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Potential of Water Hyacinth for Phytoremediation in Low Temperature Environment

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Water hyacinth, i.e., Eichhornia crassipes *Solms can remove a large variety of different pollutants from water and are considered an excellent candidate for phytoremediation of contaminated waters. Nevertheless, exploitation of water hyacinth for phytoremediation has so far been limited to tropical or subtropical regions. In this study, the influence of temperature on the morphology, growth, and nutrient removal of water hyacinths was studied in a relatively low temperature range, 12–25°C, in the form of three night/day temperature cycles, i.e., 12/15°C, 15/20°C, and 15/25°C. It was shown that water hyacinth is very sensitive to temperature in the tested range and 15/25°C is the most favorable condition among the tested. A biomass productivity of 10.7 tons DW/ha/yr, total nitrogen removal rate of 400 kg N/ha/yr and total phosphorous removal rate of 65 kg P/ha/yr were estimated according to the experimental results on the basis of five operational months per year. No visible morphological changes were observed when the plants were cultivated at 15/20°C or 15/25°C but significant morphological changes were observed when the plants were transferred from a rooftop greenhouse to a growth chamber controlled at 12/15°C with plant size shrinking significantly. Nevertheless, substantial plant growth and nutrient removal were observed at 12/15°C, although with a prolonged adaptation phase of 2 weeks. Results of this research suggest good potential of water hyacinths for water phytoremediation in relatively cold regions such as Canada.* © 2013 American Institute of Chemical Engineers Environ Prog, 32: 976–981, 2013

Keywords: water hyacinth, Eichhornia crassipes *Solms, temperature, phytoremediation, nutrient removal*

INTRODUCTION

Phytoremediation is to mitigate the level of pollutants in contaminated soil, water, and air using plants capable of assimilating or decomposing inorganic or organic pollutants such as nitrogen, phosphorus, metal ions, pesticides, solvents, oil, polycyclic aromatics (PCA). Phytoremediation is carried out *in situ*, offering the opportunity to reduce the costs of treatment in comparison with that of traditional processes. Furthermore, phytoremediation employs naturally occurring organisms and preserves the environment in a more natural state. It is potentially the most environmental friendly method for soil and water remediation [1,2].

Water hyacinth, i.e., *Eichhornia crassipes* Solms, is a free-floating perennial aquatic plant, native to tropical South America. It grows rapidly in favorable conditions and forms dense, interlocking mats. The ability of water hyacinth to grow rapidly in heavily polluted water has attracted considerable attention as a potential agent for phytoremediation of contaminated waters. It has been widely exploited for treatment of sewage effluents, agricultural drainage waters [3], industrial effluents, and eutrophic lake waters [4].

Some of the advantages of using water hyacinth for phytoremediation of eutrophic water include: (1) water hyacinth is a fast growing, prolific and productive aquatic plant that can uptake large quantities of nutrients from water; (2) it can control the growth of algae and submerged macrophytes by shading and nutrients competition; (3) it is easy to harvest; and (4) it is an attractive crop for production of biofuels, fertilizers, and animal feed.

Nevertheless, water hyacinths are considered an invasive species and an environmental hazard in tropical and subtropical regions. Due to its fast growing rate, water hyacinth may impose a severe threat to the survival of native species. Furthermore, massive water hyacinths often block the water ways and their lengthy and dense roots systems may also form thick mats that blocks the oxygen transfer from the atmosphere to the water covered by the plants, causing hypoxia during night time. In these scenarios, controlling the spread and growth of water hyacinth to minimize its negative impacts on economy, transportation, and the ecosystem often become a very challenging task.

To this end, employing water hyacinths in relatively cold regions such as Canada may incur some unique advantages. Owing to the decreased growth rate in colder environment, controlling the overgrowth of water hyacinths become a much easier task to accomplish. Furthermore, it is difficult for water hyacinths to survive the severe winter in cold regions such as most parts of Canada, making the control of its spread to unwanted water bodies relatively easy to achieve. However, the cold climate in these areas could greatly shorten the operational seasons and reduce the efficiency should water hyacinths be employed for phytoremediation. Therefore, it is of relevance to investigate the impacts of temperature on the growth and nutrient removal of water hyacinths in a relatively low temperature range to help define the lower end of operational temperature and estimate the potentials of phytoremediation using water hyacinths in such regions.

MATERIALS AND METHODS

Plants

Water hyacinth plants, *Eichhornia crassipes* Solms, were purchased from a local plant store (Moore Water Gardens, Port Stanley, Ontario, Canada). Water hyacinth plants were planted in plastic containers containing 30 L culture medium, after being rinsed using distilled water for several times and all plants were cultivated in a growth chamber at 20/25°C (night/day) for 1 month before being moved to a rooftop greenhouse. These procedures were followed to minimize the possibility of carrying diseases or insects in to the greenhouse. The plants used in all experiments were initially cultivated in the rooftop greenhouse.

Cultivation Medium

The culture medium was prepared based on modified 1/10 Hoagland's solution (contain 50.5 mg/L KNO_3, 49.3 mg/L $MgSO_4{\cdot}7H_2O$, 13.6 mg/L KH_2PO_4, 118 mg/L $Ca(NO_3)_2{\cdot}4H_2O$, 8 mg/L NH_4NO_3, 1.5 mg/L Fe-EDTA, and micronutrients). Initial pH of the media was 7.5–7.9.

Experiment Conditions

The experiments were performed in a ventilated temperature and solar radiation environment controlled growing chamber (CONVION, MOD E15). The temperature was programed to start ramping up at 6:00 am for 4 h in the morning until 10:00 am to the highest setting point and start ramping down at 19:00 pm for 4 h until 23:00 pm to the lowest set point every day. The artificial light used was fluorescence lights, given at a constant radiation level of around 630 μEn/m^2s to simulate the day time, which was started from 6:00 am in the morning and ended at 19:00 pm at night.

Plants are cultivated in plastic boxes with a surface area of 0.112 m^2 containing 38 L of medium. The medium was replaced completely at weekly intervals with a fresh nutrient solution. All the plants in the container were taken out in a mesh basket and drained for 10 min following the method to measure plant weight by Reddy [5]. The fresh weight of the plant was measured by using a laboratory balance (Ohaus Scout Pro, 2000 ± 0.1 g, Scout Pro Ohaus Canada). The total fresh weight of water hyacinth plants was recorded at a weekly interval. Every temperature cycle was tested twice following the same procedures for reproducibility.

Water Chemistry Analysis

Water samples were collected twice a week, on the 4th and 7th day of the week, and stored in sealed plastic bottle at 4°C. Water total nitrogen (TN) was analyzed using Persulfate Method [6] and total phosphorous (TP) using Ascorbic Acid colorimetric method [6], following the Standard Methods for the Examination of Water and Wastewater [6]. The chemical analysis test kits were purchased from Hach. The absorbance for TN and TP was taken at wavelength of 345 nm and 890 nm, respectively. The standard concentrations and absorbance calibration curve were manually repeated for accuracy check.

Calculation

The plant growth rate and productivity were determined using the following equations.

The plant growth rate (R) was calculated as

$$R=\frac{W-W_0}{\Delta t} \tag{1}$$

or by regression in MSExcel to obtain the growth rate as the slope of the plant fresh weight vs. time curve in the growth phase. Biomass productivity was calculated by dividing the plant growth rate with A (m^2), the surface area of the growth container.

Nutrient (N or P) removal, %, was calculated as

$$Removal,\ \%=\frac{(C_0-C)}{C_0}\times 100\% \tag{2}$$

and annual nutrient removal rate, kg/ha/year, was calculated as

$$Removal\ rate=\frac{(C_0-C)}{\Delta t\times A}\times V\times 365\times 0.01 \tag{3}$$

Furthermore, the specific removal rate, kg/kg W/yr, was calculated as

$$k=\quad\frac{(C_0-C)\times V}{\Delta t\ \ W}\times 10^{-3}\times 365 \tag{4}$$

where C was the nutrient (N or P) concentration (mg/L) of water sampled at time t (day), C_0 was the initial nutrient concentration added to the container, V (L) was the volume of the nutrient medium, W was the plant fresh weight (kg) at the sampling time t, and Δt was the time period of time (day) between the latest change of medium and the time of sampling.

RESULTS AND DISCUSSION

Effects of Temperature on Plant Growth

Three different night/day temperature cycles in the range of 12–25°C, i.e., 12/15°C, 15/20°C, and 15/25°C (night/day), were tested for the effects of temperature on water hyacinth growth for a period of 1 month. In the chamber at 15/25°C, plants started to grow rapidly after transfer without visible adaptation phase. The surface area of the container was half covered at the beginning of the experiment and was completely covered by the end of the 2nd week.

At 15/25°C, plant leaves and stems and bulb showed healthy green and reproduced by stolon spreading and started blossom in 2 weeks. Falling off of some roots was observed in the second week. The morphology of the leaves, including their size, did not change significantly during the 4 weeks of cultivation. However, the roots system was enlarged with longer roots at the end of the 4-week period.

For plants transferred to the chamber at 15/20°C, an adaptation phase was observed in the first week. The plant growth was substantially slower in the first week than in the later weeks. Similar to that at 15/25°C, plants growing at 15/20°C did not show significant morphology changes.

For plants growing at 12/15°C, a prolonged 2-week adaptation phase was observed. Furthermore, as shown in Figure 1, plants experienced dramatic morphological changes. The plant shown in picture Figure 1 was grown in the rooftop greenhouse with a minimal temperature of 20°C. It was a large plant with green healthy leaves. On the other hand, Figure 1 shows the plant after cultivation at 12/15°C for 4 weeks. It is clear that the plant was much smaller and it was with brown- and crispy-margined leaves. When the large plants grown in the greenhouse were transferred to the chamber at 12/15°C, the leaf margins gradually turned crispy and leaf color turned to brown and eventually died off. New leaves grew out but they were of much smaller size with leaves of brown and crispy edges. As shown in Figure 1, the large green bulb belonged to an old large leaf that already died off. It is interested to notice that after the first 2 weeks, the plant started to spread by producing stolon. A stolon is

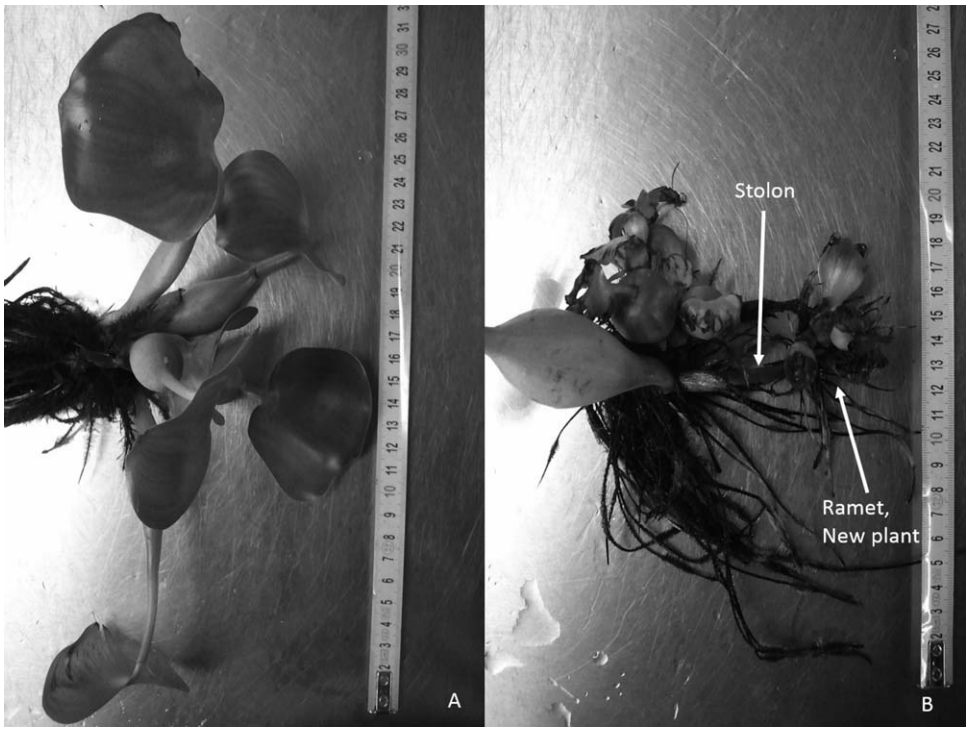

Figure 1. Water hyacinth morphology changes with time at 12/15°C: A, Plant grown in greenhouse before transferred to 12/15°C; B, Plant grown at 12/15°C after 4 weeks).

highlighted in Figure 1. These results indicate that water hyacinth has the ability to adapt to temperature environment, although with significantly reduced plant size and growth rate.

The morphology change of the plants at the low temperature seems to be a way for the plants to survive the low temperature condition. While the reduced plant growth rate was apparently due to the reduced photosynthesis efficiency of the plants at the low temperature environment, the adaption advantages of the reduced plant size remains unknown. One possible explanation is that the reduced plant size makes it less demanding on bioenergy for plants to maintain their viability and therefore could direct more of the limited bioenergy generated in the reduced photosynthesis to reproducing new plants.

As shwon in Figure 2, the plant fresh biomass profiles show linear growth with a constant growth rate in the growth phase at all the three temperature cycles. At the lowest temperature cycle, i.e.,12/15°C (night/day), as shown in Figure 2A, a prolonged adaptation phase of approximately 2 weeks was observed in both tests, in which no increase of plant fresh weight was recorded and the growth rate in the last 2 weeks was was 3.8 ± 0.4 g/day. In the process of adapting from the more favorable greenhouse condition (temperature 20°C or above) to the low temperature environment, some roots and leaves of the plants die off and detached from the plants. The weight of these parts that fell off the plants during the entire period of experiments were measured and included in the calculations.

The plants also had visible adaptation phase at 15/20°C, although with a shorter period of less than 1 week. As shown in Figure 2B, the growth rate, i.e., the slope of the fresh weight vs. time curve, was significantly smaller than that in the later 3 weeks. The growth rate in the growth phase was a constant of 9.4 ± 0.8 g/day, which is higher than at lower temperature cycle of 12/15°C. As discussed before, dramatic morphological changes were observed at 12/15°C while the plant morphology at 15/20°C was not significantly affected.

There was no visible adaptation phase for plants being cultivated in the chamber at 15/25°C. The plants started to grow the first week and the growth rate was constant throughout the 28 days. It is worth noting that the only difference between the 15/20°C cycle and the 15/25°C cycle was that the day time temperature increased from 20°C to 25°C while the night time temperature was controlled both at 15°C. The five-degree day temperature increase resulted in significant increase of growth rate from 9.4 ± 0.8 to 15.7 ± 2.0 g/day. The fact that the plants, although required lengthy adapation time, could grow at temperature cycle as low as 12/15°C is quite encouraging. Since in the Canadian environment, the temperature could be fluctuating between such a low temperature and some higher and more favorable temperature for a lengthy period in the late spring and in the early autumn seasons, the ability of the plants to survive and grow in such temperature cycles has significant practical relevance.

The plants showed linear growth in the growth phase in all three temperatures. The growth rate of plants at the three temperature cycles were calculated by taking linear regression of the plant fresh weight vs. time plot in the growth phase and are listed in Table 1. As shown in Table 1, the plant growth rate was 3.8 ± 0.4, 9.4 ± 0.8, and 15.7 ± 2.0 g/day for cycles 12/15°C, 15/20°C, and 15/25°C, respectively. It is worth mentioning that the reduction of day time temperature from 25 to 20°C while mataining the night time temperature at 15°C resulted in a reduction of growth rate by 40%. Furthermore, when the temperature was further decreased to 12/15°C, drastic morphology changes were observed and the reduction of growth rate was 59% compared with 15/20°C cycle and 76% compared with 15/25°C cycle. In other word, the growth at 12/15°C was less than a quarter of that at 15/25°C. As also shown in Table 1, the fresh weight of plants

increased by 1.2, 1.6, and 2.3 times at temperature cycle of 12/15°C, 15/20°C, and 15/25°C, respectively, during the 1-month experimental period. The productivity of water hyacinth plant for a growth duration of 1 month at three temperatures range was 34.4 ± 4.8, 80.8 ± 4.9 and 123.4 ± 17.3 g/d.m^2, respectively. The productivity at temperature 15/20°C was 2.3 times compared with that at temperature 12/15°C. The highest productivity was obtained at temperature cycle of 15/25°C.

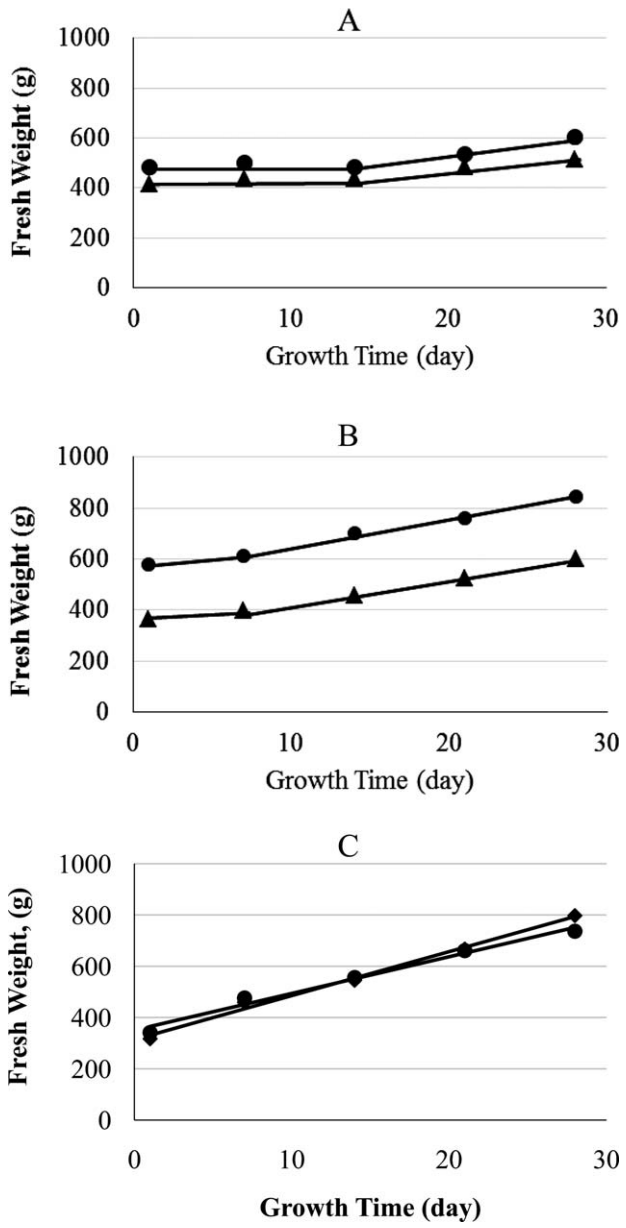

Figure 2. Water hyacinth fresh biomass profiles at three temperature cycles: A, 12/15°C; B, 15/20°C, and C: 15/25°C.

Effects of Temperature on Nutrient Removal of Water Hyacinth

The weekly nutrient removal of TP and TN are presented in Figures 3 and 4, respectively. Water samples were taken on the 7th day of every week, and the culture was replaced with fresh medium immediately after water sampling. To check the accuracy of TN and TP measurements using the Hach kits, standard TN solution (Fisher Scientific Canada) containing 10 mg N/L and 5 mg N/L and KH_2PO_4 solution containing 1 mg/L PO_4^{3-} (i.e., 0.33 mg P/L) were used to calibrate the methods. The corresponding mean values of the triplet measurements of 0.33 mg P/L solution, 10 mg N/L solution, and 5 mg N/L solution were 0.312 ± 0.0098 mg P/L, 10.1 ± 0.29 mg N/L, and 5.06 ± 0.036 mg N/L, respectively.

As shown in Figure 3, at temperature 15/25°C, the weekly phosphorus removal was approximately 69%, which increased to approximately 84% in the second week and was constant afterward. At temperature 15/20°C, a low phosphorus removal of 35% was observed in the first week, which increased to 50% in the second week and 68% in the third and was constant afterward. It was a surprise to observe that the phosphorus removal at 12/15°C was almost constant at level of approximately 65% throughout the 4 weeks.

Comparing the phosphorus removal at the three temperature conditions, it seems to be clear that the phosphorus removal increases with temperature in the tested temperature. This is reasonable because phosphorus assimilation is directly related to plant growth, which increases with temperature in the tested range. It is worth noting that at 15/20°C and 15/25°C temperature conditions, the phosphorus removals were both at a relatively low level in the first week and eventually increased to a stable level. It took one more week for stable level to be reached at 15/20°C than at 15/25°C, corresponding to the observation that there was an adaptation phase of 1 week at 15/20°C but no adaptation phase at 15/25°C in terms of plant growth. It should be pointed out, however, that the phosphorus removal at 12/15°C did not follow this trend and was constant throughout the 4 weeks of cultivation period. It should be pointed out that TP measurements using the Hach kits are associated with significant errors, which were particularly evident with the samples at 12/15°C (Figure 3). The experimental error may have contributed to the different trend observed with the 12/15°C in comparison with that observed with the other two temperature conditions.

The weekly nitrogen removal data are presented in Figure 4, which shows that there was no clear trend in the weekly nitrogen removal profiles at different temperatures although nitrogen removal seemed to be slightly higher at 15/25°C than in the other two temperature conditions. These results

Table 1. Summary of water hyacinth growth data for 28 days experiment at three temperature cycles (Mean values of two parallel tests)

Temperature cycle		Initial fresh weight (g)	Final fresh weight (g)	Biomass multiplication, times	*R* (g/day)
12–15°C	Test 1	415.5	512.6	1.2 ± 0.01	3.8 ± 0.4
	Test 2	482.7	603.4		
15–20°C	Test 1	580.2	844.4	1.6 ± 0.1	9.4 ± 0.8
	Test 2	363.5	600.0		
15–25°C	Test 1	341.2	736.7	2.3 ± 0.3	15.7 ± 2.0
	Test 2	317.2	797.6		

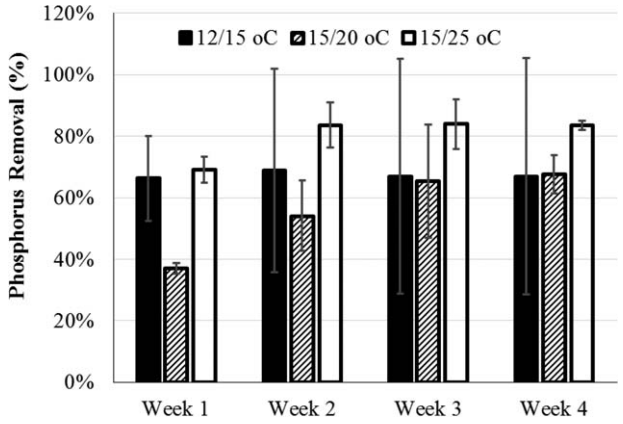

Figure 3. Weekly TP removal at three temperature cycles.

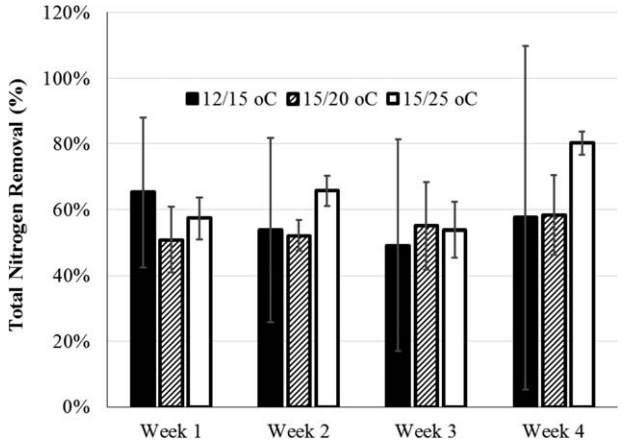

Figure 4. Weekly TN removal at three temperature cycles.

seem to suggest that nitrogen removal, unlike phosphorus removal, is less dependent on plant growth rate in the tested condition. This could be tentatively explained by the capacity of plants to accumulate nitrogen when the conditions do not favour growth or when the environment contains abundant nitrogen sources. For instance, it was observed that green alga *Neochloris oleoabundans* could use chlorophyll, a nitrogen-rich photosynthetic pigment, as an intracellular nitrogen reservoir [7], which could be the case of other plants too. The content of chlorophyll varies in water hyacinth not only with the pigment contents of different parts (i.e., leave, bulb, stem, and roots) but also with the ratio of these parts of individual plants, both of which changes with temperature and other conditions. It is worthwhile to point out that the standard deviations of TN measurement using the Hach kits was also quite large, which is particularly evident with the results obtained at 12/15°C (Figure 4).

Operation Window of Phytoremediation Using Water Hyacinths In Low Temperature Environment

The results of this study show that water hyacinths can grow and function well as phytoremediation agents in a night temperature of 12°C and daytime temperature of 15°C or above. Using the Ottawa region as an example, as shown in Figure 5, the monthly average tempearture in the region is above 12°C from middle April to middle September, leaving an operation window of approximately 5 months, i.e., May, June, July, August, and September. Among these 5 months,

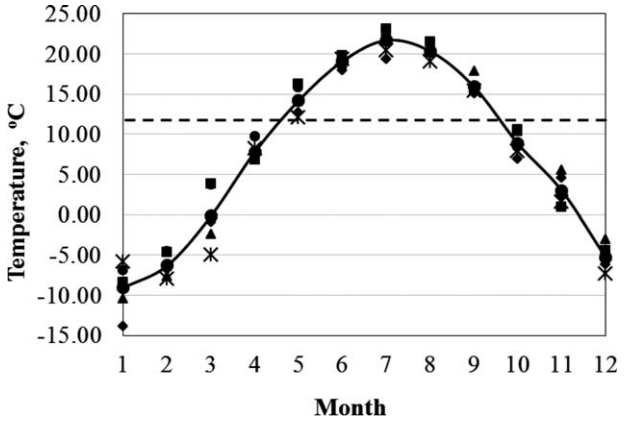

Figure 5. Ottawa 5-year monthly temperature average (2008–2012) from Environment Canada.

May, June, July, and August have an average temperature of 15°C or higher. June and July, with an average temperature of 20°C or above, are expected to be the most productive seasons for both plant growth and nutrient removal.

The fact that only 5 of the 12 months of a year might be suitable for phytoremediation using water hyacinths in areas such as the Ottawa region are factored into the estimation of the annual biomass productivities and nutrient removal. As shown in Table 2, the annual biomass productivity on the basis of five productive months per year is 2.6, 6.4, and 10.7 tons DW/ha/yr at temperatures of 12/15°C, 15/20°C, and 15/25°C, respectively; the annual nitrogen removal rates are 313, 353, and 400 kg N/ha/yr at 12/15°C, 15/20°C, and 15/25°C, respectively and that of phosphorus removal are 51, 45, and 65 kg P/ha/yr at 12/15°C, 15/20°C, and 15/25°C, respectively. The annual plant biomass productivities were significantly lower than the literature data, which are typically in the range of 100–700 ton DW/hectare/year [9]. This is not surprising since the literature data are collected from tropical or subtropical environments with year-round plant growth at significantly higher temperature.

It should be cautioned that the nutrition, temperature, light intensity, and many other environmental parameters would be very different from the well-controlled laboratory conditions used in this study. For instance, solar radiance as the energy source for photosynthesis is another important factor affecting plant growth and nutrient removal. As shown in Figure 6, the solar radiance as recorded at the roof of the greenhouse at the main campus of University of Ottawa fluctuates with the seasons. It is worth noting that the monthly average solar radiance during the entire operational season, i.e., April-September, was either close to (i.e., April and September) or much higher than 630 $\mu En/m^2s$, the radiance level used in the growth chambers for all experiments. It is therefore reasonable to expect the plant growth and nutrient removal of water hyacinths could well exceed their performance as observed in this study.

Although the actual capacities of water hyacinth for biomass production and nutrient removal in cold regions such as Canada remains to be verified by outdoor experiments, the data do point to the excellent phytoremediation capacity of water hyacinths in the operational seasons. This could be particularly advantageous taking into consideration that the plants are much less aggressive in relatively cold environments and the fact that the control of their spread as an invasive species to unwanted waters is less a problem since it is difficult for the vegetation of water hyacinths to survive the severe winter in these regions, which is characterized by long period of time with temperature below the freezing point.

Table 2. Estimation of water hyacinth annual plant biomass productivity and nutrient removal capacity at the three temperature cycles on 5-month basis

Temperature cycle	Plant biomass (Ton DW/ha.yr)*	Total nitrogen (Kg N/ha.yr)	Total phosphorous (Kg P/ha.yr)
12–15°C	2.6	313	51
15–20°C	6.4	353	45
15–25°C	10.7	400	65

*Calculated according to the data shown in Table 1 with the assumption of 95% moisture content of water hyacinth [8].

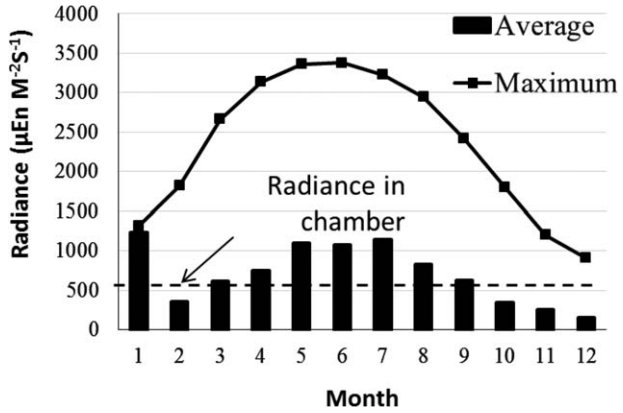

Figure 6. Solar radiance of 2012 recorded at the rooftop greenhouse on the main campus of the University of Ottawa, Ottawa, ON, Canada. The dashed line represents the radiance used in the environment growth chamber, i.e., 630 μEn M^{-2} S^{-1}.

CONCLUSION

Results presented in this study demonstrated in laboratory conditions that temperature has strong impacts on plant growth and nutrients removal of water hyacinths. For plants growing at low temperature cycle of 12/15°C, drastic changes in plant morphology were observed. As temperature increased, the plant growth and nutrient removal rate increased as well. At the favorable growth condition of 15/25°C, the TP removal can reach up to 84% and TN removal was up to 80%. Adaptation phase, in which the plants do not grow but adapt themselves to the new environment, were observed when the temperature was 15/20° or lower and the lower the temperature, the longer the adaptation phase. Despite of a prolonged adapting phase of 2 weeks, substantial plant growth and nutrient removal was observed at temperature as low as 12/15°C (night/day). This research showed water hyacinth is a promising agent for phytoremediation in cold regions such as the Ottawa region in Ontario, Canada.

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ABBREVIATIONS

PCA polycyclic aromatics
TN total nitrogen
TP total phosphorous

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