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Growth and kinetic studies of tropical aquatic plants in the presence of macro-nutrients accumulation

by

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Nitrate and phosphate pollution is a worldwide problem as it affects the ecology of water bodies and public health. Phytoremediation is an emerging technology using aquatic plants to remove toxic contaminants from water. In this study, six selected aquatic plants were studied to determine their phytoremediation potential in reducing nitrate and phosphate levels from water.

Abstract – This phytoremediation study explored the use of six selected macrophytes to decrease the nitrate and phosphate levels from water. The kinetics of nitrate and phosphate removal and growth rate of *Ipomoea aquatica*, *Ludwigia hyssopifolia*, *Melastoma malabathricum*, *Eichhornia crassipes*, *Pistia stratiotes* and *Salvinia molesta* were studied to determine their phytoremediation application potential. *I. aquatica* exhibited the greatest rate of nitrate and phosphate removal at 15.1μM $g^{-1}_{dry\ wt}$ day^{-1} and 4.4μM $g^{-1}_{dry\ wt}$ day^{-1} respectively. *S. molesta* and *M. malabathricum* showed the highest growth rate, increasing by an average of 76.0% day^{-1} for fresh mass and 84.6% day^{-1} for dry mass, respectively. All six macrophytes investigated showed good potential in reducing the nitrate and phosphate levels from water.

1 INTRODUCTION

Nitrogen (N) and phosphorus (P) are the two most important macronutrients for the growth and physiological development of plants[1][2]. N is required for photosynthesis, growth and reproduction while P is critical in energy transfer and storage. N and P pollution in water bodies adversely affects both the ecology of water bodies and public health[3][4]. It is responsible for excessive algal growth in surface waters and limiting primary productivity in freshwater streams, rivers, lakes and reservoirs[5]. Nitrate (NO_3^-) and phosphate (PO_4^{3-}) contamination of groundwater is a worldwide problem [6] and nitrate and phosphate contamination of aquifers is associated with non-agricultural[7] and agricultural sources[8]. The removal of control targets for N and P is desirable to restore eutrophic water bodies, such as lakes, streams, reservoirs, ponds and marshes[9]. Technologies developed to remove nitrate and/or phosphate from water include activated carbon process[10], catalytic reduction[11], electrodialysis[12], land disposal[13], reverse osmosis[14], ion exchange[15], chemical denitrification[16] and microbial treatment[17]. Despite the fact that such technologies are effective, pilot scale operation is expensive[18]. Locally, reverse osmosis is commonly used but it consumes a lot of energy, is relatively expensive and environmentally unfriendly[19].

Phytoremediation is an emerging plant-based technology for the removal of toxic contaminants from water. Plants provide phytoremediation treatment 'services'[20] and are efficient in remediating contaminated soil water[19] with high nutrient removal capability[4]. The concept of phytoremediation constructed on wetland models has been implemented in various countries for heavy agricultural runoff and wastewater and stormwater treatment[21][22][23][24]. Stormwater runoff in tropical climates usually contains significant levels of N and P. In Singapore, the average concentration of nitrate in rainwater was reportedly *ca*. 1.4 mg L^{-1}[25]; wastewater contained about 1-5 mg L^{-1} of organic phosphate and 3-10 mg L^{-1} of inorganic phosphate[26]. As stormwater collection is an important water resource in Singapore, the appropriate selection of phytoremediating aquatic plants (macrophytes) is the most versatile and eco-friendly technology, and therefore the most desirable solution for removing N and P.

2 AIMS / OBJECTIVES

This project aims to study the kinetics of nitrate and phosphate removal and the growth rate of six macrophytes to determine their phytoremediation application potential in Singapore's water bodies. These macrophytes were selected from two categories of functional groups, namely emergent plants (*Ipomoea aquatica*, *Ludwigia hyssopifolia* and *Melastoma malabathricum*) and floating plants (*Eichhornia crassipes*, *Pistia stratiotes* and *Salvinia molesta*).

3 METHODOLOGY / MATERIALS

Representative samples of studied macrophyte species were collected from various typical habitats in Singapore. Emergent plants were sown into black bags containing sand washed with deionised water and incubated for at least 3 weeks so as to allow them to acclimatize to the following experimental laboratory conditions: 30/25°C day/night temperatures, 65-85% relative humidity, 12-hour photoperiod and average irradiance (Photosynthetically Active Radiation, PAR) of 300μmol $_{quanta}$ m^{-2} s^{-1}.

To measure nutrients uptake kinetics, a modified depletion method was used[27]. The measurements were done in the same controlled-environment room for plant establishment. Plants of uniform size were selected and placed into nutrient solutions, but without N and P for 2 days. This was to elicit starvation-induced maximal uptake response during the subsequent measurements. To simulate wastewater, a modified form of Hewitt's solution[28] was used to prepare the nutrient solution (Appendix A). The solution contained macronutrients: N as NO_3-N and P as KH_2PO_4, K^+, Ca^{2+}, Mg^{2+} and SO_4^{2-}; and micronutrients: Zn^{2+}, Cu^{2+}, Fe^{2+}, Mn^{2+}, BO_3^{3-} and MoO_4^{2-}. After 2 days of starvation, three replicates per plant species were weighed to obtain an average fresh mass per species (Appendix B) and dried in a forced convention drying oven (FineTech, Korea) at 80°C for 3 days. They were then reweighed again to determine the dry mass: fresh mass ratio for each species. At the end of the experiment, the fresh mass and dry mass of the experimented plants were recorded. The plants were placed in 20 pre-cleaned plastic containers (length 45cm × breadth 30cm × height 31cm) with a capacity of 35L. In each plastic container, 10L of nutrient solution (with N and P) were added. Emergent plants were sown into black bags containing sand re-washed with deionised water. The controls were with the same nutrient solution but without any plants. Control 1 contained only the nutrient solution while Control 2 contained the nutrient solution with a bag of sand. Sampling was conducted on Days 0, 2, 4, 7, 11 and 14.

The pH of nutrient solution was measured using a portable probe (PH-22, Lutron Electronic Enterprise Co. Ltd, Taiwan). Li-Cor 250A (LI-COR Biosciences, Lincoln, U. S. A.) and a hygrometer (Sper Scientific Ltd, Arizona) were used to measure light intensity and humidity respectively. Temperature was also monitored.The water level of each nutrient sample was marked on each container at the start of the experiment. Before sampling, deionised water was added to the containers up to the marked water level, in order to account for water loss through evapotranspiration[29] and the pH of solutions were measured and adjusted to between 5.5 and 6.5 with HCl and NaOH. 25 mL of nutrient samples were collected from random points of each container using a 5 mL micropipette (HTL Labmate). The nitrate and phosphate concentrations in the nutrient samples were analyzed using Flow Injection Analysis (Quick Chem 8000 Flow Injection Analyzer 13-107-04-1B, Hach Company, Colorado, U. S. A.). Before analysis, standard solutions of concentration ranging from 0.02 to 2.0 mg N L^{-1} were prepared from KNO_3; standard solutions of concentration ranging from 0.03 to 0.12 mg P L^{-1} were prepared from $NaH_2PO_4 \cdot 2H_2O$. Results given were then used to calculate the concentrations of nitrate and phosphate. A one-way analysis of variance (ANOVA) test at a significance level of $p<0.05$ determined the significant differences between the control nutrient samples and the experimented nutrient samples.

After the experiments, the plants were harvested. All plant samples were dried in a forced convention drying oven at 80°C for 3 days then weighed to obtain dry masses.

4 RESULTS

The performance of nitrate and phosphate removal by each plant was evaluated using the decrease in the initial nitrate and phosphate concentration in the culture solution and the respective increase in dry and fresh mass. The average rate of nutrient [*X*] removal was calculated by $\frac{[X]_{initial} - [X]_{final}}{biomass_{final} - biomass_{initial}} \times \frac{1}{no.of\ days}$.

Fig. 1(A) showed that *L. hyssopifolia* and *I. aquatica* had high nitrate removal rate among the three emergent macrophytes. The removal of nitrate by *M. malabathricum* was more gradual. Fig. 2(A) showed that the floating macrophytes had similar and high nitrate removal rates. For these five plants, the nitrate concentration decreased steadily from 0.17mM to about 0.01mM over the 14 days. The steeper decrease in nitrate concentration at higher concentrations, with respect to that at lower nitrate concentrations, indicates that the rate of nitrate removal is concentration dependent. In the control setup, the nitrate concentration decreased by about 0.02mM. From Fig. 1(B) and Fig. 2(B), all six macrophytes follow the zero order kinetics for phosphate removal. This implied that phosphate uptake rate was independent of concentration, even when phosphate was depleted.

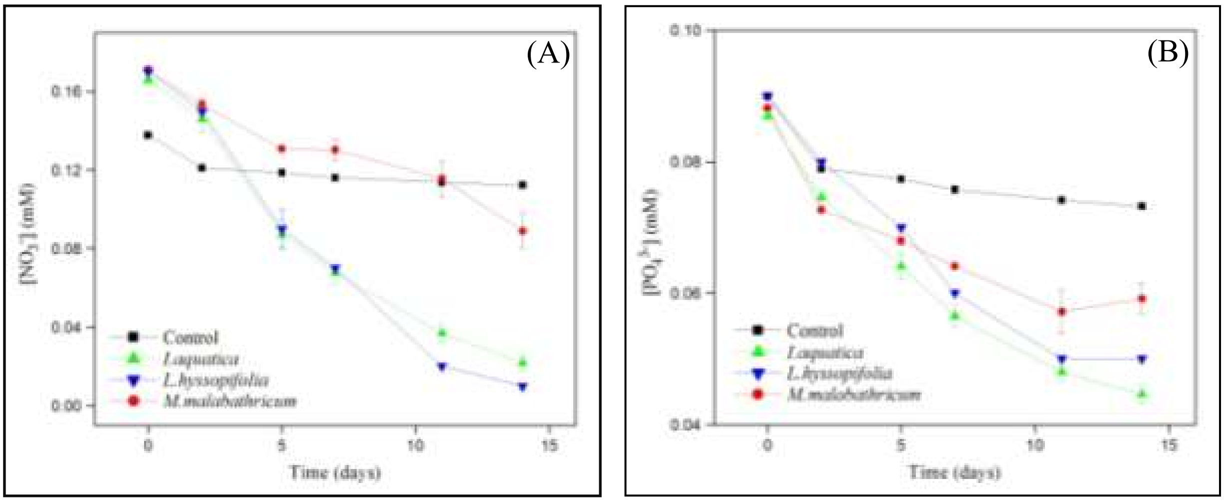

Figure 1. Kinetics of the removal of (A) nitrate and (B) phosphate by emergent aquatic macrophytes

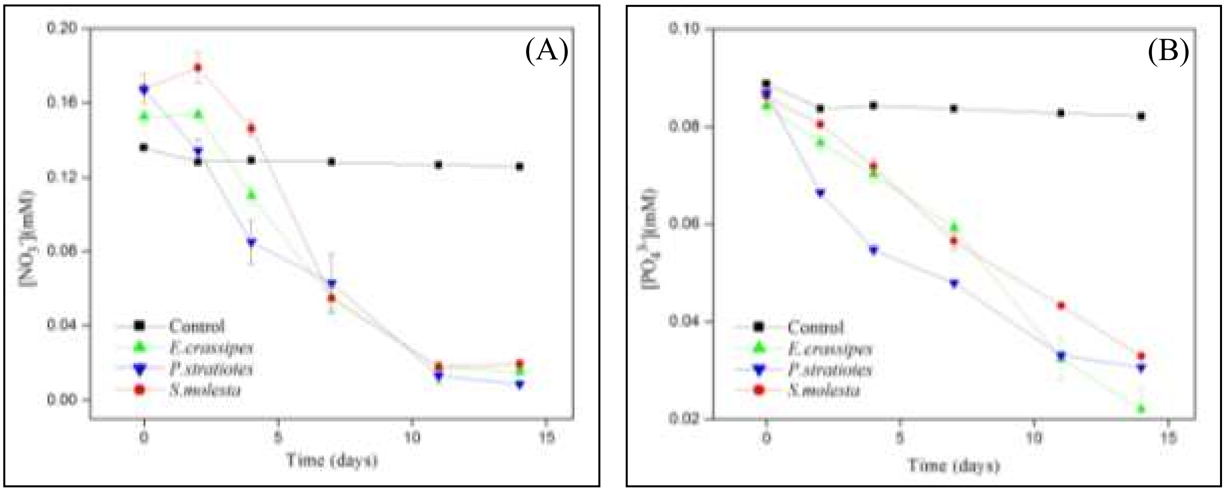

Figure 2. Kinetics of the removal of (A) nitrate and (B) phosphate by floating macrophytes

TABLE 1: NITRATE AND PHOSPHATE REMOVAL RATES OF MACROPHYTES*

Plant Species	NO_3^- Removal Rate		$PO4^{3-}$ Removal Rate	
	(µM g^{-1} $_{fresh\ wt}$ day^{-1})	(µM g^{-1} $_{dry\ wt}$ day^{-1})	(µM g^{-1} $_{fresh\ wt}$ day^{-1})	(µM g^{-1} $_{dry\ wt}$ day^{-1})
I. aquatica	2.0 ± 0.3	15.1 ± 1.2	0.6 ± 0.1	4.4 ± 0.4
L. hyssopifolia	3.3 ± 0.5	12.0 ± 1.0	0.7 ± 0.1	2.7 ± 0.2
M. malabathricum	6.4 ± 2.3	3.1 ± 0.2	0.9 ± 1.6	1.1 ± 0.0
E. crassipes	0.21 ± 0.01	3.74 ± 0.1	0.03 ± 0.01	1.5 ± 0.04
P. stratiotes	0.8 ± 0.1	7.6 ± 0.4	0.3 ± 0.0	2.7 ± 0.1
S. molesta	0.1 ± 0.01	4.28 ± 0.2	0.07 ± 0.01	1.2 ± 0.02

* Values are the mean (± standard error) of n=3 determination over an experimental period of 14 days.

TABLE 2: THE PERCENTAGE GROWTH RATE FOR FRESH AND DRY MASS OF MACROPHYTES*

Plant Species	Percentage Increase in Biomass (%)		Growth Rate per Day (% day^{-1})	
	Fresh Mass	Dry Mass	Fresh Mass	Dry Mass
I. aquatica	82.0 ± 11.6	431.8 ± 35.5	5.9 ± 0.8	30.8 ± 2.5
L. hyssopifolia	56.9 ± 8.4	607.0 ± 38.3	4.1 ± 0.6	43.4 ± 2.7
M. malabathricum	17.0 ± 7.0	1184.1 ± 105.4	1.2 ± 0.5	84.6 ± 7.5
E. crassipes	105 ± 7.3	79 ± 3.2	7.5 ± 0.5	5.6 ± 0.2
P. stratiotes	223.9 ± 30.7	934.6 ±49.9	16.0 ± 2.2	66.8 ± 3.6
S. molesta	1065 ± 5.0	306 ± 5.6	76 ± 0.4	21.9 ± 0.4

* Values are the mean (± standard error) of n=3 determination over an experimental period of 14 days.

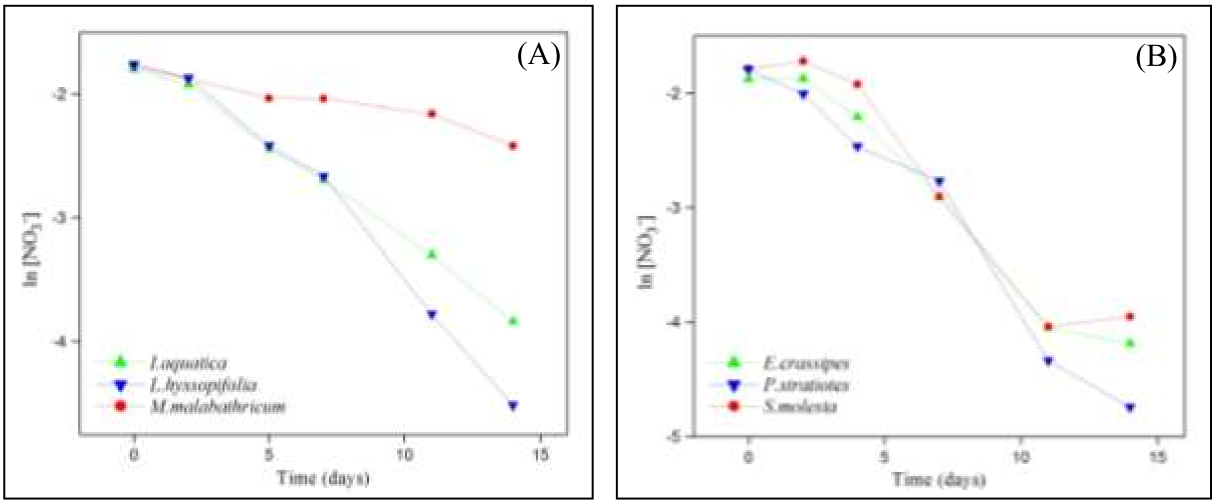

Figure 3. Kinetic analysis of nitrate removal by (A) emergent and (B) floating macrophytes

TABLE 3: NITRATE UPTAKE KINETICS FOR THE FIVE MACROPHYTES*

Plant Species	P Value	Correlation Coefficient	Slope	K_m (ppm)	V_{max} (ppm day^{-1})
I. aquatica	<0.05	0.99	1.35	1.60	1.18
L. hyssopifolia	<0.05	0.97	0.95	1.16	1.22
E. crassipes	<0.05	0.99	0.06	0.144	1.80
P. stratiotes	<0.05	0.97	0.69	0.95	1.37
S. molesta	<0.05	0.99	0.06	0.211	1.70

* *M. malabathricum* is omitted as the nitrate uptake followed the zero order kinetics, meaning that it is independent of nitrate concentration.

Summarising Tables 1 and 2, *I. aquatica* had the greatest removal rate and lowest increase in biomass among the emergent macrophytes. *M. malabathricum* displayed the highest growth in biomass but had the lowest removal rate. *L. hyssopifolia* had an intermediate removal rate and growth. Among the floating macrophytes, *S. molesta* had a relatively high removal rate for nitrate, intermediate removal rate for phosphate and low increase in biomass. *P. stratiotes* had a relatively high growth rate and intermediate removal rate. *E. crassipes* had a low removal rate and slow growth rate.

Based on the Lineweaver-Burk plot (double reciprocal plot), i.e. $\frac{1}{V} = \frac{K_m}{V_{max}} \cdot \frac{1}{[S]} + \frac{1}{V_{max}}$, it was possible to determine K_m (Michaelis constant which is equivalent to nitrate concentration $[S]$ at which the rate of conversion is half of V_{max}) and V_{max} (maximum nitrate uptake rate) of these plants. The Michaelis-Menton expression describes the relationship between rate of nutrient uptake (V) and nutrient concentration $[S]$. Table 3 showed *E. crassipes* have the smallest K_m value, indicating that it will approach V_{max} more quickly than the rest. On the other hand, *I. aquatica* had the highest K_m value. Nitrate uptake by *M. malabathricum* followed the zero order kinetics where nitrate uptake is independent of nitrate concentration.

5 DISCUSSIONS

All six plants were effective in decreasing the levels of nitrate and phosphate in the culture solution (Table 1). Phytoremediation is more useful when highly productive plants are used[30]. The rate of increase in fresh mass of plants can give an indication of how fast the plants would colonize their application site. This will provide an idea of the degree of maintenance needed to keep the plants in check, thereby preventing excessive plant growth which may affect the aesthetics and possibly, some aspects of aquatic ecology. Since the roots of emergent and floating plants are exposed to different water depths, the concurrent use of both types may maximise the efficiency of nitrate and phosphate removal in the various zonations of our local waterways.

Among the three floating macrophytes, *P. stratiotes* exhibited the highest removal rate for nitrate and phosphate (Table 1), the highest increase in dry mass and second highest increase in fresh mass (Table 2). This high growth rate would mean that it would be able to respond quickly to any sudden increase in nitrate and phosphate level in the water body, e.g. in a tropical storm. *S. molesta* has intermediate nutrient removal rate but the highest growth rate for fresh mass while *E. crassipes* had similar nutrient removal ability as *S. molesta,* but a lower nitrate removal rate and much slower growth rate in comparison. It should be noted that the experiments were done in the NIE greenhouse environment where parameters like temperature, light intensity and humidity were less variable than in a real natural environment. While the experimental conditions were within the range of growth conditions for all plants, they may not be optimal for each plant. Urbanc-Bercic and Gaberscik[31] reported that the net photosynthetic rate of *E. crassipes* increased with increasing light intensity and light saturation occurred at a PAR of 800μmol m^{-2} s^{-1}. However, in the current experiment, the average irradiance was only 300μmol m^{-2} s^{-1}. Hence, the rate of photosynthesis could have been limited by the relatively low light intensity at the experimental site, resulting in a lower growth rate for *E.crassipes*. Regardless, it could be postulated that with the appropriate harvesting and containment strategy in place, the floaters are potentially useful candidates for phytoremediation of nitrate and phosphate, provided that the periodic removal of excess floaters are performed. These harvested floaters can then be used as compost, providing some economic returns[32].

Among the emergent amacrophytes, *M. malabathricum* and *I. aquatica* had the fastest growth for dry and fresh mass respectively (Table 2). While *I. aquatica* had a high average removal rate (Table 1), it had one of the lowest increases in growth of dry mass (Table 2). Hence, the total amount of nitrate and phosphate that it can remove would be limited by its low intrinsic growth rate. However, this also means that less maintenance work would be needed for *I. aquatica*. *L. hyssopifolia* exhibited intermediate average removal rate for both nutrients and showed intermediate increase in dry mass. *L. hyssopifolia* can be used in agricultural water bodies with high nitrate concentration and pesticides because Bouldin *et al.*[33] showed that *Ludwigia* species could absorb pesticides. *M. malabathricum* exhibited the lowest nutrient removal rate and the highest growth rate pertaining to dry mass. Thus, it is the least effective plant for phytoremediation of nitrate and phosphate. However, this marginal shrub was reported to be suitable for removing aluminium from contaminated soil[34]. It should be noted that one of the limitations of the project is that the experimental environmental conditions may not be appropriate to each plant's optimal growth conditions. Regardless, all six macrophytes still showed positive results in nutrient uptake and exhibit significant growth rate. Therefore, with the proper implementation and planting of the macrophytes in the environments that are the closest to its optimal conditions, the macrophytes can perform even better nutrient uptake abilities.

From Table 3, plants with low K_m for nitrate, which includes all the floating macrophytes, are able to reach V_{max} faster than the emergent macrophytes. This is because the nitrate reductase enzyme, needed for the assimilation of nitrate, could be induced by nitrate more effectively for these plants[1]. Therefore, the floating macrophytes can be used to remove the nitrate from wastewater at the beginning of the phytoremediation treatment, while the emergent macrophytes will be used at the later part of phytoremediation treatment. By putting the floating macrophytes first into the wastewater system, they will come into contact with wastewater that is of high nutrient concentration. Their low K_m value helps them to absorb nitrate at a faster rate and aid their growth, which is favourable for phytoremediation. This is supported by the high growth rate in terms of dry mass in Table 2. By continually removing a portion of the floater population, it is possible to maintain a proportion of rapidly growing plants within the population. This ensures that at least a portion of the floaters is growing and absorbing nutrients at all times. Hence, the judicious use of very fast-growing floaters has its merits in mopping up rapidly the excessive nutrients present in certain eutrophic water bodies (canals and lakes) in Singapore's context. The floaters will therefore complement the sustainable but slower nutrient uptake capacities provided by the emergents.

6 CONCLUSIONS

This study was successful in utilizing and refining past methods to screen the nitrate and phosphate removal capacity of six macrophytes, namely *Ipomoea aquatica*, *Ludwigia hyssopifolia*, *Melastoma malabathricum*, *Eichhornia crassipes*, *Pistia stratiotes* and *Salvinia molesta*. The study was able to provide a good gauge of the phytoremediation potential of the six macrophytes. *P. stratiotes* had the highest nitrate and phosphate removal rate and the highest increase in dry mass for floating macrophytes. *I. aquatica* had the fastest growth for fresh mass and a high average removal rate for emergent macrophytes. In order to clean up Singapore's water bodies, the concurrent use of these two plants will potentially be efficient in decreasing the nitrate and phosphate levels of water bodies. *S. molesta*, *E. crassipes* and *L. hyssopifolia* were also found to be relatively effective as they had intermediate removal rates. Additionally *I. aquatica*, *E. crassipes* and *L.* Hyssopifolia, with their attractive flowers, will not only be effective at phytoremediating water bodies, these plants will also increase the plant diversity and aesthetic appeal of the waterways.

Future works can be done to match the macrophytes to the sites of application with suitable environmental conditions, so as to optimize growth and removal rates of N and P. *E. crassipes* and *S. molesta* are considered to be aquatic weeds[35], and may be breeding sites for vectors of human diseases and habitats for venomous snakes[36]. Therefore, proper management and harvesting should be investigated to avoid such complications. Stormwater runoff not only contains nitrate and phosphate but also other dissolved ions like ammonium. Hence macrophytes with ammonium phytoremediation properties should be considered as well. *Cyperus papyrus* is reportedly efficient in this aspect[37], and hence an ideal candidate for future study. Studies can also be done on submerged macrophytes, such as *Hydrilla verticillata*, which grows optimally in water of approximately 1 to 3 metres deep[38], since only submerged macrophytes can affect dissolved nitrate and phosphate at these depths.

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STATEMENT OF CONTRIBUTION

All student authors contributed to this work. Dr Tan and Dr Yong came up with the initial ideas for this research. Kai Hui and Yinghui worked together to finalize the details of the research. Kai Hui carried out a large part of the experimental work while Yinghui contributed most of the kinetics analysis. The results and discussion of this paper was done by Yinghui while the rest of the report was done and edited by Kai Hui. Yinghui plotted the graphs for this paper using the software Origin and Kai Hui drew the tables using Microsoft Excel. Dr Tan and Dr Yong provided access to the equipment and assisted us with the experiments.

APPENDICES

APPENDIX A: CHEMICAL COMPOSITION OF VARIOUS STOCK NUTRIENT SOLUTIONS

Stock Solution*	Chemical Compound	Amount Used (g)^	Concentration (µM)
A	$Ca(NO_3)_2 \cdot 4H_2O$	4.24	17.8
	Fe Na EDTA	6.70	18.3
B	KNO_3	14.44	14.3
C	$NaH_2PO_4 \cdot 2H_2O$	4.11	26.3
	$MnSO_4 \cdot 1H_2O$	8.92	52.8
	$ZnSO_4 \cdot 7H_2O$	1.16	0.403
	$CuSO_4 \cdot 5H_2O$	1.00	4.00
	H_3BO_3	12.40	201
	$Na_2MoO_4 \cdot 2H_2O$	0.48	1.98
	NaCl	23.40	394
	$CoSO_4 \cdot 7H_2O$	0.21	0.747

* For every 1L of stimulated wastewater, 2.5mL of Solution A, 0.5mL of Solution B and 4.0mL of Solution C were used.

^ The amount of salt used to prepare 1L of stock solution.

APPENDIX B: BIOLOGICAL CHARACTERISTICS OF PLANTS IN EACH EXPERIMENTAL TANK

Plant Species	Average Fresh Mass (g)	Average Dry Mass (g)	No. of plant(s)
I. aquatica	6.9 ± 0.2	1.0 ± 0.1	1
L. hyssopifolia	2.0 ± 0.2	0.3 ± 0.0	1
M. malabathricum	9.2 ± 1.6	2.6 ± 0.6	1
E. crassipes	64.6 ± 0.8	4.2 ± 0.1	2
P. stratiotes	10.8 ± 0.9	0.6 ± 0.0	2
S. molesta	13.0 ± 0.0	1.0 ± 0.0	5 clumps*

* Each clump was palm-sized

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12

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