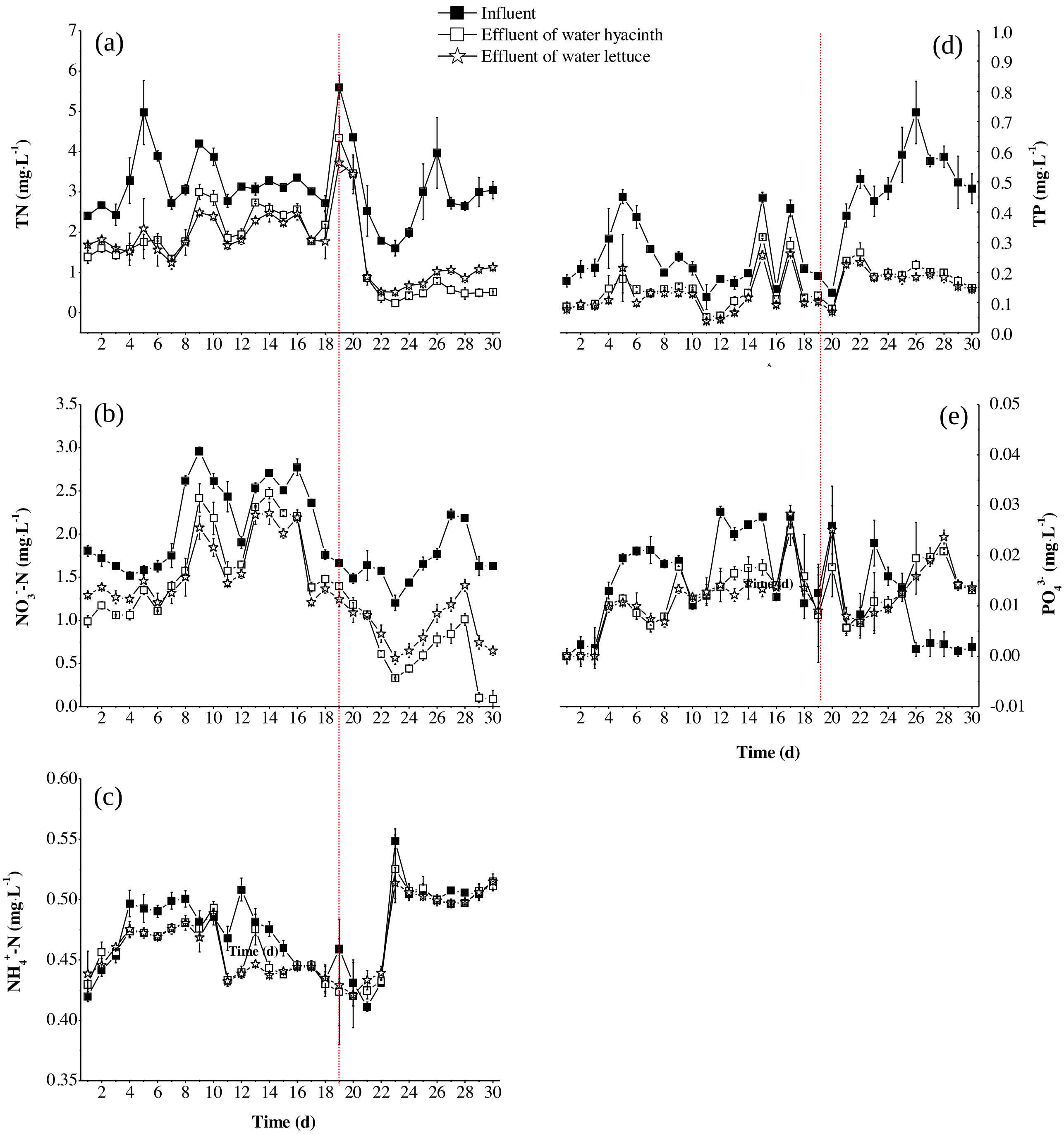

Fig. 3. Changes of N and P concentrations ((a) TN; (b) NO_3^--N; (c) NH_4^+-N; (d) TP; (e) PO_4^{3-}) of water samples.

($P < 0.05$). However, the effluent concentrations of the water lettuce group (0.26–0.06 mg L^{-1}, average 0.14 mg L^{-1}) were slightly lower than those of the water hyacinth group (0.29–0.09 mg L^{-1}, average 0.16 mg L^{-1}) throughout the entire experimental period, although no statistically significant difference was found ($P > 0.05$) (Fig. 3d). Complex changes in PO_4^{3-} concentration were exhibited during the entire experimental procedure. The influent PO_4^{3-} concentrations were markedly higher than their effluent counterparts during the two intermittent periods, i.e., 4–8 days and 12–15 days of the test, but were obviously lower than their effluent counterparts at the end (26–30 days) of the test ($P < 0.05$).

3.2. Physicochemical properties of plant samples

Table S1 shows the morphological characteristics of the water lettuce and water hyacinth samples, which were investigated at a 5-day interval. The root length of the water lettuce samples (49.00 cm) was obviously longer at the effluent sites than at the effluent sites (33.50 cm) ($P < 0.05$). Similar changes were observed on the water hyacinth group, whereas the root length (41.63 cm) of the water hyacinth group was markedly shorter than that of the water lettuce group ($P < 0.05$). However, the shoot lengths of both macrophytes were opposite to their root lengths. Eventually, the root–shoot ratio (cm/cm) of the water lettuce group was up to 6.88 and was significantly higher than that of the water hyacinth group

(1.50) at the effluent sites ($P<0.05$). Moreover, the root–shoot ratio at the influent sites (cm/cm) of the water lettuce group (1.63) was significantly lower than that at the effluent sites and was significantly higher than that of the water hyacinth group at the influent sites (0.74) ($P<0.05$).

The root weight of the water lettuce group was higher at the influent sites (41.76 g plant^{-1}) than at the effluent sites (25.93 g plant^{-1}). Other parameters based on weight, such as shoot weight and root–shoot ratio, at all study sites were consistent with the changes in length parameters mentioned above (Table S1).

The Pn, Cond, Ci, Tr, and leaf area were measured at the end of the experiment to evaluate the photosynthetic characteristics of the two macrophytes (Table 1). The Pn values of the water hyacinth samples harvested from the influent and effluent sites were 27.90 and 20.28 μmol CO_2 m^{-2} S^{-1}, respectively, whereas those of the water lettuce samples were 16.50 and 8.64 μmol CO_2 m^{-2} S^{-1}, respectively. The leaf areas of the water hyacinth samples were 80.77 and 34.41 cm^2 at the influent and effluent sites, respectively, which were significantly higher than those of the water lettuce samples (75.29 and 29.82 cm^2, respectively) ($P<0.05$). No significant difference in Cond, Ci, and Tr was observed among all the sampling sites ($P>0.05$).

Changes in TSA, AAA, and root activity in different investigated sites were determined regularly (Fig. 4). All of the three parameters of water hyacinth were markedly higher than those of water lettuce ($P<0.05$) (Figs. 4a–c). The TSA range of the water lettuce samples was 0.59–0.72 m^2 g^{-1} fresh weight at the influent sinks and 0.70–0.88 m^2 g^{-1} fresh weight at the effluent sinks ($P<0.05$). Meanwhile, the data for the water hyacinth samples were 0.76–1.03 and 0.97–1.10 m^2 g^{-1} fresh weight, respectively (Fig. 4a). The AAAs of the water hyacinth samples were slightly lower at the effluent sites than at the influent sites; however, the AAAs of the water lettuce samples were slightly higher at the effluent sites than at the influent sites (Fig. 4b).

Compared with TSA, similar changes in root activity are shown in Fig. 4c. The root activity of the water lettuce group ranged between 31.34 and 48.83 μg g^{-1} h^{-1} at the effluent sites and between 12.27 and 42.24 μg g^{-1} h^{-1} at the influent sites. Moreover, the root activity of the water hyacinth group was significantly higher than that of the water lettuce group ($P<0.05$), and their values ranged between 71.79 and 98.34 μg g^{-1} h^{-1} at the effluent sites and between 43.61 and 56.11 μg g^{-1} h^{-1} at the influent sites.

3.3. Assessment of nitrogen and phosphorus mass balance

The TN, TP and COD_{Mn} reductions (Table 2), plant absorptions (Table S2), root mat adsorptions (Table S3), and sediments, as well as TN and TP in duckweeds growing in sinks (Table S4), were calculated and analyzed to analyze the fate of main nutrients (N and P) in experimental sinks. No significant difference in TN reduce efficiency was found between the water hyacinth and water lettuce groups ($P>0.05$). However, a significantly higher TP and COD_{Mn} reduce efficiency were observed in the water lettuce group (58.27%, 45.71%) than in the water hyacinth group (53.44%, 40.44%) ($P<0.05$). The same removal rates on TN (0.75 g m^{-2} d^{-1}) was shown of the two plants. For TP removal rate, 0.09 g m^{-2} d^{-1} and 0.10 g m^{-2} d^{-1} were shown in water hyacinth and water lettuce group. Water lettuce group (1.84 g m^{-2} d^{-1}) showed significantly higher removal rate on COD_{Mn} than water hyacinth group (1.57 g m^{-2} d^{-1}) (Table 2). The water hyacinth group (132.52 g) absorbed significantly higher TN level than the water lettuce group (87.62 g) ($P<0.05$). Meanwhile, the water hyacinth group (11.85 g) absorbed lower TP level than the water lettuce group (13.68 g), but the difference was not significant ($P>0.05$) (Table S2). The TN and TP adsorbed by the root mats of the water hyacinth group were 14.28 and 12.35 g, respectively, and were significantly higher than those of the water lettuce group (10.78 and 9.49 g, respectively) ($P<0.05$) (Table S3).

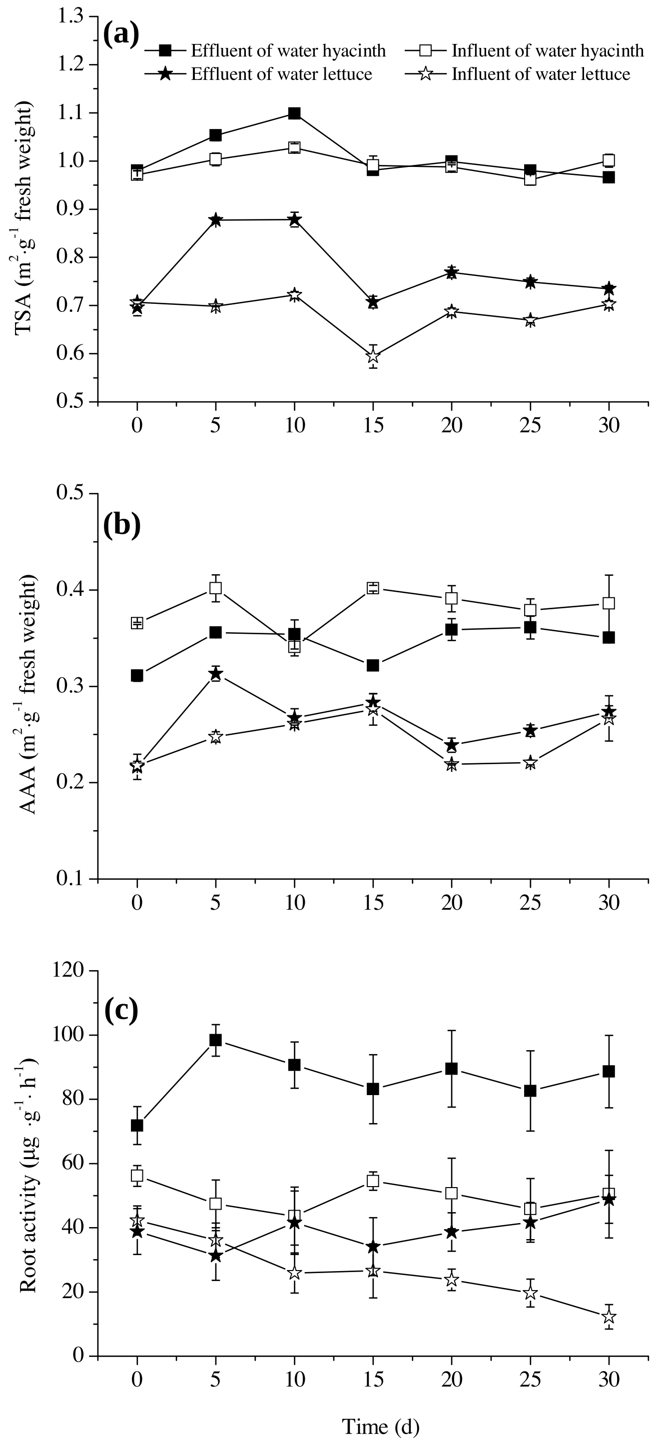

Fig. 4. Changes of TSA (total root surface area), AAA (active absorption area) and root activity in different investigated sites.

We then determined the amounts of TN and TP in the sediments of all the sinks and duckweeds growing in the water lettuce sinks (Table S4). The total TN content of the sediments collected from the water hyacinth sinks (26.75 g) was significantly lower than that of the sediments collected from the water lettuce sinks (35.58 g) ($P<0.05$), but no significant difference in TP content was noted (4.29 g for the water hyacinth and 5.53 g for the water lettuce) ($P>0.05$).

Table 1
Photosynthetic characteristics of the two floating macrophytes.

	Pn*(μmol CO_2 m^{-2} S^{-1})	Cond(mol H_2O m^{-2} S^{-1})	Ci(μmol CO_2 mol^{-1})	Tr(mmol H_2O m^{-2} S^{-1})	Leaf area(cm^2)
Influentwater hyacinth	27.90 ± 0.66	0.57 ± 0.04	263.00 ± 6.24	4.91 ± 0.18	80.77 ± 18.00
water lettuce	16.50 ± 1.01	0.50 ± 0.03	296.33 ± 1.15	4.81 ± 0.20	75.29 ± 19.74
Effluentwater hyacinth	20.28 ± 1.20	0.49 ± 0.09	300.25 ± 43.96	4.37 ± 0.30	34.41 ± 5.64
water lettuce	8.64 ± 1.52	0.51 ± 0.01	336.67 ± 8.08	4.58 ± 0.27	29.82 ± 9.58

*Pn: Net photosynthetic rate; Cond: Stomatal conductance; Ci: Intercellular CO_2 concentration; Tr: Transpiration rate.

Table 2
The concentrations of TN, TP and COD_{Mn} at the influent and effluent of sinks.

	Influent WH (g)	Influent WL (g)	Effluent WH (g)	Effluent WL (g)	Removal rates WH (g m^{-2} d^{-1})	Removal rates WL (g m^{-2} d^{-1})	Reduce efficiency WH (%)	Reduce efficiency WL (%)
TN	476.56 ± 13.76	481.79 ± 14.00	250.58 ± 6.23	257.45 ± 5.93	0.75 ± 0.03	0.75 ± 0.02	47.42 ± 1.48	46.56 ± 1.81
TP	51.40 ± 1.48	51.76 ± 1.32	23.93 ± 0.91	21.60 ± 1.35	0.09 ± 0.00	0.10 ± 0.00	53.44 ± 0.68	58.27 ± 0.84
COD_{Mn}	1164.93 ± 110.37	1204.93 ± 110.37	693.84 ± 58.42	654.19 ± 60.72	1.57 ± 0.18	1.84 ± 0.14	40.44 ± 3.67	45.71 ± 4.02

*WH: water hyacinth; WL: water lettuce.
Influent (g) = 30d × Average water influent concentration (mg/L) × Pump flow (5 m^3/d).
Effluent (g) = 30d × Average water effluent concentration (mg/L) × Pump flow (5 m^3/d).
Removal rates (g m^{-2} d^{-1}) = (Influent(g) – Effluent(g))/10m^2/30d.
Reduce efficiency (%) = 100% × (Influent – Effluent)/Influent.

Table 3
The N and P balance of phytoremediation system with water hyacinth and water lettuce.

			Reduction	Absorption	Adsorption	Sediment	Duckweed	Difference
WH	Nitrogen balance	(g)	225.98	132.52	14.28	26.75	0.25	52.18
		(%)	100	58.64	6.32	11.84	0.11	23.09
	Phosphorus balance	(g)	27.47	11.85	12.35	4.29	0.03	−1.05
		(%)	100	43.13	44.96	15.62	0.11	−3.81
WL	Nitrogen balance	(g)	224.34	87.62	10.78	35.58	--	90.36
		(%)	100	39.05	4.81	15.86	--	40.28
	Phosphorus balance	(g)	30.16	13.68	9.49	5.53	--	1.46
		(%)	100	45.35	31.47	18.34	--	4.84

*The nitrogen and phosphorus balance were calculated based on values of Tables 2, S2, S3, S4 as follows:
(1) Nitrogen balance for water hyacinth (WH).
Difference value = Reduction (225.98 g, 100%) – Absorption (132.52 g, 58.64%) – Adsorption (14.28 g, 6.32%) – Sediment (26.75 g, 11.84%) – Duckweed (0.25 g, 0.11%) = (52.18 g, 23.09%).
(2) Phosphorus balance for water hyacinth (WH).
Difference value = Reduction (27.47 g, 100%) – Absorption (11.85 g, 43.13%) – Adsorption (12.35 g, 44.96%) – Sediment (4.29 g, 15.62%)-Duckweed (0.03 g, 0.11%) = (−1.05 g–3.81%).
(3) Nitrogen balance for water lettuce (WL).
Adsorption (g) = 3.33 × (1.60 + 1.98 + 1.37) – 10 × 0.57 = 10.78 (4.81%); Sediment (g) = 35.58 (15.86%)
Difference value = Reduction (224.34 g, 100%) – Absorption (87.62 g, 39.05%) – Adsorption (10.78 g, 4.81%) – Sediment (35.58 g, 15.86%) = (90.36 g, 40.28%)
(4) Phosphorus balance for water lettuce (WL).
Difference value = Reduction (30.16 g, 100%) – Absorption (13.68, 45.35%) – Adsorption (9.49 g, 31.47%) – Sediment (5.53 g, 18.34%) – Duckweed = (1.46 g, 4.84%).

In view of nitrogen balance, approximately 23.09% (52.18 g) of TN, which refers to the reduction difference subtracted from the TN absorbed, adsorbed, in the sediments, and in the duckweeds, was unclear in fate in the water hyacinth sinks. Meanwhile, 90.36 g (40.28%) TN was unclear in fate in the water lettuce sinks (Table 3). For phosphorus balance, the difference (−1.05 g) was −3.81% of the TP amount (27.47 g), including those obtained from absorptions, adsorptions, sediments, and duckweeds, for the phytoremediation system using water hyacinth. Meanwhile, the difference (1.46 g) was 4.84% of the TP amount (30.16 g) for the system using water lettuce. However, both difference values ranged between 5% and −5%.

4. Discussion

Phytoremediation, which involves green and environment-friendly technologies that employ plants to remove pollutants from the surrounding environment, has attracted increasing attention in ecological studies (Hanks et al., 2015) because of its safety, high efficiency, low cost, and recyclability of plant harvests. However, the technology is often limited by its time-consuming feature because the life cycle of most plants used for phytoremediation is excessively long (Agunbiade et al., 2009). However, this disadvantage may not be apparent in the alien plants water hyacinth and water lettuce because of their enormous biomass production.

Aquatic plants are highly sensitive to the temperature and pH of the growing media (Ansari et al., 2014); both parameters significantly control the bioremoval of nutrients. The optimum growth temperature for the plants ranges from 20 °C to 31 °C (Cayuela et al., 2007). We measured the water temperature to lie between 24.9 °C and 32.2 °C throughout the entire experimental procedure. Thus, the study was carried out under the appropriate temperature for the test plants water hyacinth and water lettuce.

According to Raju et al. (2015), the maximum increase in biomass production occurred at neutral and slightly alkaline pH (pH 7 and 8), which may be due to the increase in nutrient uptake and immobilization capacity of the water hyacinth samples. The pH of influent water ranged from pH 7.0 to 8.7, and the suitable pH controlled the nutrient absorption and biochemical reactions that took place in living organisms (Ansari et al., 2011). Overall, the experiments were conducted under optimal environmental conditions, although rainy days were frequently experienced in the initial 19 days.

The DO sources in water may mainly include the following three pathways (Qin et al., 2015): (1) water reaeration, (2) oxygen release by aquatic photosynthetic organisms, and (3) root-excreted oxygen. The decreased DO in the sinks was mainly due to the following notions: (1) dense shoots of macrophytes could reduce water reaeration, block sunlight, and consequently reduce oxygen release from the photosynthesis of aquatic photosynthetic organisms, (2) the corruption and decomposition of phytoplankton filtered and intercepted by root mats could consume large amounts of oxygen and (3) oxygen consumption caused by respiration or aerobic microbiodegradation. So, additional aeration may be considered to meet surface water quality during later scale-up of remediation. Thus, we suggest that the enclosures that confined the macrophytes could be designed in a patch style for oxygen shelters of high oxygen demand species (Wang et al., 2012). Under anaerobic conditions, the CO_2 released by microbial respiration as well as the organic acids released by organic matter decomposition decreased the pH, a phenomenon consistent with other studies (Rommens et al., 2003).

Macrophytes with long and dense roots are beneficial for absorbing nutrients and serve as media for intercepting and adsorbing suspended particulate matter, including cyanobacteria (Kim and Kim, 2000). Rhizofiltration, the process of using plant root systems to intercept or degrade contaminants in wastewater, is one of the five types of major processes involved in phytoremediation (Clayton, 2007; Ansari et al., 2014). Moreover, a system with free-floating aquatic plants has been used extensively to treat municipal and industrial wastewater in recent years (Jayaweera et al., 2008). The earlier discussion revealed that both test plants (water hyacinth and water lettuce) served as rhizofiltration plants in phytoremediation technology because of their large roots. In this study, the rhizofiltration of both plants played important roles in intercepting and filtering particulate matter, including algae and particulate organic matter.

Roots play an important role in assimilating nutrients (Yang et al., 2012), and the physiological parameter root activity is commonly used to evaluate ion and water uptake (Wang et al., 2006). The increased root activity in the phytoremediation system employed in the water with lower nutrient (N and P) concentration suggests that macrophyte root activity may be stimulated for nutrient absorption even under such low nutrient conditions. The result indicates that water hyacinth was more suitable for the deep purification of contaminated water with domestic sewage. In addition, positive correlations among DO concentration, root weight and Pn values were found in this study, which suggested that root weight of macrophytes might be affected by DO concentration in water and Pn value of their leaves.

The parameters TSA and AAA are crucial in root function (Yang et al., 2008). Although larger TSA and AAA along with higher root biomass (kg m^{-2}) were found in the water hyacinth, shorter root length was the key limiting factor for algal and Chl *a* removal. This result is consistent with the findings of Hodko et al. (2000) in which contaminant removal efficiency was shown to be dependent on the extension of plant roots in water. Moreover, Putra et al. (2015) reported that phytoremediation application is strictly determined by plant root length.

The potential applications of alien free-floating aquatic plants water hyacinth and water lettuce for nutrient removal from wastewater have been widely reported (Jayaweera and Kasturiarachchi, 2004; Lu et al., 2010). These potential applications were based on the plants' rapid growth and high biomass yield, as well as their hyperactive accumulating (N and P) abilities especially in tropical or subtropical areas (Putra et al., 2015). However, only a few successful examples on nutrient (N, P) mass balance have been demonstrated by *in situ* experiment because of the limitation of flow-controllable experimental devices in a field situation. The results of this study suggested that the self-designed experimental device could achieve flow control, automatic floating, and internal closure. The device provided the guarantee for studies on simulating *in situ* water purification and phytoremediation. Our results indicate that both water hyacinth and water lettuce are highly efficient in removing N and P. This finding is consistent with that of Lu et al. (2010), who demonstrated the efficient N and P removal by water lettuce from eutrophic stormwater in constructed water detention systems, and that of Ayyasamy et al. (2009), who showed that water hyacinth can attain the highest nitrate removal efficiency from synthetic solutions compared with other test plants. Both floating macrophytes could serve as effective phytoremediation plants, while compared with water hyacinth, water lettuce provided a higher TP removal capability (58.27%) and similar TN removal capability (46.56%) on phytoremediation of contaminated water with domestic sewage.

However, our study showed that differences in N and P removal efficiency and the main fates of nutrient elements existed between the water hyacinth and water lettuce samples. Although no significant difference in TN reduction was found between the water hyacinth and water lettuce samples, a higher uptake capability was noted in the water hyacinth samples (58.64% in TN) than in the water lettuce samples (39.05% in TN) during the 30 d experiment (Table 3). Our results suggest that water hyacinth is more suitable for *in situ* phytoremediation by nitrogen removal from wastewater in terms of plant absorption.

In previous studies, 22%–55% of N were unclear in fate during eutrophic water restoration with water hyacinth (Zhang, 2009). According to Gao et al. (2013), the ebullition of N_2O contributed to the minor fraction of total gaseous nitrogen released to air, and the nitrogen loss was quantified in the eutrophic pond containing water hyacinths growing in confined enclosures through an improved bubble trap device. Our results indicate that a great proportion of nitrogen was released by nitrification and denitrification reactions from the phytoremediation system. Moreover, the proportion was similar to absorption by water lettuce, in which the nitrification and denitrification reactions were much more important than the water hyacinth system. Nitrification and denitrification are associated with bacterial biomass and vitality. The enhanced nitrogen release by nitrification and denitrification may be ascribed to the much longer roots and much higher root–shoot ratio of the water lettuce, which could provide larger attachment space and reproduction location for nitrifying and denitrifying bacteria.

The fate of P in the water mainly includes absorption, adsorption, and precipitation (A.L.-Nozaily et al., 2000). In addition, the much higher TP removal efficiency of the water lettuce than the water hyacinth is beneficial for higher P accumulation capability and higher particulate P adsorption and precipitation when intercepted by longer roots. The results suggest that water lettuce is more suitable for the phytoremediation of phosphors from domestic sewage.

Compared with the water lettuce group, the water hyacinth group exhibited a higher efficiency of assimilating greenhouse gases, such as CO_2 by shoot photosynthesis in the atmosphere, because of its higher Pn and larger leaf area when used for contaminant removal from wastewater. The larger dry weight of the water hyacinth group than the water lettuce group may have resulted from the higher efficiency in nutrient (e.g., C and N) assimilation in the former than in the latter. The results also demonstrated that approximately 23% TN was released as gaseous nitrogen, whereas the value from the phytoremediation system with water lettuce was over 40%. Compared with water hyacinth, water lettuce was higher in moisture content, which served as a main issue during the later disposal and resource utilization of the harvested aquatic macrophyte.

Basing from the above analyses, we conclude that the use of water hyacinth for phytoremediation would result in greater environmental benefits and involve easier handling when wastewater contaminants are mainly composed of nitrogen. However, both N and P exceeded the emission standards for most wastewaters, the major issue that puzzled administrations and researchers. Previous studies suggested that the desired ecological management effect is difficult to achieve if only one floating aquatic plant water hyacinth is used (Hu et al., 2007). For dual-contaminant-polluted waters, a combination of water hyacinth and water lettuce is highly recommended in phytoremediation, whereas the confined growth area and relative proportions of these two aquatic macrophytes must be further confirmed in future studies.

5. Conclusions

(1) The phytoremediation potential of water hyacinth and water lettuce for nutrient (N and P) removal from eutrophic domestic sewage was evaluated using self-designed experimental devices, which achieved aims on flow control, automatic floating, and internal closure.
(2) The rhizofiltration of water hyacinth and water lettuce roots played important roles in intercepting and filtering algae, which resulted in significant removal efficiency in algal biomass (WH: 93.50%, WL: 96.37%), algal Chl *a* (WH: 93.94%, WL: 97.66%), and COD_{Mn} (WH: 40.44%, WL: 43.84%), especially for water lettuce with much longer roots.
(3) Water hyacinth is more suitable than water lettuce for the deep purification of water contaminated with domestic sewage with high nitrogen concentrations because of the former plant's larger TSA, AAA, and leaf area and higher root biomass ($kg\,m^{-2}$) and Pn even if the plant was cultivated in oligotrophic water with TN lower than 1.0 mg/L
(4) Similar high TN removal efficiency was shown between water hyacinth (47.42%) and water lettuce (46.56%). And compared with water hyacinth (53.44%), water lettuce provided a higher TP removal efficiency (58.27%) in domestic sewage, which consequently increased P accumulation, adsorption, and precipitation when water flowed through the longer roots.
(5) A combined pattern involving both water hyacinth and water lettuce was recommended for water phytoremediation, although the relative proportions of these two aquatic macrophytes remain to be confirmed in future studies.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ecoleng.2016.07.022.

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