Fate of Nitrogen and Phosphorus in a Waste-water Retention Reservoir Containing Aquatic Macrophytes[1]

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ABSTRACT

Potential use of retention/detention reservoirs stocked with vascular aquatic macrophytes was evaluated, using a microcosm reservoir for reducing the N and P levels of agricultural drainage effluents (waste water). The treatments evaluated were reservoirs stocked with (i) pennywort (*Hydrocotyle umbellata* L.), (ii) water hyacinth (*Eichhornia crassipes* [Mart] Solms), (iii) cattails (*Typha latifolia* L.) and elodea (*Egeria densa* P), and (iv) control (no macrophytes). Labeled ^{15}N was used to differentiate preferential uptake of $^{15}NH_4^+$ and $^{15}NO_3^-$, and to follow the fate of added $^{15}NH_4^+$ and $^{15}NO_3^-$.

Results showed that 34 to 40% of the added inorganic ^{15}N ($^{15}NH_4^+ + {}^{15}NO_3^-$) was removed through plant uptake, while 45 to 52% of the added ^{15}N was unaccounted for, presumably lost through NH_3 volatilization and nitrification-denitrification processes. In the control reservoir, algal biomass removed 4.4% of added ^{15}N, while 41% of the added ^{15}N was not accounted. Pennywort and cattail-elodea systems were found to be most effective, with about 50% inorganic N removal in a 4-day detention period. All aquatic macrophytes preferred $^{15}NH_4^+$ over $^{15}NO_3^-$, but the difference in uptake was not significant, except for pennywort and cattails, which removed 84 and 92% of the added $^{15}NH_4^+$ as compared to 16 and 8% of the added $^{15}NO_3^-$, respectively. About 25 to 29 d were required by the systems with macrophytes to remove 50% of the wastewater P. Plant removal of P was in the range of 3 to 65% of added P, while 7 to 87% of the added P was lost through precipitation and adsorption reactions.

Additional Index Words: **water hyacinth, pennywort, cattails, elodea, aquatic system.**

Reddy, K. R. 1983. Fate of nitrogen and phosphorus in a waste water retention reservoir containing aquatic macrophytes. J. Environ. qual. 12:137-141.

Vascular aquatic macrophytes such as water hyacinths (*Eichhornia crassipes* [Mart] Solms), duckweed (*Lemna minor*), and cattails (*Typha* sp.), cultured in ponds and reservoirs, offer potential alternatives for treating sewage and industrial effluents (Boyd, 1969; Wooten and Dodd, 1979; Wolverton and McDonald, 1979), and agricultural effluents (Reddy et al., 1982). The capacity of vascular plants to assimilate nutrients from polluted waters has been recognized for several years (Rogers and Davis, 1972; Stewart, 1970; Boyd, 1976). Nutrient removal efficiency of a system containing plants will depend on the type of aquatic plant, growth rates of plants, nutrient composition of the water, and physicochemical environment in the water. Studies reported by several researchers (Clock, 1968; Scarsbrook and Davis, 1971; Cornwell et al., 1977) calculate the nutrient removal rates, based on the changes in concentrations at the inflow and outflow of a pond or reservoir. Although these calculations provide information on the nutrient removal efficiency from waste water, they provide very little understanding on the rate of N and P removal in these systems.

Presence of aquatic macrophytes in a pond alters the physicochemical environment of the water, and the role of these changes are often ignored in evaluating the efficiency of a biological treatment system. The dense cover of floating water hyacinths depletes dissolved O_2 of the underlying water, thus creating anaerobic conditions (Reddy, 1981). These conditions favor the denitrification process, thus maximizing NO_3^- removal. Presence of submersed plants, such as elodea or algae, can deplete dissolved CO_2 in the water during the periods of high photosynthetic activity (mid-afternoon) and increase the dissolved O_2 of the water, thus resulting in increased water pH (Reddy, 1981). This condition can maximize NH_4^+ removal through volatilization and soluble ortho-P removal by chemical precipitation. The role of underlying sediments of a pond or reservoir as a nutrient source or sink to the overlying waters is often ignored in calculating the nutrient removal efficiencies.

In central Florida, organic soils (Histosols) planted with vegetable crops are artificially drained during wet seasons, and the drainage effluent discharged from these fields is being pumped into retention/detention reservoirs and subsequently into Lake Apopka. These retention reservoirs are needed to reduce the nutrient

[1] Florida Agricultural Experiment Stations Journal Series no. 3766. Received 6 Mar. 1982.

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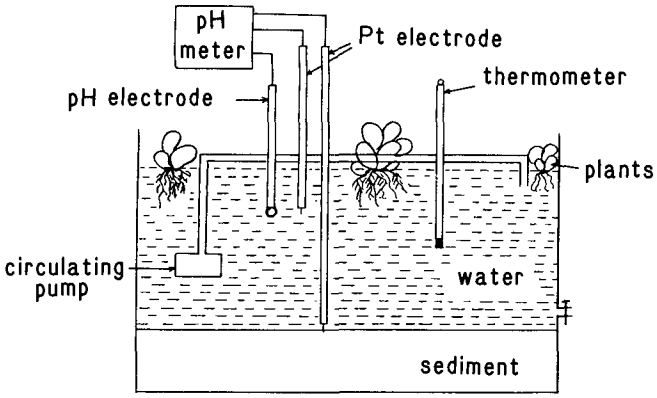

Fig. 1—Schematic presentation of the apparatus used in the study.

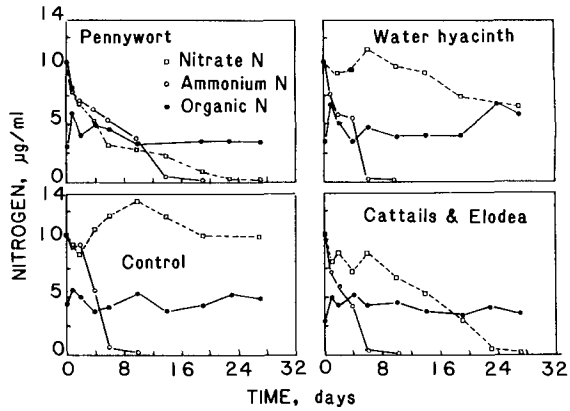

Fig. 2—Changes in the total N conc. of the floodwater in various aquatic systems.

loads to Lake Apopka, which is currently highly eutrophic. There are eight retention reservoirs (≅20 ha each) in operation on the north shore of Lake Apopka. Some of these reservoirs contain natural stands of water hyacinths, cattails, and pennywort. The study reported in this paper is part of a large project (Reddy et al., 1982) to design a low-cost, energy-integrated biological treatment system to reduce the nutrient levels of agricultural drainage effluents.

The purposes of this experiment were to determine the fate of waste water ^{15}N and P in a simulated retention/detention reservoir containing vascular aquatic macrophytes, identify the major N and P removal mechanisms, and relate these processes to physicochemical environment of the water.

MATERIALS AND METHODS

Galvanized tubs measuring 1.2 m (length) by 0.6 m (width) by 0.6 m (depth) were used to simulate retention reservoirs. The tubs were coated with white epoxy paint and lined with two layers of 6-mm polyethylene sheet. Schematic presentation of the experimental setup is shown in Fig. 1. Calcareous marly clay loam sediment obtained from the field reservoir was placed in the tub to obtain a depth of 15 cm at a bulk density of 1.1 g/cm³. The soil had a total N content of 0.30%, total C content of 3.9%, and pH of 7.2.

Agricultural drainage effluent was obtained from the drainage canals located in organic soil areas planted to vegetable crops. Drainage effluent was pumped into each tub to a depth of 40 cm and stocked with aquatic macrophytes. Each treatment was replicated two times. Treatments evlauated include the reservoir stocked with (i) water hyacinths; (ii) pennywort; (iii) cattails and elodea; and (iv) control (no macrophytes). Initial plant densities used were 65.6, 78.7, 122.5, and 365.8 g/m² for water hyacinths, pennywort, elodea, and cattails, respectively. Water in one set of tubs was enriched with 10 µg N/mL each as $^{15}NH_4$-N and $^{14}NO_3^-$-N (2.0 atom % ^{15}N excess), while the water in the second set of tubs was enriched with 10 µg N/mL each as $^{14}NH_4^+$-N and $^{15}NO_3^-$-N (2.0 atom % ^{15}N excess), and all tubs received 5 µg P/mL. Ammonium sulfate and KNO_3 were used as NH_4^+ and NO_3^- sources, respectively; and KH_2PO_4 was used as P source. Water in the tubs was continuously mixed by a submersible pump.

Tubs were placed in a greenhouse, where air temperature was maintained at the same level as encountered outside the greenhouse by cross ventilation and using an electric fan. Average ambient air temperature during the experimental period was 21°C (max 29°C; min 16°C). Dissolved O_2 and pH of the water were monitored continuously on two typical days for each treatment, while on remaining days, dissolved O_2 and pH values were measured at 1000 once each 2 d. Redox potential of the water and the underlying sediments was measured once a week. Water samples were obtained at 0, 1, 2, 4, 6, 10, 14, 19, 23, and 27 d, and analyzed for NH_4^+-N, NO_3^--N, total Kjeldahl N (TKN), ortho-P, total P, and total organic C (TOC). It should be noted that the data obtained on the fate of added $^{15}NH_4$-N and $^{15}NO_3$-N was based on single tub (with four subsamples for each tub) for each treatment, while data for the fate of inorganic N ($NH_4^+ + NO_3^-$) and P was based on repliated tubs (with four subsamples for each tub) for each treatment. At the end of the study period, the surface area of each tub was divided into four sections. Plant, water, and sediment samples were obtained from all four sections and analyzed separately. Plants from four sections of each tub were harvested separately, and both fresh and dry weights were obtained. For each treatment receiving either $^{15}NH_4$-N or $^{15}NO_3$-N, these four sections of each tub were considered as four replications within each treatment. All plant samples were dried at 70°C for a period of 48 h, ground to pass through a 20-mesh sieve, and analyzed for total N and P. Sediment samples were obtained at 0 to 5 and 5 to 15 cm and analyzed for N and P. All water, soil, and plant samples was analyzed for labeled N content.

Analytical Methods

Dissolved O_2 was measured by a YSI (Yellow Springs Instruments) O_2 meter. A glass electrode and a recording pH meter monitored pH. Redox potential (E_h) was measured by platinum electrode and a calomel half cell. Ammonium N and NO_3^--N were analzyed by steam distillation (Bremner, 1965). Total Kjeldahl N was determined by digestion (APHA, 1971) followed by steam distillation (Bremner, 1965). Soluble ortho-P was determined on water samples filtered through 0.2-µm filter paper (Murphy and Riley, 1962). Total P was determined by persulfate digestion, followed by the ascorbic acid reduction method (APHA, 1971). Sediments were extracted by 2*M* KCl at a sediment-to-solution ratio of 1:5, and analyzed for NH_4^+-N (Bremner, 1965). Sediments were also extracted by dilute acid (0.05*N* HCl + 0.025*N* H_2SO_4) at a sediment-to-solution ratio of 1:5, and extracted solutions were analzyed for ortho-P (APHA, 1971). Sediment and plant samples were digested with nitric-perchloric acid, and P in the digested samples was determined by the ascorbic acid reduction method. Labeled N was analyzed on an isotope ratio (Micromass 602) mass spectrometer. All data were subjected to analysis of variance and the multiple range test to separate means, by assuming four subsections of each tub as additional replications.

Calculations

Rate of N and P loss in the controlled experiments was expressed using the following first-order equation:

$$C_t = C_o [1 - \exp(-kt)],$$

where

C_t = nutrient (NH_4^+-N, NO_3^--N, PO_4-P, or TP) loss from the water in the tub, µg/mL;
C_o = initial nutrient concentration, µg/mL;
k = nutrient loss rate constant, d^{-1}; and
t = time, d.

Using least-square fit of the data to the above shown equation, k values were estimated for several treatments. The k value reflects the

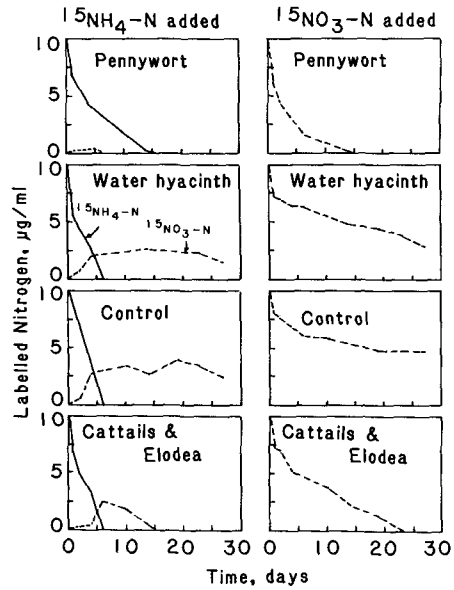

Fig. 3—Changes in the added $^{15}NH_4^+$-N and $^{15}NO_3^-$-N in the flood-water of various aquatic systems.

nutrient removal as a result of biochemical transformations and plant uptake.

RESULTS AND DISCUSSION

Inorganic N Removal

Ammonium concentration of the water reached negligible values in 6 d in the reservoirs with water hyacinths, cattails plus elodea, and control (no macrophytes), while about 14 d were required to remove the same amount of NH_4^+ in pennywort system (Fig. 2). In the pennywort system, both NH_4^+ and NO_3^- of the water disappeared at approximately the same rate with complete removal in 23 d. Nitrate removal was slower in the remaining three aquatic systems, apparently due to nitrification of NH_4^+, which has resulted in increased levels of NO_3^- in the water. Data on the tracer $^{15}NH_4^+$ and $^{15}NO_3^-$ also showed similar results (Fig. 3). Rapid depletion of $^{15}NH_4^+$ resulted in the formation of significant quantities of $^{15}NO_3^-$. Disappearance of added $^{15}NO_3^-$ was also found to be slower in water hyacinths, cattails plus elodea, and control (no plants) systems. Organic N of the water showed very little variation in all four systems.

Inorganic N ($NH_4^+ + NO_3^-$) removal was found to be faster in pennywort and cattail-elodea systems, with a removal rate constant of 0.188 and 0.184 d^{-1}, respectively. About 4 d detention time was needed for 50% reduction in inorganic N removal, while 18 and 28 d detention time was needed for water hyacinth (0.039 d^{-1}) and the control (0.025 d^{-1}) reservoir, respectively. Even though adequate N was present in NO_3^- form, water hyacinths exhibited chlorosis after a 6-d growth period, thus resulting in slow growth of the plants. At that time, all plants were sprayed with micronutrient solution containing Fe to correct for deficiencies. In other experiments conducted in our laboratory, it was found that the chlorosis was due to Fe deficiency.

Floating aquatic macrophytes (pennywort and water hyacinths) removed maximum amount of N (0.19 to 0.20 g N/m²-d), as compared with submersed (elodea) or emersed (cattail) plants (0.05 to 0.07 g N/m²-d) (Table 1). Floating plants also produced maximum amounts of plant biomass. The presence of cattails and elodea in the same system did not increase N removal efficiency, but submersed elodea was found to be more effective for N removal from water than cattails. Although cattails were rooted in the sediment, significant amounts of N were probably absorbed through shoots and leaves.

Table 1—Rate of biomass production and nutrient uptake by aquatic macrophytes in a simulated retention reservoir.

Aquatic plant	Biomass (dry matter)	Uptake	
		Nitrogen	Phosphorus
	g/m² per d		
Pennywort	4.20 c†	0.19 b	0.050 b
Water hyacinth	4.26 c	0.20 b	0.024 b
Cattail—elodea	3.60 bc	0.12 ab	0.004 a
Cattails	2.92 b	0.05 a	0.001 a
Elodea	0.67 a	0.07 a	0.003 a
Algae	0.76 a	0.02 a	0.003 a

† Values with same letter are not significant at 0.05 level of probability.

Table 2—Uptake of added ^{15}N and native waste water plus sediment N by several aquatic macrophytes at the end of a 27-d study period.†

Aquatic plant	Total N	Labeled N			Native sediment + waste-water N
		$^{15}NH_4$-N	$^{15}NO_3$-N	Total	
	g N/m²				
Pennywort	5.10 c‡	2.69 d	0.52 ab	3.21 c	1.89 b
Water hyacinth	5.44 c	1.65 c	1.57 c	3.22 c	2.22 b
Cattails	1.42 ab	0.84 b	0.07 a	0.91 a	0.51 a
Elodea	1.92 b	0.91 b	0.88 b	1.79 b	0.13 a
Algae	0.54 a	0.18 a	0.17 a	0.35 a	0.16 a

† Inorganic N (added + native waste-water N) at the beginning of the study was 8.46 g N/m² (4.0 g N/m² each as $^{15}NH_4$-N and $^{15}NO_3$-N, and 0.46 g N/m² native waste-water N).
‡ Values with same letter are not significant at 0.05 level of probability.

Data in Table 2 show the uptake of added ^{15}N by different plants. When plants were supplied with equal quantities of NH_4^+ and NO_3^-, pennywort plants preferred NH_4^+ over NO_3^-, with about 84% of the total ^{15}N uptake derived from NH_4^+ and 16% of the total ^{15}N uptake from NO_3^-. Water hyacinths showed very little or no preference for NH_4^+, with about 51% of the total ^{15}N uptake from NH_4^+ and 49% of the total ^{15}N uptake from NO_3^-. Cattails, elodea, and algae preferred NH_4^+ over NO_3^-. In this study, a significant portion of added $^{15}NH_4^+$ was converted to $^{15}NO_3^-$ resulting in less availability of NH_4^+ to the plants.

About 60–64% of the total N assimilated by pennywort, water hyacinths, and cattails was derived from the added ^{15}N, while 36–40% was derived from native waste-water N, sediment N, and from decomposition of dead plant portions (Table 2). About 93% of the total N assimilated by elodea was derived from the added ^{15}N, while only 7% was derived from other sources.

Data on (Table 3) mass balance of the added $^{15}NH_4^+$ show that in three systems with macrophytes, a major portion of the added $^{15}NH_4^+$ was recovered in the plant tissue (41–67% of added $^{15}NH_4^+$). In the control reservoir (with no macrophytes), algae assimilated only 4.6% of the added $^{15}NH_4^+$. A small portion of added

Table 3—Mass balance of added ^{15}N in four types of reservoir systems, with a waste-water detention period of 27 d.

Reservoir system	Water	Sediment	Plant	N unaccounted for
	% of added $^{15}NH_4^+$-N†			
Pennywort	0.0 a§	8.9 a	67.3 c	23.8 a
Water hyacinth	3.4 a	8.5 a	41.2 b	46.9 b
Cattail—elodea	0.1 a	8.0 a	43.8 b	48.1 b
Control (algae)	21.0 b	20.9 b	4.6 d	53.5 b
	% of added $^{15}NO_3$-N‡			
Pennywort	0.0 a	6.2 a	13.0 b	80.8 c
Water hyacinth	11.5 b	5.9 a	39.3 d	43.3 b
Cattail—elodea	0.1 a	28.5 b	23.8 c	47.6 b
Control (algae)	35.8 c	30.9 b	4.3 a	28.9 a

† Each system received 4.0 g N/m² each as $^{15}NH_4$-N and $^{14}NO_3$-N, respectively.
‡ Each system received 4.9 g N/m² each as $^{14}NH_4$-N and $^{15}NO_3$-N, respectively. ^{15}N in water was recovered as $^{15}NO_3$-N only.
§ Values with same letter are not significant at 0.05 level of probability.

$^{15}NH_4^+$ was recovered in the surface 5 cm of the sediment layer (8–21% of added $^{15}NH_4^+$). In water hyacinths and control reservoirs, about 8 and 21% of the added $^{15}NH_4^+$ were recovered as $^{15}NO_3^-$. Ammonium ^{15}N not accounted for (loss) was found to be minimum (24% added $^{15}NH_4$-N) in the pennywort system, while 54% of the added $^{15}NH_4^+$ was unaccounted for in the control reservoir with algae.

A major portion of added $^{15}NO_3^-$ was unaccounted for (43–81% of added $^{15}NO_3^-$) in the reservoirs with macrophytes. Unaccounted $^{15}NO_3^-$ was found to be maximum in the pennywort system and minimum in the system with algae. Plant uptake accounted for 13–39% of added $^{15}NO_3^-$ in the reservoirs with macrophytes, while algae in the control reservoir assimilated only 4.3% of added $^{15}NO_3^-$. In water hyacinth and control reservoirs about 11.5–36% of added $^{15}NO_3^-$ remained in the water after a 4-week detention period. In our system, water hyacinth plants were found to be less efficient in assimilating $^{15}NO_3^-$, probably due to an inadequate supply of micronutrients such as Fe. Studies conducted by Rogers and David (1972) and Reddy and Tucker (1982; unpublished results, Univ. of Fla.) have shown that water hyacinth plants were very productive when grown in a balanced nutrient solution containing NO_3^- and Fe.

Major loss mechanisms functioning in the reservoir systems are NH_3 volatilization and nitrification-denitrification. Ammonia volatilization was probably more active in the reservoirs (Mikkelsen et al., 1978) with elodea and algae because pH of the floodwater (Fig. 4) increased to a maximum of 9–9.5 between 1600 and 2000 h. In water hyacinth and pennywort systems, pH values remained relatively constant (6.7–7.0) indicating less favorable conditions for NH_3 volatilization. Labeled N data showed significant quantities of $^{15}NH_4^+$ converted to $^{15}NO_3^-$ indicating active nitrification in all systems (Fig. 3). Average dissolved O_2 values of the water were in the range of 2.5 to 4.7 μg/mL for pennywort and water hyacinth systems, 6.6 to 9.8 μg/mL for cattail-elodea and control reservoir systems. Under field conditions, dense cover of floating macrophytes (Reddy, 1981) depletes the dissolved O_2 level of the water to <1 μg/mL, creating less favorable conditions for nitrification. However, in our system, circulating the overlying

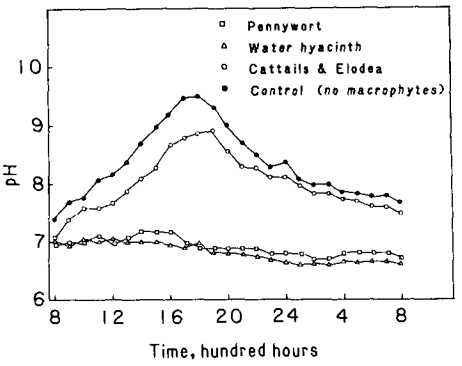

Fig. 4—Diel variations in pH of the floodwater in various aquatic systems.

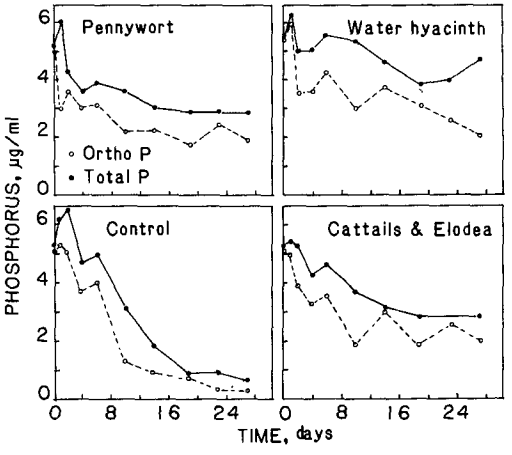

Fig. 5—Changes in the total and ortho-P conc. of the floodwater in various aquatic systems.

water resulted in elevated levels of dissolved O_2. Results obtained in this study suggest that NH_3 volatilization was probably active during the periods of high pH (>8.0), and nitrification was functioning during most of the day, except at times when pH >9.0 may have resulted in decreasing the activity of nitrifying organisms.

Denitrification was probably the most important mechanism functioning in the reservoir system in reducing NO_3^- levels of the water. Denitrification primarily occurs in the underlying sediments (Reddy et al., 1980), with very little or no denitrification occurring in the overlying waters (Engler and Patrick, 1974). In aquatic systems with dense cover of floating plants (water hyacinth or pennywort), denitrifiation can possibly occur in the root zone of floating plants. Currently, studies are in progress in our laboratory to evaluate denitrification in the root zone of floating plants. Redox potential values of the sediments used in our study were in the range of -100 to 50 mV, indicating favorable conditions for denitrifiation. Although in this study, no direct evidence for denitrification is available, large quantities of $^{15}NO_3$ unaccounted for (Table 3) suggest the possibility of this process.

Phosphorus Removal

Rapid removal of solube ortho-P and total P was observed in the control reservoir with no macrophytes as compared with the other three systems studied (Fig. 5).

Table 4—Mass balance of P in a simulated retention reservoir with a waste-water detention period of 27 d.

Reservoir system	P remaining in the water		P removed from the system	
	Soluble P	Insoluble P	Plant uptake	Unaccounted for
	% of added P			
Pennywort	27.6 b†	13.7 b	64.5 c	−5.8 a
Water hyacinth	27.5 b	36.5 c	28.6 b	7.4 a
Cattails—elodea	29.3 b	11.1 b	4.4 a	55.2 b
Cattails	--	--	1.0 a	--
Elodea	--	--	3.4 a	--
Control (algae)	4.5 a	4.8 a	3.4 a	87.3 c

† Values with same letter are not significant at 0.05 level of probability.

Loss of P in the control reservoir was probably due to the uptake by algae and precipitation with Ca compounds at high pH levels. No significant differences were observed among pennywort (0.025 d^{-1}), water hyacinth (0.024 d^{-1}), and cattail-elodea (0.028 d^{-1}) systems in the removal of P from the water column. About 25 to 29 d were required by the systems with macrophytes to reduce ortho-P concentration of the water by 50%, while only 6 d were needed for the control reservoir containing algae to remove the same amount of P.

The mass balance of P indicates that about 55–87% of added P was not accounted for in cattails-elodea, and control reservoirs, respectively (Table 4). The water hyacinth reservoir lost 7.5% added P, while in the pennywort reservoir P recovery was about 106%. Pennyworth plants were found to be most effective, with about 65% of the added P recovered in plant tissue, while in the remaining three systems, plant uptake resulted in the removal of 3.4–28.6% of added P. Water hyacinths were found to be less efficient in removing P than N (Stewart, 1970; Dunigan et al., 1975; Boyd, 1976). In the cattail-elodea and control reservoirs, pH of the water reached a max of 9.8 in the mid-afternoon (Fig. 4), which probably resulted in the precipitation of P with Ca compounds, and the precipitate thus formed probably deposited on the sediment surface (Reddy, 1982). Diel variations in water pH can play an important role in controlling P availability in calcareous water bodies.

In conclusion, results obtained in this study showed that N removal due to plant uptake accounted for 34–40% of the added N, while 45–52% of the added N was unaccounted for, presumably lost through NH_3 volatilization and denitrification. Use of low initial plant densities for pennywort and water hyacinth systems resulted in poor utilization of added N. All systems functioned effectively for NH_4^+ removal with 100% removal in less than 6 d. Nitrification of NH_4^+ resulted in increased levels of NO_3^-, and all systems removed NO_3^- at a slower rate. The water hyacinth system was found to be less efficient in removing NO_3^-, primarily due to less active growth of plants as a result of chlorosis caused by Fe deficiency. The results reported on the water hyacinth system should be applied with some caution. Control reservoir (algae) and cattail-elodea systems were found to be most effective for P removal, primarily due to alkaline water pH, which has resulted in the precipitation of P. Pennywort was found to be more efficient in P uptake than water hyacinths. Longer detention times were needed for P removal than N removal.

ACKNOWLEDGMENTS

This research was supported, in part, by the funds from the Center for Environmental and Natural Resources Programs, Institute of Food and Agricultural Sciences, University of Florida, Gainesville. This paper also reports results from a project that contributes to 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." The assistance of Dr. D. A. Graetz and Mr. W. G. Portier for ^{15}N analysis is gratefully acknowledged.

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