J. Aquat. Plant Manage. 23: 35-40

Growth and Nutrient Uptake of Pennywort (*Hydrocotyle umbellata* L.), as Influenced by the Nitrogen Concentration of the Water[1]

K. R. REDDY AND J. C. TUCKER[2]

ABSTRACT

The effect of varying levels of nitrogen (N) additions on the growth and nutrient removal of pennywort (*Hydrocotyle umbellata* L.) was evaluated using microcosm aquaculture systems. Biomass yields of pennywort increased from 11.7 to 15.7 g (dw) when^{-2} day^{-1} when plant available N in the growth media was increased from 303 to 1513 mg N m^{-2} day^{-1} (4 to 20 mg N l^{-1}). Further increase to 40 mg l^{-1} did not increase the yields. Nitrogen release from underlying sediments supported a biomass yield of 5.4 g (dw) m^{-2} day^{-1}. Nitrogen concentration of the plant tissue was also increased with each increment level of N in the culture medium of up to 20 mg N l^{-1} (1513 mg N m^{-2} day^{-1}). This represents a maximum N uptake rate of 41 mg N g^{-1} (dw) of plant tissue. Nitrogen uptake by the plants cultured in the systems with underlying sediments was 20 mg N g^{-1} (dw) of plant tissue. Phosphorus uptake was in the range of 9.2 to 10.2 mg P g^{-1} (dw) of plant tissue at all levels of N additions. Unlerlying sediments supported a P uptake rate of

[1]Florida Agricultural Experiment Stations Journal Series No. 6119.

[2]Professor and Biological Scientist, respectively. University of Florida, IFAS, Central Florida Research and Education Center, Sanford, FL 32771.

4.3 mg P g^{-1} (dw) of plant tissue. The potential N and P removal rates by the pennywort system during winter months were found to be 321 to 645 mg N m^{-2} day^{-1} and 103 to 106 mg P m^{-2} day^{-1}, respectively. Results of this study indicate that pennywort can be successfully grown during cooler months and can be used as a substitute for water hyacinth in a water hyacinth-based wastewater treatment system.

Key words: Aquatic plant, water treatment, nitrogen uptake, biomass, wastewater, water hyacinth.

INTRODUCTION

Aquatic plants have been found to play a significant role in removing nutrients from polluted waters (Boyd, 1970; Steward, 1970). Among many aquatic plants commonly found in tropical and sub-tropical freshwater ecosystems, water hyacinth plants were successfully employed in wastewater treatment systems and were found to remove up to 90% of the N and 70% of the P, depending on the residence time of wastewater (Cornwell et al., 1977; Wolverton and McDonald, 1979; Reddy et al. 1982). The recent energy crisis prompted several researchers to evaluate the potential of plant biomass produced in the wastewater treatment systems for conversion to methane.

Although water hyacinth ranked highest in biomass yield among freshwater aquatic plants (Reddy et al., 1983), its growth is severely affected by frost during winter months (Reddy and DeBusk, 1984), resulting in poor nutrient removal efficiency. The sensitivity of water hyacinth to cold temperatures has prompted the search for plants tolerant to winter temperatures. Such alternative plants need to exhibit relatively high productivities and nutrient removal capacities. Among the three large-leaf aquatic macrophytes evaluated for productivity during winter months (Reddy and DeBusk, 1984), pennywort (*Hydrocotyle umbellata* L.) had the highest growth rate. Like water hyacinth, pennywort is a floating aquatic plant and grows through the year in freshwater habitats of Florida.

Nitrogen probably is the most limiting plant nutrient affecting the biomass yields of aquatic plants and pennywort is no exception. In order to optimize the biomass yields and water treatment potential of the pennywort system, data on N levels critical to achieving maximum growth and nutrient uptake in the water are needed. This study was designed to evaluate 1) the effect of varying levels of N in the culture media on growth and nutrient uptake of pennywort, and 2) the underlying sediment of a pond as a source of nutrients for pennywort.

MATERIALS AND METHODS

Productivity and nutrient uptake of pennywort were monitored in controlled aquaculture systems. Pennywort was collected from the St. Johns River and cultured in 1000-*l* concrete vaults having a surface area of 1.7 m^2. Pennywort was grown in media containing two sources of nutrients i.e., 1) modified Hoagland's nutrient solution containing varying levels of N (4, 10, 20, and 40 mg N l^{-1} as NH_4NO_3), and 2) underlying sediments (this treatment consisted of a layer of St. Johns River sediment). To evaluate the effect of varying levels of N, 900 *l* of nutrient medium were placed in each vault (55 cm deep). Phosphorus and secondary and minor elements were added at a concentration shown in Table 1. To simulate field conditions in one system sediment obtained from the St. Johns marsh was placed in the vault to a depth of 15 cm and flooded to a depth of 40 cm with water containing no added nutrients. Water in the vaults was mixed constantly with the aid of a submersible pump to avoid any stratification in the water.

Each treatment was replicated two times. Starting plant density for each treatment was 4.5 kg wet wt m^{-2}. Two 0.25 m^2-Vexar mesh baskets containing plants were placed in each vault at the same starting density as outside the basket. Each week the baskets were removed, allowed to drain for 5 min, weighed, and placed back in the respective vaults. This procedure was continued until maximum plant density was attained and no measurable growth was recorded. Each vault contained two baskets; therefore, the productivity data are based on four replications. On the same day productivity was measured, nutrient medium was drained from the vaults, and replaced with fresh solution. Water was not disturbed in the system containing sediment, but additional tap water was added to compensate for the water loss due to evapotranspiration.

Water samples were taken at the start and every 7 days throughout the experiment and analyzed for NH_4^+, NO_3^-, and ortho-P on an autonanalyzer using standard methods (A.P.H.A., 1980). Dissolved O_2 was measured by a YSI oxygen meter and pH by a glass electrode. Total N in the plant tissue was determined by digestion followed by analysis on an autoanalyzer. Plant tissue was digested with nitric-perchloric acid and analyzed for total P using an autoanalyzer.

RESULTS AND DISCUSSION

Biomass Yield. Data on standing crop yields of pennywort during the months of December 31, 1981, to March 22, 1982, are shown in Figure 1. Biomass yields increased when N levels of the water were increased up to 20 mg N l^{-1}. Further increases in N concentration of the water up to 40 mg N l^{-1} did not increase the yields. Although penny-

TABLE 1. SELECTED PLANT NUTRIENTS IN MODIFIED HOAGLAND NUTRIENT SOLUTION.

Plant nutrient	Concentration (mg l^{-1})
Phosphorus	3.1
Potassium	23.5
Calcium	20.0
Magnesium	4.8
Sulfur	6.4
Iron	0.4 (Fe-EDTA)
Mn	0.27[1]
B	0.27[1]
Zn	0.13[1]
Cu	0.03[1]
Mo	0.01[1]

[1]Added as 'Nutrispray'—Sunniland—Manufactured by Chase & Co., Sanford, FL 32771.

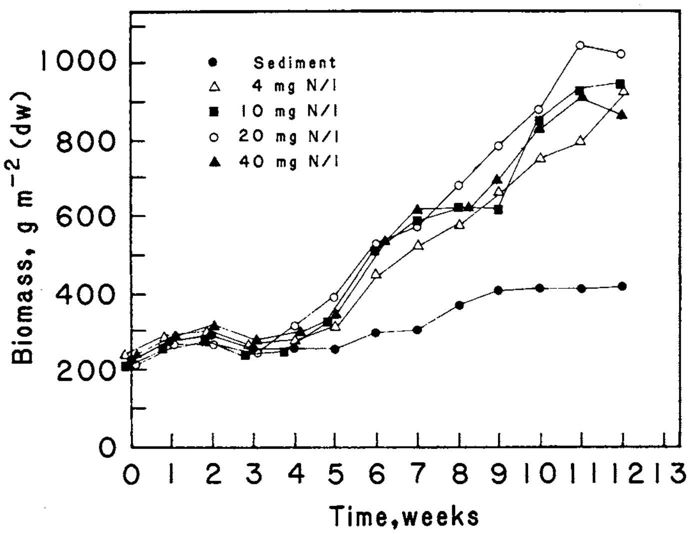

Figure 1. Growth curves of pennywort at various concentrations of nitrogen in the culture medium. Study period 12/30/81-3/22/1982.

wort performs well during cooler months compared to many aquatic plants, its growth was also affected by the hard freeze during the week of January 15-25, 1982 (Table 2), thus, low temperature stress decreased yields. During this period, yield increase was in the range of 11 to 19% for various N levels of the culture medium. After the plants recovered from the freeze, biomass yields increased at a linear rate until maximum plant density was reached. Yield increase of 5.5% per day was observed for plants cultured in the water containing 20 mg N l^{-1}, while a yield increase of 4.5 to 5% per day was recorded when plants were cultured in the water containing 4, 10, and 40 mg N l^{-1}. At all levels of N additions, net increase in pennywort growth was observed until a maximum plant density of 920 to 1046 g (dw) m^{-2} was observed. Growth rate was found to be significantly lower (2.2% increase per day) when pennywort plants derived nutrients solely from sediments. In this system, pennywort reached a maximum plant density of 416 g (dw) m^{-2} after which no additional increase in growth was recorded.

Table 2. Weekly average values for ambient air temperature (C) and solar radiation (KCal·m^{-2} day^{-1}).

Dates	Temperature			Water temp.	Solar radiation
	High	Low	Mean		
12/30/81-1/6/82	18.6	12.7	15.7	14.4	2071
1/7 -1/14[1]	16.9	4.6	10.8	—	—
1/15 -1/17[2]	14.6	3.5	9.1	6.3	2501
1/18 -1/24	23.6	9.9	16.8	11.3	3004
1/25 -1/31	21.0	7.9	14.5	17.4	3350
2/1 -2/7	26.4	15.9	21.2	14.6	2512
2/8 -2/14	23.9	13.0	18.5	15.0	2381
2/15 -2/21	25.8	12.8	19.3	14.0	3325
2/22 -2/28	24.2	10.7	17.5	15.7	3800
3/1 -3/7	24.8	12.6	18.7	13.1	3058
3/8 -3/14	25.4	13.5	19.5	22.3	4893
3/15 -3/21	30.6	16.5	23.5	22.3	4861
3/22 -3/28	26.3	15.0	20.7	—	3725

[1]Low temperature dropped below freezing for three consecutive nights (−17 C, −2.8 C, −2.8 C), respectively.
[2]Low temperature dropped to freezing point on one night.

Maximum growth was observed in the linear phase of the growth curve (4th week to 11th week) and the slope of this phase was calculated using the least square fit of the data and is presented in Figure 2. Maximum growth rate of 15.7 g (dw) m^{-2} day^{-1} was obtained when plants were cultured in the water containing 20 mg N l^{-1}. Slopes of the linear phase of the growth curve for plants cultured in the water containing 4, 10, and 40 mg N l^{-1} were found to be 11.7, 12.8, and 12.1 g (dw) m^{-2} day^{-1}, respectively. These growth rates were obtained during the months of February and March. Linear increase in growth was observed in a plant density range of 275 to 1000 g (dw) m^{-2}. A wide range in optimum density will allow flexibility in scheduling harvesting frequency without affecting the biomass yields. Plants deriving nutrients from the underlying sediment recorded a maximum growth of 5.4 g (dw) m^{-2} day^{-1}, in a plant density range of 250 to 406 g (dw) m^{-2}.

Growth was found to be significantly lower during the month of January. Average growth during this period was found to be in the range of 3.2 to 6.1 g (dw) m^{-2} day^{-1} for the plants cultured in the N enriched water. Plants deriving nutrients from sediment recorded an average growth rate of 1.5 g (dw) m^{-2} day^{-1}.

Biomass yield of pennywort based on the productivity of the month of January ranged from 6 to 22 mt (dw) ha^{-1} yr^{-1}, while during the months of February and March, calculated biomass yields were in the range of 20 to 57 mt (dw) ha^{-1} yr^{-1}.

Root length of pennywort was found to be inversely related to the available N present in the water. Shoot length was found to be approximately the same at all levels of N additions (4 to 40 mg N l^{-1}). Shoot/root (length) ratios of pennywort cultured in N enriched media were in the range of 0.94 to 8.9. Shoot/root ratio was much lower (0.25) for the plants deriving nutrients only from the underlying sediments. Shoot/root ratio of water hyacinth was also found to be influenced by the plant available N in the culture medium (Reddy and Tucker, 1983). Under nutrient

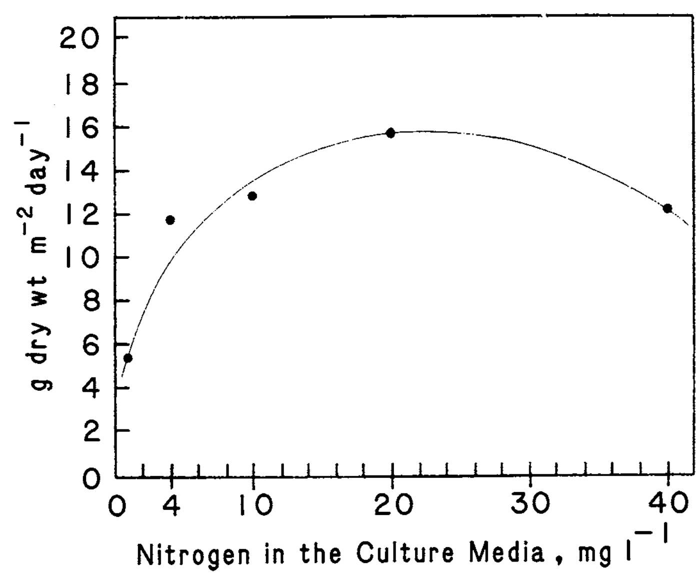

Figure 2. Maximum growth rate (linear phase of the growth curve) as influenced by the nitrogen concentration of the culture medium.

limiting conditions, N-deficient plants tend to decrease their shoot growth more than its effect on photosynthesis. This results in more new carbohydrate availability for export to the roots, thus resulting in increased root growth in N-deficient plants (Boote, 1977).

Nutrient Composition of the Plant Tissue. Nitrogen concentration of the plant tissue was also increased with each increment level of N (up to 20 mg N l^{-1}) in the culture medium (Figure 3). Data presented in Figure 3 represent the average N content of the plant samples collected weekly during the growth cycle. The vertical bars represent the range in plant tissue N as influenced by the standing crop of biomass. Values on the lower end of the bar represent the plant samples collected from the second half of the growth cycle (high standing crop), while the upper end of the bar represents the first half of the growth cycle (low standing crop). Further increase in N concentration did not result in increased tissue N. This represents a maximum N uptake rate of 41 mg N g^{-1} (dw) of plant tissue. Plants cultured in the systems containing underlying sediment but no added N resulted in a tissue concentration of 20 mg N g^{-1} (dw) of plant tissue. In general, plant tissue N decreased as the standing crop biomass increased. This decrease was more striking in the plants cultured at 4 mg N l^{-1} of added N and for plants deriving N solely from the sediments. Increased standing crop biomass had minimal effect on plant tissue N when plants were cultured at 40 mg N l^{-1} of added N, since the culture medium contained more N than plants can use.

Data on the relationship between plant tissue N content and the growth rates are prsented in Figure 4. Increasing the plant tissue N from 20 mg N g^{-1} to 41 mg N g^{-1} (dw) by adding N to culture media increased the biomass yields by about 2.5 times. A striking yield increase (115%) was observed when plant tissue N content was increased from 20 to 27 mg N g^{-1} (dw). Further increase in tissue N content to 41 mg g^{-1} (dw) resulted in an additional yield increase of about 34%

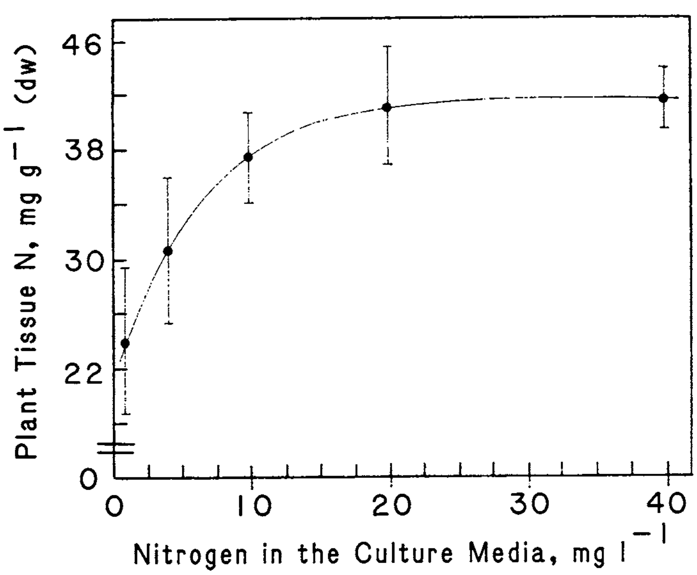

Figure 3. Plant tissue nitrogen (average of all growth phases) at varying nitrogen levels of the culture medium. Vertical bar at each data point represents the range in plant tissue nitrogen at varying plant densities.

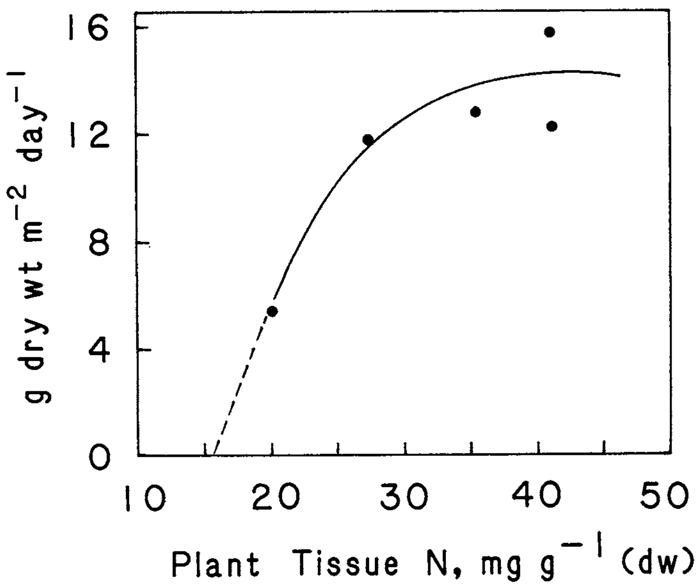

Figure 4. Relationship between plant tissue nitrogen and the growth rate of pennywort.

Phosphorus concentration of the plant tissue was found to increase up to 10.2 mg P g^{-1} of plant tissue when N concentration of the culture medium was increased to 20 mg N l^{-1}. Further increase in N concentration of the culture medium decreased the P concentration of plant tissue to 9.2 mg P g^{-1} (dw) (Figure 5). Ower et al. (1981) and Shiralipour et al. (1981) also observed that increased N concentration of the culture medium decreased P uptake by water hyacinths. Underlying sediments support a P uptake rate of 4.3 mg P g^{-1} (dw) of plant tissue. Although the N/P ratio of the culture medium was in the range of 1.3 to 12.9, the plants removed N and P at a N/P ratio of 3.0 to 5.1 (Figure 6). Percent ash content of the plant tissue was found to be the same in all treatments (12.0 ± 1.2%).

Nutrient Removal of the Pennywort System. Dissolved O_2 and pH of water under the pennywort mat were found to

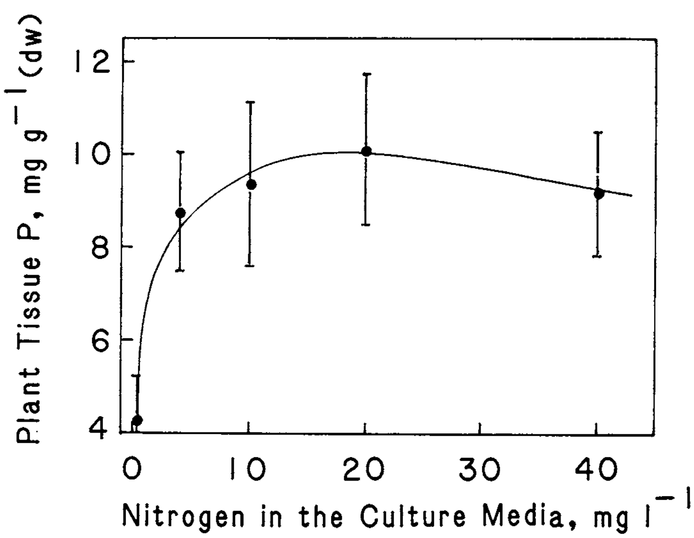

Figure 5. Plant tissue phosphorus (average of all growth phases) at varying nitrogen levels of the culture medium. Vertical bar at each data point represents the range in plant tissue phosphorus at varying plant densities.

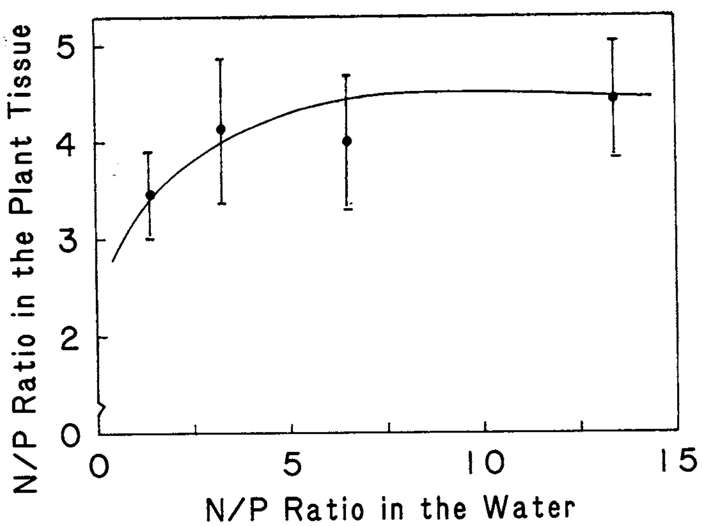

Figure 6. Relationship between N/P ratio of the plant tissue and N/P ratio of the culture medium.

be the same in all treatments (DO = 4.1 ± 2.0 mg l^{-1}; pH = 6.60 ± 0.19). Mass balance of N in a pennywort system (Table 3) indicates that plant uptake is the dominant process involved in removing N from the system. This was expected because the system enriched with N did not have the underlying sediments, thus decreasing the losses of NO_3^- through denitrification. Nitrogen removal due to plant uptake ranged from 284 to 415 mg N m^{-2} day^{-1}. This represents the average value of all growth phases observed in the growth cycle. Nitrogen removal rates were found to be significantly higher (321 to 645 mg N m^{-2} day^{-1}) in the linear phase of the growth curve (Table 4). Tucker (1981) also observed that water hyacinth cultured at a low level of N removed about 100 mg N m^{-2} day^{-1}, while the plants cultured at high levels removed 700 mg N m^{-2} day^{-1}.

TABLE 3. MASS BALANCE OF NITROGEN (AVERAGE OF WEEKLY SAMPLES TAKEN DURING A 12-WEEK GROWTH PERIOD).

Nitrogen added	Plant uptake	N-remaining in the water	Total	Nitrogen unaccounted for
mg N m^{-2} day^{-1}		% of added N		
303	93.8	20.3	114.1	+14.1
756	46.5	26.1	72.6	27.4
1513	27.4	70.8	98.2	1.8
3026	11.4	86.6	98.0	2.0

TABLE 4. PLANT DENSITY (g (dw) m^{-2}), GROWTH RATE (g (dw) m^{-2} day^{-1}), AND NUTRIENT REMOVAL POTENTIAL (mg m^{-2} day^{-1}) OF PENNYWORT IN THE LINEAR PHASE OF THE GROWTH CURVE.

Treatment	Plant density[1]	Growth rate[2]	Nutrient removal potential		
			N	P	N/P
Sediment	324	5.43	109	23	4.7
303 mg N m^{-2} day^{-1}	625	11.70	321	103	3.1
756 "	633	12.79	455	119	3.8
1513 "	633	15.70	645	160	4.0
3026 "	606	12.09	497	111	4.5

[1]Average plant density of the linear phase of the growth curve.
[2]Slope of the linear phase of the growth curve.

TABLE 5. MASS BALANCE OF PHOSPHORUS (AVERAGE OF WEEKLY SAMPLES TAKEN DURING A 12-WEEK GROWTH PERIOD).

Nitrogen added	Plant uptake	P-remaining in the water	Total	Phosphorus unaccounted for
mg N m^{-2} day^{-1}		% of added P		
303	34.8	34.1	68.9	31.1
756	37.8	34.7	72.5	27.5
1513	43.9	41.5	85.4	14.6
3026	32.3	26.8	59.1	40.9

Phosphorus added = 234 mg P m^{-2} day^{-1}.

Although P removal rates were found to be in the range of 59 to 85% of added P, plant uptake accounted for about 32 to 44% of added P, with the remaining 15 to 41% of added P unaccounted for (Table 5). Maximum P removal was observed when N/P ratio of the plant tissue was about 6. Average P removal rates for the whole growth cycle were found to be in the range of 76 to 103 mg P m^{-2} day^{-1}, while the linear phase of the growth curve removal rates were in the range of 103 to 160 mg P m^{-2} day^{-1} (Table 4).

Results obtained in this study indicate that biomass yields of pennywort did not increase in direct proportion to the N availability. In the linear phase of the growth curve, biomass yields increased from 11.7 to 15.7g (dw) m^{-2} day^{-1} indicating luxury uptake of N which did not result in dry matter production. Similar results were also observed for water hyacinths (Boyd and Scarsbrook, 1975; Tucker, 1981). Under the best growth conditions (linear phase of the growth curve), N availability in the range of 750 to 1500 mg N m^{-2} day^{-1} with a N/P ratio of about 6 appears to produce highest biomass yields under our experimental conditions (Table 4). Considering winter conditions (January-March), growth rates and nutrient uptake potential of pennywort are comparable to that of aquatic plants grown during warmer months of the growing season. The calculated potential N and P removal rates were found to be 1172 to 2354 kg N ha^{-1} yr^{-1} and 376 to 584 kg P ha^{-1} yr^{-1}. These values are of the same order of magnitude as the values reported for water hyacinth by Boyd (1970); Rogers and Davis (1972); Sato and Kondo (1981); Reddy and Tucker (1983). In conclusion, this study has shown that pennywort can be successfully grown under cool weather conditions and still maintain high biomass yields and nutrient removal efficiency.

ACKNOWLEDGMENTS

This paper is a contribution of a cooperative program between the Institute of Food and Agricultural Sciences (IFAS) of the University of Florida and the Gas Research Institute (GRI), entitled, "Methane from Biomass and Waste."

REFERENCES

A.P.H.A. 1980. Standard methods for the examination of water and wastewater. 15th Ed. Byrd PrePress, Springfield, VA p. 1134.

Boote, K. G. 1977. Root:shoot relationships. Proc. Soil Crop Sci. Soc. Fla. 36:15-22.

Boyd, Claude E. 1970. Vascular aquatic plants for mineral nutrient removal from polluted waters. Econ. Bot. 23:95-103.

Cornwell, D. A., Zoltek, Jr., J., Patrinely, C. D., Furman, T. des., Kim, J. I. 1977. Nutrient removal by water hyacinths. J. Water Pollut. Cont. Fed. 49:57-65.

Ower, J., C. F. Cresswell, and G. C. Bate. 1981. The effects of varying culture nitrogen and phosphorus levels on nutrient uptake and storage by the water hyacinth, *Eichhornia crassipes* (Mart) Solms. Hydrobiologia 85:17-22.

Reddy, K. R., and J. C. Tucker. 1983. Effect of nitrogen source on productivity and nutrient uptake of water hyacinth (*Eichhornia crassipes*). Econ. Bot. 37:236-246.

Reddy, K. R., and W. F. DeBusk. 1984. Growth characteristics of aquatic macrophytes cultured in nutrient enriched water: I. Water hyacinth, water lettuce, and pennywort. Econ. Bot. 38:225-235.

Reddy, K. R., D. L. Sutton, and G. E. Bowes. 1983. Biomass production of freshwater aquatic plants in Florida. Soil & Crop Sci. Soc. Fla. Proc. 42:28-40.

Reddy, K. R., K. L. Campbell, D. A. Graetz, and K. M. Portier. 1982. Use of biological filters for agricultural drainage water treatment. J. Environ. Qual. 11:591-595.

Rogers, H. H., and D. E. Davis. 1972. Nutrient removal by water hyacinths. Weed Sci. 20:423-428.

Sato, H., and T. Kondo. 1981. Biomass production of water hyacinth and its ability to remove inorganic minerals from water. I. Effect of the concentration of culture solution on the rates of plant growth and nutrient uptake. Jap. J. Ecol. 31:257-267.

Shiralipour, A., L. A. Garrard, and W. T. Haller. 1981. Nitrogen source, biomass production, and phosphorus uptake in water hyacinth. J. Aquat. Plant Manage. 19:40-43.

Steward, K. K. 1970. Nutrient removal potentials of various aquatic plants. Hyacinth Contr. J. 8:34-35.

Tucker, C. S. 1981. The effect of ionic form and level of nitrogen on the growth and composition of *Eichhornia crassipes* (Mart) Solms Hydrobiologia 83:517-522.

Wolverton, B. C., and R. C. McDonald. 1979. Upgrading facultative wastewater lagoons with vascular aquatic plants. J. Water Pollut. Contr. Fed. 51:305-313.