RESEARCH PROJECT FINAL TECHNICAL

COMPLETION REPORT

OWRR PROJECT NO. A-023 OKLAHOMA

NITROGEN TURNOVER IN IMPOUNDMENTS

SUBMITTED TO
THE OKLAHOMA WATER RESOURCES RESEARCH INSTITUTE
OKLAHOMA STATE UNIVERSITY
STILLWATER, OKLAHOMA

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The research upon which the report is based was supported in part by funds provided by the United States Department of the Interior, Office of Water Resources Research as authorized under the Water Resources Research Act of 1964

ABSTRACT

The relationship between growth of aquatic plants and their nutrient supply is poorly understood. One object of this research was to learn if the Michaelis-Menten model describes the relationship between the uptake of NH_4 and the concentration of NH_4 in solution. A hyperbola resulted when uptake of NH_4 by Ceratophyllum (v) was plotted against the concentration of NH_4 in solution (S). Linearity between v and S existed at concentrations less than 7 mg NH_4-N$(l)^{-1}$, suggested that a model for first order saturation kinetics might be appropriate. However, uptake was depressed by one-half at low temperatures and some of the uptake was apparently related to processes which are not completely passive.

Another objective was to determine if hydrophytes could absorb ammonia through the root and rhizome system and, subsequently, translocate the absorbed nitrogen to stems and leaves. Uptake and translocation of ^{15}N occurred in three species of hydrophytes. In the laboratory 90% of Potemogeton nodosus tested took up the isotope by roots and rhizomes and approximately 50% had the isotope in stem and leaf organs. Specific uptake rates for plants with enriched parts were highly variable: 0.02 to 40.08 μg NH_4-N (mg N Root-Rhizome day)$^{-1}$. Root, rhizomes, stems and leaves of P. nodosus became enriched with ^{15}N labeled ammonia which had been injected into the lake sediments.

(A) Research Project Accomplishments

The original objective of this research was to learn if the uptake of inorganic nitrogen by algae and aquatic macrophytes could be described by a model for enzyme kinetics. The ecological importance of such a finding would be that it would provide another means to evaluate competition for nutrients among various components of the flora, predict turnover rates and provide a convenient entry into the study of nutrient limitation.

The original objectives were:

1. To learn if the Michaelis-Menten expression applies to the phytoplankton, filamentous algae and to the Ceratophyllum-periphyton complex for two nutrients, nitrate and ammonia.
2. To determine the influence of nitrate concentration and ammonia concentration on the maximum uptake velocity (V_{max}) and the half-saturation constant (K_s) for Ceratophyllum.
3. To determine the rate at which assimilated nitrate and ammonia is lost to the environment as dissolved nitrogen compounds, by the Ceratophyllum-periphyton complex.

During FY 1972, Objective 1 was completed. Analysis of preliminary data revealed that uptake of nitrate and ammonia could not be described for Ceratophyllum or Spirogyra according to the Michaelis-Menten expression. However, a linear modal describing uptake as a function of concentration appeared to be appropriate. This suggested that uptake was due to diffusion. This hypothesis was examined for uptake of ammonia by Ceratophyllum in the laboratory and was rejected. The following narrative describes observations leading to this conclusion. Details can be found in Toetz (1973).

Ammonia Uptake by Ceratophyllum

Field Observations

Plants were obtained for both field and laboratory research from a pond described by Toetz (1971). Initial observations of NH_4 uptake were made using ^{15}N. Ceratophyllum was obtained from the surface of the pond. Water samples were obtained by holding a plastic bottle 40 cm below the surface. The water was used to fill 12 glass reagent bottles with a capacity of two liters. Then 99% $^{15}NH_4$-N as NH_4Cl was serially added to the bottles. The bottles were incubated for four hours in the pond horizontally on a metal frame at 30 cm. At the end of incubation, each bottle was poisoned with 2 ml formalin. Subsequent analyses follow Toetz (1971) exactly.

Uptake was also estimated as the disappearance of NH_4 from solution, using replicates containing no plant material as controls. Pond water was obtained as above. In Experiments 2 and 3, five sub-samples of 5 l were measured into 7.5 l polypropylene bottles. These were serially enriched with $(NH_4)_2SO_4$. Six quart glass bottles were filled with 800 ml of the water from each polypropylene bottle. Three of the quart bottles were used as controls at each concentration, while Ceratophyllum was added to the others. Plants in healthy condition were collected 15 minutes before the experiment from the surface of the pond. A stem about 15 to 20 cm long with one or more apical ends was added to each bottle, the exact time of addition being carefully noted. All bottles were placed linearly into steel racks and moved into pond water where they were suspended from wooden floats (Toetz, 1973) and orientated toward the sun.

After a period of incubation of four hours, the racks were removed from the pond. Each bottle was shaken and 100 ml was poured through cheesecloth into an acid-washed Erlenmeyer flask.

In field and laboratory experiments NH_4 analyses followed Solórzano (1969) and plants were dried in tared alumimum pans. Most of the plant was removed intact, but broken stems and leaves were retained on tared filter paper. The above material was dried at 105°C for 24 hours and then reweighed to obtain an estimate of the dry weight.

Laboratory Observations

Conditions common to all laboratory experiments are set out below and then details of each experiment are described. A rake was used to bring up plant material from the bottom at 1 m. Ceratophyllum was placed in plastic basins filled to a depth of 5 cm with N-free artificial lake water (Table 1). The basins were placed in the environment where the observations were subsequently made and acclimated there for 12 to 24 hours.

The object of Experiment 4 was to learn if agitation of the water in the quart bottles used in laboratory experiments might be necessary. Sixteen glass quart bottles were filled with 800 ml of artificial lake water with 20 mg $(l)^{-1}$ NH_4-N and another sixteen with 800 ml artificial lake water containing 0.1 mg $(l)^{-1}$ NH_4-N. The pH is all bottles was 9.3. About 1 gm (dry weight) Ceratophyllum was added to each of eight bottles at each concentration; the other were controls. Four controls and four with plant material at each concentration were shaken by hand every 10 minutes while the others were not. Incubation was at 20°C with illumination from 75 watt incandescent lamps at 59 cm. After 1 hour, the contents of all bottles were shaken and poured through cheesecloth into acid-washed Erlenmeyer flasks. Subsequent analyses in laboratory experiments followed the procedure used for field experiments.

In Experiment 5, I observed the effect of substrate concentration on velocity of uptake to confirm field observations. Here 25 liters of artificial lake water were equally divided among 5 polypropylene bottles. Each subsample

was enriched with a different quantity of NH_4Cl and the pH of each adjusted to 9.30 with HCl. Six glass quart bottles were then filled with 800 ml from each polypropylene bottle. At the outset of the experiment, about 1 g (dry weight) of Ceratophyllum was added to three respective bottles at each concentration; three bottles served as controls. Incubation was for 1 hour, at 21°C and under 16,000 lux (from fluorescent lights). The experiment was terminated as in Experiment 4. The same procedure was used in Experiment 6 to determine the rate of uptake at 5 concentrations of NH_4 in the dark and in the light, respectively. Here pH was adjusted to 7.52 with HCl to observe uptake at the usual pH of the pond water where Ceratophyllum was obtained. Incubation in the dark was accomplished in a light-tight room, while plants were incubated in the light at 16,000 lux. The temperature was 19°C. Both parts of Experiment 6 were performed simultaneously.

The effect of temperature on NH_4 uptake was observed in Experiment 7. Thirty-six quart bottles were prepared with 800 ml of artificial lake water with 10 mg $(l)^{-1}$ NH_4-N as $(NH_4)_2SO_4$ at pH 8.90. Eighteen were incubated at 6 to 10°C, another eighteen at 20°C. Illumination was 16,000 lux. Half of the bottles at each temperature served as controls and contained no plants, while half contained about 1 gm (dry weight) Ceratophyllum. After an incubation period of 12 and 24 hours, control bottles and three experimental bottles were removed from each chamber and the experiment terminated. The experiment was repeated, but at lower illumination in Experiment 8. Here sixteen quart bottles were filled with 800 ml culture solution with 10 mg NH_4-$N(l)^{-1}$ at pH 9.00. Ceratophyllum was added to eight. Eight (four experimental and four controls) were incubated at 2°C, the others at 20°C. Illumination from a 75-watt incandescent lamp at 21 cm was used at both temperatures. After 6, 12, and 24 hours, each bottle was shaken and an aliquot of 3 ml removed with a pipette.

Results

The results of field experiments demonstrate that a hyperbola results when NH_4-N uptake (gm dry weight)$^{-1}$ by Ceratophyllum is plotted against the initial substrate concentration. Asymptotic response was achieved when substrate concentrations reached 4 to 7 mg NH_4-N (l)$^{-1}$.

Experiment 4 was performed to learn if shaking bottles would be necessary to obtain reproducible results. No difference could be found in the uptake of NH_4-N (g dry wt plant)$^{-1}$ in agitated versus nonagitated bottles ($P>0.4$) at both high and low concentration of substrate. Fitzgerald (1968) states that the rates of NH_4 uptake by different masses of Ceratophyllum are essentially the same, when normalized to a dry weight basis. In Experiment 7, which lasted more than one hour, the plant biomass in each bottle was the same. In this experiment it was assumed that any error due to diffusion gradients would be systematic. In addition, a piece of Ceratophyllum weighing about 1 g dry weight which was normally added to a quart bottle containing 800 ml, virtually filled all available space in the bottle. The conclusion was reached that lack of shaking of the experimental bottles would not significantly bias the results.

Experiments 5 and 6 also demonstrate that a hyperbola results when uptake velocity is plotted against concentration. The scatter in the data points was marked, however, and may be due to a selection of plants having different ages and/or condition. All plants selected in the field were taken from below the surface and were terminal portions. It is likely that such selection resulted in a rather homogeneous sample and, thus, a comparatively uniform response to experimental perturbations. However, plants selected for laboratory experiments were dredged up from one meter. This mode of sampling may have resulted in a selection of plants having quite different physiological properties, which would explain greater scatter in the results of laboratory observations.

Two strategies were employed to derive an expression to describe the relationship between velocity of uptake (v) and substrate concentration (S). A least squares fit was obtained for the relationship between (1) the velocity of uptake (v) and the initial substrate concentrations (S), and (2) S/v and S.

The fit between v and S was most satisfactory in Experiment 2 where S was less than 4 mg NH_4-N $(l)^{-1}$, and the correlation coefficient (r) between v and S was 0.96. In Experiment 3 where S>7 mg NH_4-N, r was 0.85. Obviously, this regression best describes the linear part of the hyperbola, but it is not satisfactory for the hyperbola when S is set very high (Table 2).

The best strategy to obtain values for the Michaelis-Menten expression is to plot S/v vs S for reasons explained by Dowd and Riggs (1965). In Table 2, V_{max} is RC and K_s is the negative intercept (I). According to these data, K_s is set between 613 and 4425 μg NH_4-N $(l)^{-1}$ and V_{max} is between 1001 and 2195 μg NH_4-N (g dry wt hour)$^{-1}$. In Experiment 6, V_{max} was almost the same in the dark as in the light but K_s was about seven times higher in the light than in the dark.

The usefulness of the regression of S/v on S will rest upon the resolution of the possibility that two or more processes are involved in uptake of NH_4 and the identification of variables which are rate limiting. If the Michaelis-Menten expression is an approximate model which describes the uptake velocity of ammonia by Ceratophyllum in terms of substrate concentration, then it could be used to calculate turnover time. Assume the supply of the substrate is equal to removal. Then, the turnover time is the interval wherein uptake of the substrate is equal to the equilibrium standing quantity of the substrate. In Experiment 2, for example K_s is 2906.0 μg NH_4-N $(l)^{-1}$ and V_{max} is 1749.5 μg NH_4-N (g dry wt hour)$^{-1}$. At 10 μg NH_4-N $(l)^{-1}$, the turnover time is 1.7 hours.

The correlation coefficients of the v vs S plots were usually higher than those for the S/v vs S plots (Table 2). The correlation between S/v and S was generally poor (Table 2). These findings suggest that an expression for diffusion can be used to describe uptake of ammonia by Ceratophyllum. However, further observations did not entirely confirm this conclusion, since low temperatures depressed NH_4-N uptake. Uptake at 6 to 10°C was lower than uptake at 20°C but the difference was not statistically significant in Experiment 7. However, low temperatures significantly depressed uptake in Experiment 8. The rate of ammonia uptake at 2°C was about 50, 64, and 73% that at 20°C after incubation of 6, 12, and 24 hours respectively. If uptake were by diffusion alone, then a decrease in temperature of 18°C should decrease the velocity of uptake by a factor of 1.1, not by factors of 1.4 to 2.0 as reported here. This suggests that movement is not entirely passive. Although expression for diffusion can describe the velocity of NH_4 uptake as a function of concentration, the movement of NH_4 into Ceratophyllum is probably more complicated. Since this research did not confirm that uptake of either NH_4 or NO_3 could be described by either a Miachaelis-Menten expression or an expression for diffusion, original Objectives 2 and 3 did not seem feasible and I selected alternatives. These alternative objectives sought to explore the nature of the NH_4 uptake mechanism in Ceratophyllum.

Laboratory Experiments on the Mechanism of Ammonia Uptake

The alternative goals were to learn (1) relationship between the uptake of ammonia and its respective concentration in the water, (2) the fate of the assimilated nitrogen under varying degrees of N repletion and, (3) the transfer rates among the intracellular pools.

Previous experiments established that a hyperbola results when the velocity of ammonia uptake by Ceratophyllum is plotted against substrate concentration.

Further work indicated that the uptake cannot be described by Monod kinetics or a model for diffusion. I hypothesized that the uptake velocity of ammonia might be related to the internal concentration of ammonia in Ceratophyllum and sought to confirm this through experimental observation. The first step in doing so was to develop a method to assay for the mass of internal ammonia in Ceratophyllum. The latter was defined operationally, that is, as the mass of ammonia leached from a given mass of Ceratophyllum in warm, artificial lake water for a period of one hour. We analyzed the products which were leached from Ceratophyllum and discovered that more nitrogen was leached from Ceratophyllum as organic nitrogen (a fraction appearing after Kjeldahl analysis) than as NH_4. The mass of organic nitrogen, however, leached from Ceratophyllum in this manner was highly variable, whereas, the amount of ammonia leached from Ceratophyllum, as measured by the Solórzano technique, was much more consistent per gram dry weight. I used the techniques described above to measure the internal concentration of ammonia of plants exposed to various concentrations of ammonia in order to relate uptake velocity to cellular concentration of NH_4. Ceratophyllum was exposed to three different concentrations of ammonia in artificial lake water: 12.8, 6.9, and 0.4 milligrams per liter ammonia nitrogen. After three days of incubation, ammonia uptake was measured as disappearance of the ammonia from solution. Each piece of Ceratophyllum was placed into warm ammonia-free artificial lake water and the internal ammonia allowed to leach out into the water. An increase in ammonia in the solution represented the amount of internal ammonia in Ceratophyllum. The dry weight of the plant was then determined after drying for 24 hours at $105^{o}C$. The wet weight and the dry weight of representative plants were determined. The mean % dry matter was 14% of the wet weight, thus, 7.2 x the dry weight of each experimental plant was the mass of water in the plant. The water content of the plants

determined this way is probably an overestimate of the actual amount of cellular water in the plant. But as a first approximation, it sufficed.

After three days of incubation the rate of uptake of ammonia as measured by disappearance from the solution was linearly related to the final internal concentration of ammonia, expressed as the mass of ammonia per milligram dry weight of the tissue (Figure 1). The correlation coefficient between these two variables was 0.59. The rate of uptake of ammonia from solution and the internal concentration of ammonia, (expressed as mass of ammonia per ml of internal water) were also linearly related, with a correlation coefficient of 0.58 (Figure 2). Thus, after three days of incubation, highest rates of uptake occurred when the internal concentration of ammonia was highest. This finding again negates a diffusion hypothesis as far as the uptake mechanism of ammonia is concerned in this plant. After three days the internal concentration of ammonia in the plant, was linearly related to the initial concentration of ammonia in the external medium (Figure 3). In this case, the correlation coefficient between the two variables was 0.83. The mass of ammonia leached into artificial lake water per mass of internal water was linearly related to initial external concentration of ammonia (Figure 4). The correlation coefficient was 0.83. Thus, the experiment indicated that in the dark, Ceratophyllum is capable of adjusting its uptake rate in direct proportion to the supply rate The variability in uptake rate from plant to plant, however, was so great that I felt that unless material of more uniform age and growth history is found, this will not be a profitable line of research to pursue. Therefore, the objectives exploring the uptake mechanism for NH_4 were abandoned and attention was focused on a related problem.

Uptake and Translocation of Labeled Nitrogen

The objective of FY 73 research was to learn if aquatic macrophytes can take up ammonia via roots and pass the nutrients to the open water. The degree

to which the vast stores of nitrogen nutrients in the sediments are exported to algae via root or root-like systems of hydrophytes is unknown. Hydrophytes could function as nutrient pumps and export nitrogen from the sediments to the open water as hydrophytes do for phosphorus (McRoy, et al., 1972 and Reimold, 1972). The first objective of this research was to show that freshwater hydrophytes take up NH_4 at the roots and translocate the labeled nitrogen to apical tissues in the laboratory using a two compartmental apparatus such as that described by Bristow and Whitcomb (1971) with the following modifications. The lower chamber was made of brown glass and the upper chamber was covered by Saran Wrap held in place with rubber bands. Incubation was at 19-20°C with 12-hour light -- 12-hour dark irradiance from a bank of Gro-Lux fluorescent lights at an illumination of 230 lux. At the outset, fifteen to twenty experimental vessels were prepared alike with 300 and 330 ml artificial lake water (Toetz, 1973) at pH 7.0 in the upper and lower chambers, respectively. The water in the bottom compartment was enriched with 99% NH_4Cl at a concentration of 500 µg N/l.

Two or three experimental units were removed from the experimental chamber after incubation and the plant parts dissected and placed in pre-weighed aluminum pans. Subsequent analyses follow Toetz (1971) except that a Coleman Nitrogen Analyzer II was used to convert organic N to N_2 (Barsdate and Dugdale, 1965) and to determine the % N in organic matter.

Elodea densa absorbed NH_4 and translocated labeled N to stems and leaves (Table 3). There is a tendency for the uptake rate to decrease with time in the first trial, indicating a possible loss of the labeled N to the water in the upper compartment. Ceratophyllum demersum also absorbed NH_4 and translocated labeled N to the shoots but Scirpus did not (Table 3). Translocation of labeled N by Potamogeton nodosus only occurred after three days of incubation

(Table 4). The results clearly demonstrated that several species of hydrophytes can take up NH_4 through their roots and holdfasts and translocate labeled N to stems and leaves (Table 4).

Further work along this line was achieved using Potamogeton nodosus as the test plant. This hydrophyte has floating leaves and is quite common in Oklahoma ponds. To learn the rate of transport of labeled nitrogen from a lower compartment to tissues and leaves, the experimental setup was essentially as above, except that the systems were aerated with ammonia-free air which had been passed through a bicarbonate solution to enrich the air with carbon dioxide (Figure 5).

Table 5 also demonstrates quite clearly that this emergent plant has the capability of translocating labeled nitrogen from the roots to leaves and stems. There is an indication that the amount transported is a function of concentration initially present in the water in the lower compartment and time. Highest rates transported generally occurred after ten days at high substrate levels in the lower compartment.

Analysis of the interstitial water in the sediment of the pond, where the plants had been growing, revealed that nitrate was completely absent and that ammonia was a dominant form of inorganic nitrogen. Table 6 gives relevant parameters of the chemistry of interstitial water of sediments of this pond.

While it was quite clear that ammonia could be absorbed by the roots of Potamogeton nodosus and the label eventually translocated to upper plant parts, it was not clear whether the plant could do this in the natural environment. Using vegetable dyes, we discovered we could inject one to two milliliters of solution into the sediments at a depth of five to six centimeters without serious problems of backflow. Thus, we injected nitrogen-15 ammonia into the sediments in the vicinity of the root of the plant and allowed the label to be taken up by the roots and translocated to upper plant parts. A control plant was suspended

in the water near the test plant, in order to learn if any ammonia might diffuse from the sediment to the open water and be taken up in this way by the plant. The control plants were not statistically different than the normal abundance of % ^{15}N in plants collected before the experiment. Thus, enrichment of the experimental plants above a normal abundance would be interrupted to mean that uptake or translocation had occurred. Table 8 shows that in three cases, uptake of ammonia and translocation of labeled nitrogen occurred. These data demonstrate conclusively that for Potamogeton nodosus, the sediments can function as a source of ammonia for a hydrophyte. It is likely that in many aquatic situations, hydrophytes function as nutrient pumps and are able to export nutrients from the sediments to the open water where they may be used by periphyton or by freely floating algae. This pathway of regeneration of nitrogen may short-circuit normal pathways. If so, this research opens a new frontier of research on nutrient cycling in lakes.

Mass Spectrometer

The first two years of this research were thwarted somewhat by the lack of a mass spectrometer on the campus of Oklahoma State University. Recently a mass spectrometer has become available and has been evaluated by me for its suitability in ^{15}N tracer study. The mass spectrometer is a CEC 21-110B mass spectrometer presently.

Dr. Stuart Scheppele, head of the mass spectrometer laboratory, suggested that time be spent evaluating instrument performance. Therefore, we ran isotope ratio blanks with tank N and standard samples. The atom % ^{15}N in tank N agreed reasonably well with the theoretical, 0.36 atom % ^{15}N and sample pressure had no effect on the isotope ratio. In the case of isotope ratio blanks of plankton retained on glass filters, the accuracy is also good (Table 8). We concluded

that we could not detect enrichment when an experimental sample had a value of less than 0.3856 atom % ^{15}N. This is satisfactory for our work on uptake. Replicates of non-enriched and enriched samples were also assayed to learn the precision of the instrument. In general, precision was less than 5% of the mean of the measured values (Table 8). The % ^{15}N in the azine samples is close to the theoretical (Table 8). We concluded that the Dumas combustion and subsequent techniques used did not introduce contaminants into gas samples, which interferred with the measurement of % ^{15}N.

The analysis of one ^{15}N sample takes about 1.3 hours after field work has been completed. The time requirements of each step are as follows:

Step	Time-Minutes
1. Conversion to N_2	30
2. Regeneration of catalyst	10
3. Mass spectrometer time	25
4. Reading peak heights	10
5. Transferring data to cards and computer processing	5
	80

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(B) Publications

Cole, B. S. 1973. Uptake and translocation of ^{15}N by Potamogeton nodosus. Dissertation for the Degree of Doctor of Education. Oklahoma State University, Stillwater, OK, 53 p.

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(C) Project Status

This project was completed June 30, 1973.

(D) Application of Research Results

The data on nitrogen uptake by aquatic plants clearly implicates the role of roots in the nutrition of these plants. This knowledge should be of major importance in any strategy to ameliorate the effects of euthrophication of lakes.

Table 1. Artificical lake water used in experiments.

NH_4-N added at NH_4Cl or $(NH_4)_2$ SO_4.

Compound	Concentration/liter
$CaCl_2$ anh.	0.054 g
$MgSO_4 . 7H_2O$	0.100 g
Na_2CO_3	0.020 g
$Na_2SiO_3 . 9H_2O$	0.023 g
KCl	0.030 g
$FeCl_3$ anh.	1.38 mg
$ZnCl_2$	0.48 mg
$MnCl_2 . 4H_2O$	5.5 µg
$CoCl_2 . 6H_2O$	8.1 µg
$CuCl_2$	8.5 µg
H_3BO_3	0.40 mg
$Na_2MoO_4 . H_2O$	0.254 mg
NTA	20.0 mg
TRIS	500.0 mg
K_2HPO_4	0.560 mg

Table 2

Least squares transformations of data relating NH_4-N uptake rate by *Ceratophyllum* (v) to substrate concentration (S). I= intercept on y axis, RC = slope and r = correlation coefficient.

	Form or Regression Equation	
	v vs S	S/v vs S
Experiment 2		
I	87.17	-2906.0
RC	0.30	1749.5
r	0.36	0.67
Experiment 3		
I	311.25	-3424.4
RC	0.12	1687.3
r	0.85	0.88
Experiment 5		
I	847.6	-3160.0
RC	0.07	2195.7
r	0.79	0.90
Experiment 6 (light)		
I	83.90	-4435.7
RC	0.04	1001.0
r	0.90	0.68
Education 6 (dark)		
I	131.64	-613.4
RC	0.04	1088.8
r	0.82	0.64

Table 3. Enrichment by ^{15}N, uptake rates by stems and leaves and incubation intervals of Elodea densa incubated with roots exposed to $^{15}NH_4$.

Root Development	Enrichment atom % excess ^{15}N	Uptake µg N/day	Incubation days
In Sand	0.1175	2.44	1
	0.1075	2.69	1
	0.3651	4.84	1
	0.0064	0.12	2
	-0-	0.00	2
	0.0169	0.31	2
	0.0092	0.02	4
	-0-	0.00	6
In Water	0.0108	0.29	1
	0.0102	0.47	1
	0.0242	0.69	2
	0.0078	0.12	2
	0.0343	0.69	3

Table 4. Enrichment by ^{15}N, uptake rates and incubation intervals of aquatic hydrophytes[1] incubated with roots[2] exposed to $^{15}NH_4$.

Species	Enrichment atom % excess ^{15}N	Uptake µg N/day	Incubation days
Ceratorphyllum demersum	-0-	0.00	2
(Apical 5 cm)	0.0313	1.70	2
	-0-	0.00	4
	0.1511	2.40	4
Potemogeton nodosus	-0-	0.00	1
(leaves only)	-0-	0.00	1
	-0-	0.00	2
	-0-	0.00	2
	0.0351	0.88	3
	0.0330	0.61	3

1. The apical 10 cm of Scirpus sp. was not enriched with ^{15}N after duplicate incubation of 2, 4 and 6 days, respectively.

2. Stems and leaves of C. demersum found in sediment.

Table 5. RATE OF UPTAKE AND TRANSLOCATION OF NITROGEN BY P. NODOSUS CULTURED IN THREE DIFFERENT SUBSTRATE (NH_4Cl), CONCENTRATIONS FOR TWO, FIVE AND TEN DAYS (UPTAKE RATES ARE EXPRESSED AS μg NH_4-N (mg N ROOT-RHIZOME DAY)$^{-1}$ AND TRANSLOCATION AS PERCENTAGE OF STEM-LEAF ^{15}N ENRICHMENTS TO TOTAL ENRICHMENT)

Incubation Intervals	Replicates (Identified by No.)	Substrate Concentration					
		Ambient*		5X Ambient		10X Ambient	
		Uptake	% Trans.	Uptake	% Trans.	Uptake	% Trans.
2 Days	(1)	0.0	0.0	0.02	0.0	0.00	0.0
	(2)	5.47	0.0	no data	0.0	2.83	26.8
	(3)	0.97	0.0	14.93	6.5	1.68**	no data
5 Days	(4)	15.04	87.3	0.00	0.0	31.13	27.4
	(5)	2.14	0.0	8.28	29.8	40.08	38.1
	(6)	0.90	0.0	4.78	14.4	9.96	21.3
10 Days	(7)	0.65	0.0	0.73	0.0	8.08	37.2
	(8)	5.46	81.5	1.47	11.5	6.65	0.0
	(9)	0.23	0.0	1.97	0.0	25.60	47.8

* Ambient concentration of substrate was 85 μg NH_4-N l^{-1}

** Rates computed from % ^{15}N excess in stem tissue only

Table 6. CHEMICAL COMPOSITION OF INTERSTITIAL WATER (n=1) COMPARED TO CHEMICAL COMPOSITION OF LAKE WATER (n=12) (Toetz, 1971)

	Interstitial HOH		Lake HOH
Constituents	mg/l	meq/l	meq/l
Ca	47.3	2.4	0.86
Mg	19.9	1.7	0.84
Na	27.3	1.2	0.84
K	5.6	0.0	-
Cl	18.0	0.5	0.43
SO_4	12.0	0.3	0.06
NO_3	4.0*	-	-
Total Dissolved Solids	350.0	-	-
HCO_3	-	-	2.06
Hardness ($CaCO_3$)	205.0	-	-

* Represents the apparent lower limit of detection for the procedure used.

(-) Those parameters were not measured or computed

Table 7. PLANT TISSUES ENRICHED BY IN SITU UPTAKE OF ^{15}N LABELED NH_4 FROM SEDIMENT (EXPRESSED AS % ^{15}N EXCESS) AND N CONTENT OF THE PLANT TISSUE: THREE REPLICATES USED FOR EACH INCUBATION INTERVAL

Tissues Enriched	Incubation Intervals					
	3 Days		7 Days		12 Days	
	% ^{15}N Excess	mg N	% ^{15}N Excess	mg N	% ^{15}N Excess	mg N
Root-Rhizome	0.356	0.082	no data	no data	0.256	2.468
Stem	0.000	3.693	1.030	1.214	0.408*	22.209
Leaves	no data	no data	1.021	2.230	0.061**	33.360

Table 8. Mean atom % ^{15}N, number of samples, 95% confidence limits (CI), and precision of measurement for replicated samples of enriched and non-enriched organic matter. All samples converted by automated Dumas procedure unless otherwise noted. Theoretical % ^{15}N = 0.36.

Source	n	Atom % ^{15}N	
		mean $\pm$ CI	5% mean
Enriched			
^{15}N labelled Ceratophyllum	4	0.9361 $\pm$ 0.0191	0.0468
^{15}N labelled Ceratophyllum*	4	0.9324 $\pm$ 0.0946	0.0466
Non-enriched			
acetanilide	3	0.3471 $\pm$ 0.0213	0.0174
azine	2	0.3669	0.0183
tank nitrogen	11	0.3645 $\pm$ 0.0068	0.0181
plankton on glass filters	3	0.3649 $\pm$ 0.0179	0.0182

* Preparation involved Kjeldahl destruction and hypobromite conversion.

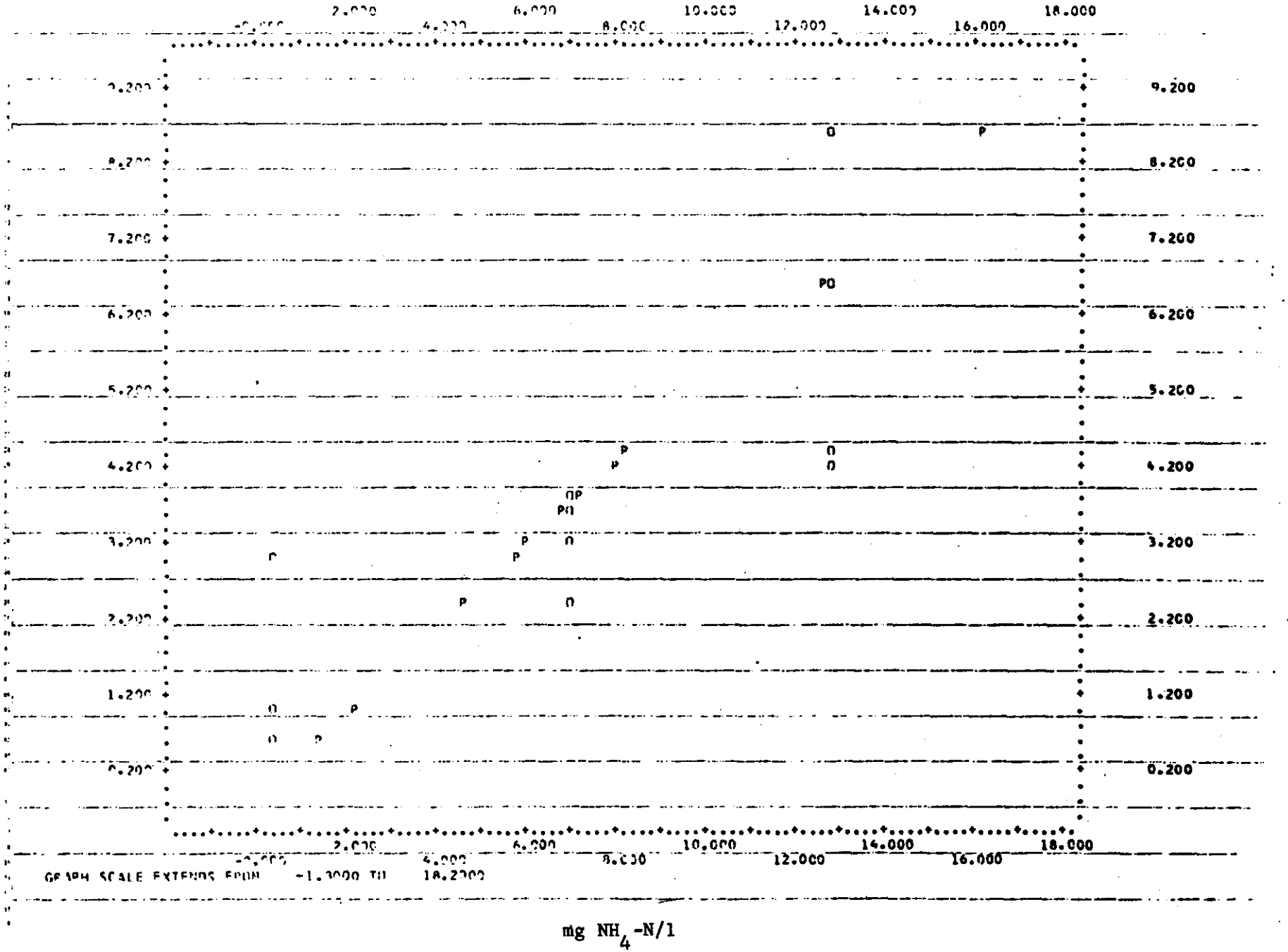

Figure 1. Internal concentration of ammonia in Ceratophyllum as a function of initial ammonia concentration in the culture medium. P = predicted, O = observed.

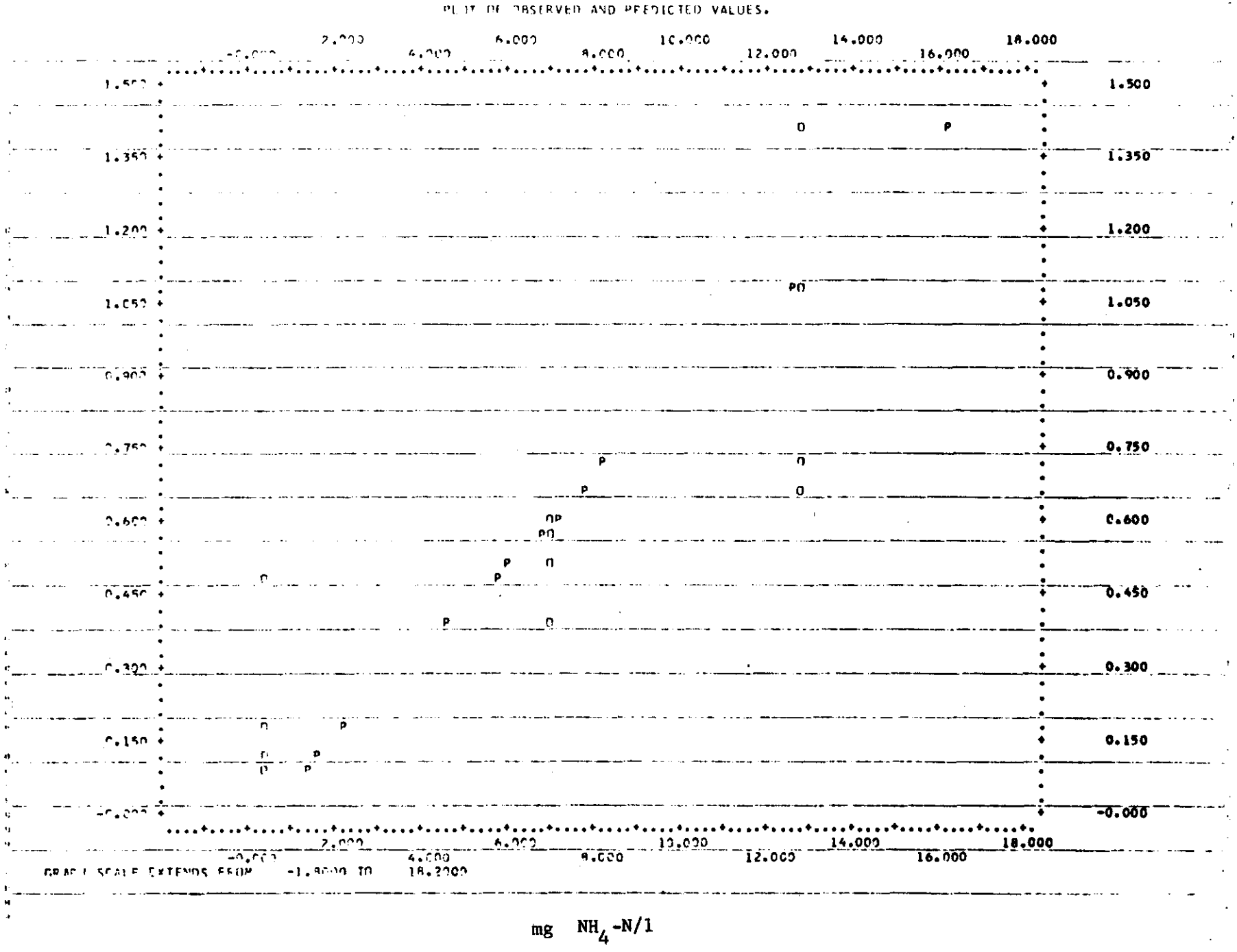

Figure 2. Internal concentration of ammonia in Ceratophyllum as a function of initial ammonia concentration in the culture medium. P = predicted, O = observed.

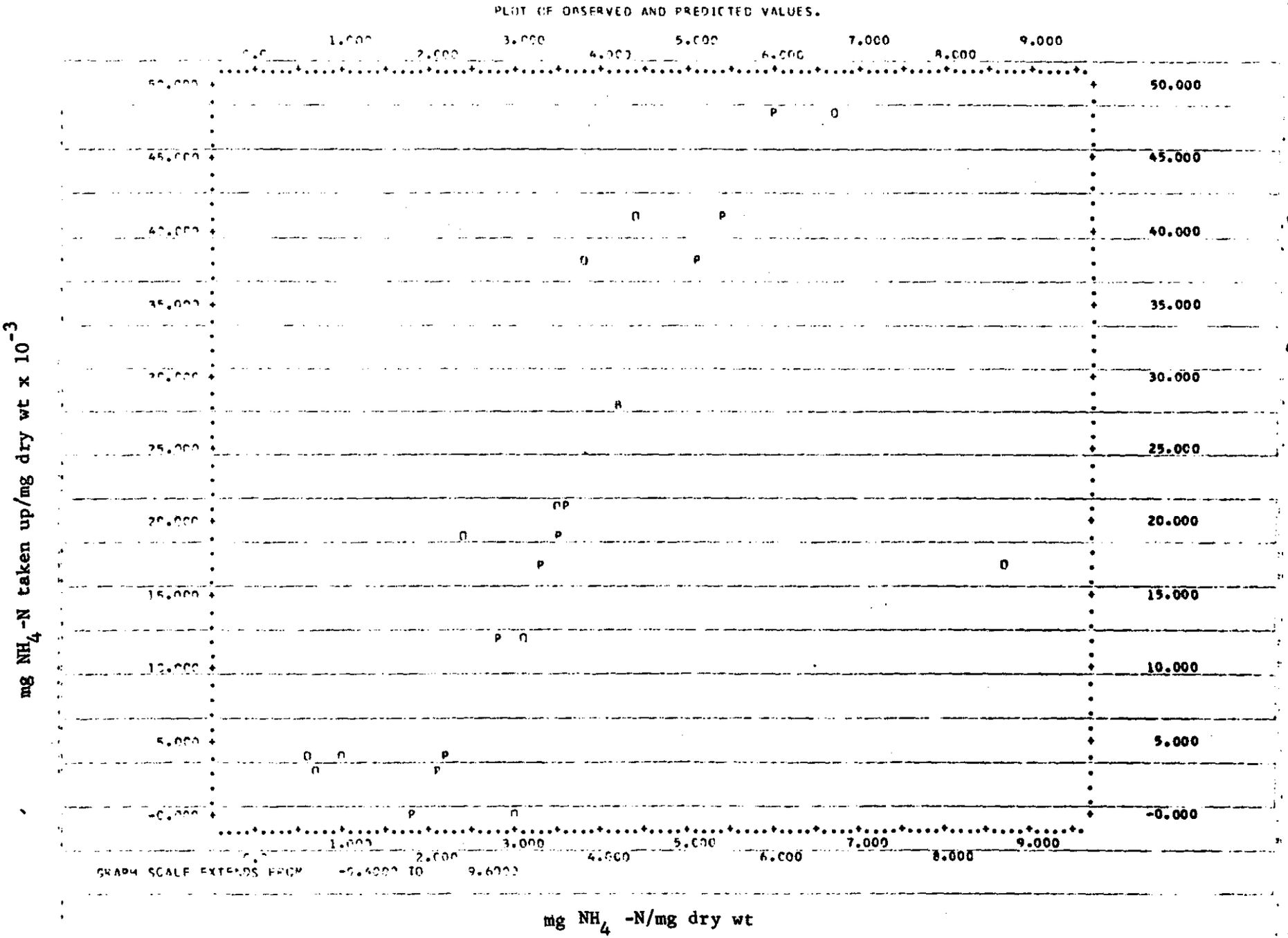

Figure 3. Uptake rate of ammonia during 3 days as a function of internal concentration of ammonia in Ceratophyllum. P = predicted, O = observed.

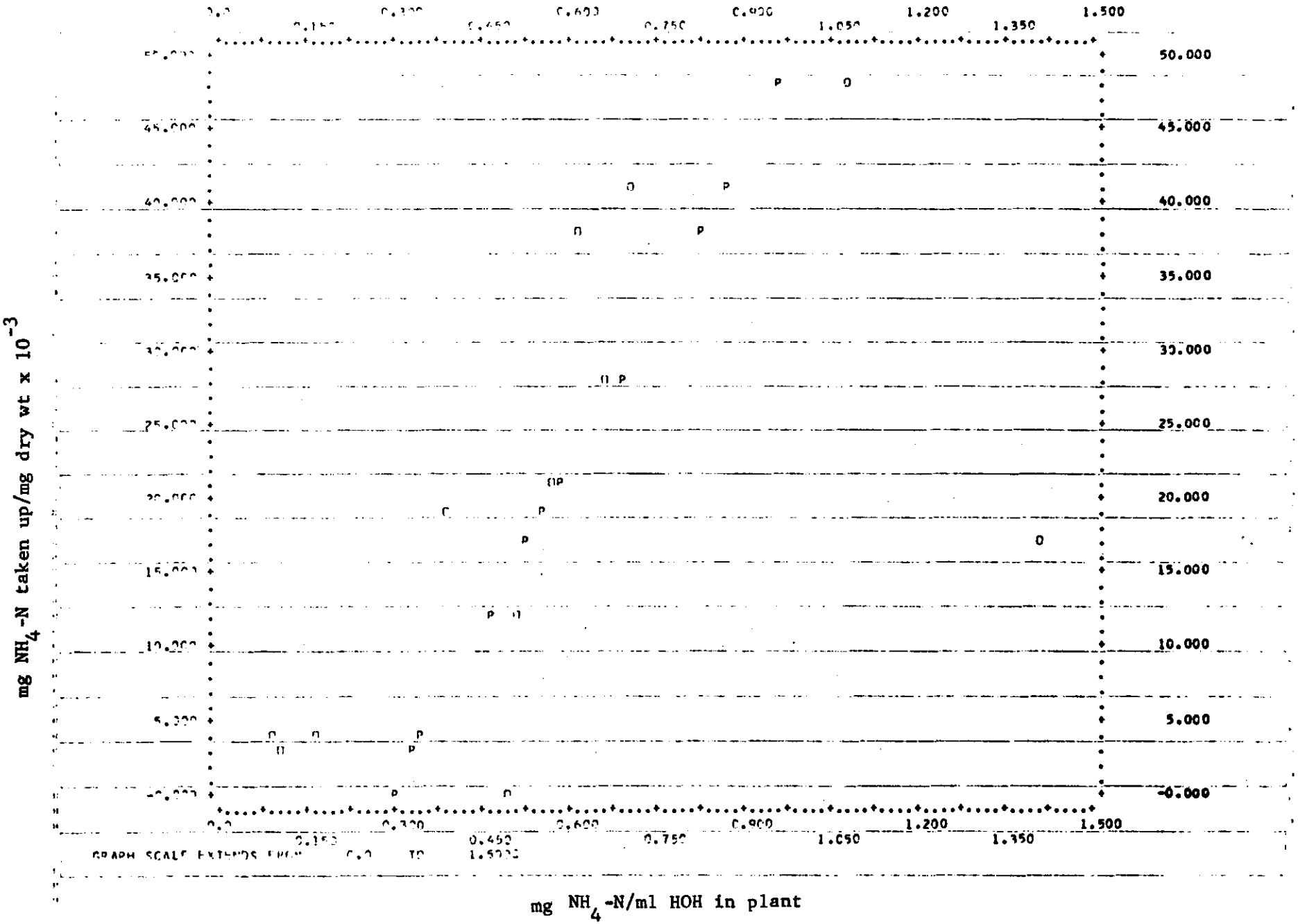

Figure 4. Uptake rate of ammonia during 3 days as a function of internal concentration of ammonia in Ceratophyllum. P = predicted, O = observed.

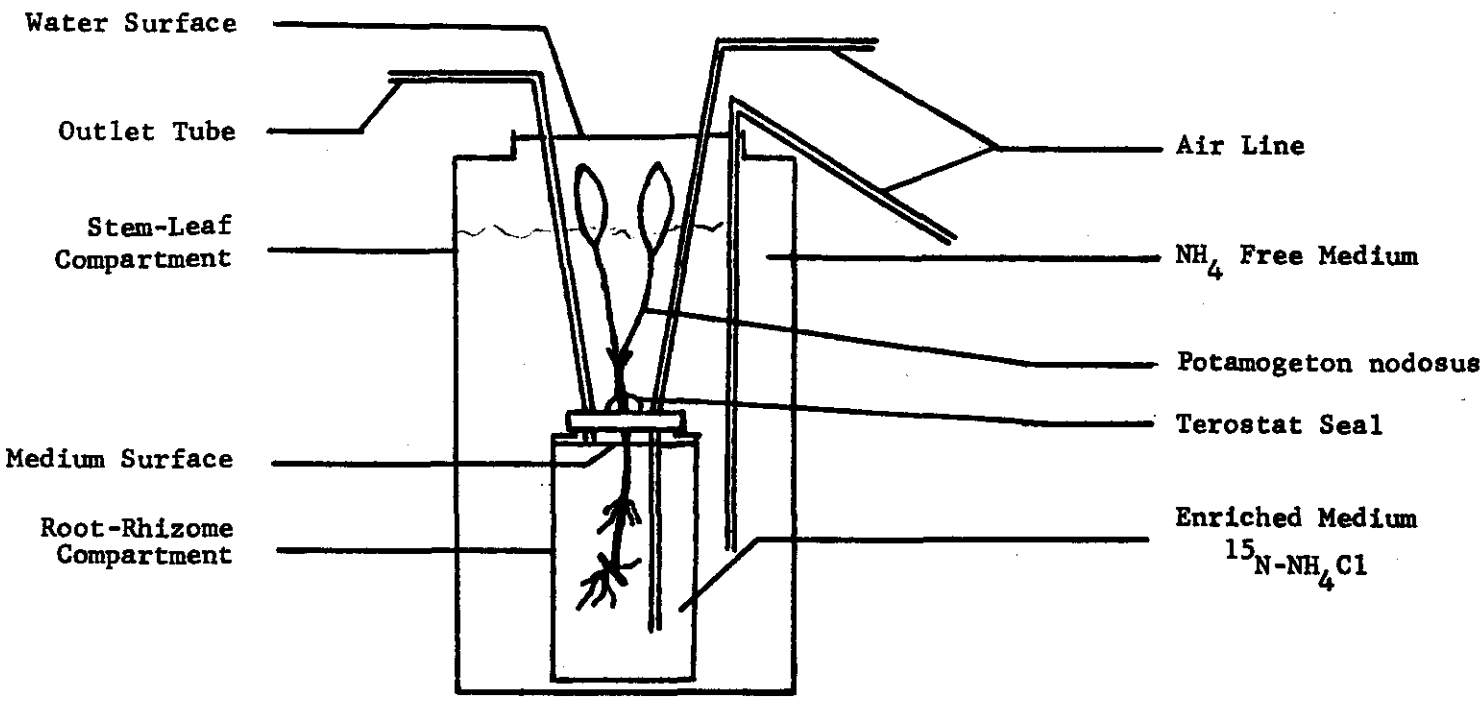

Figure 5. Two compartment Experimental Vessel for the Laboratory Uptake Experiment