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AMMONIUM AND NITRATE UPTAKE IN HUNGARIAN SHALLOW LAKES

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ABSTRACT

Observations were made on the rate of ammonium and nitrate uptake by freshwater phytoplankton and macrophytes according to Michaelis - Menten kinetics. The uptake of ammonium and nitrate was estimated for 5 different concentrations using the methods of Eppley et al., (1969). A hyperbola results when the uptake velocity (v) of ammonium or nitrate is plotted against its concentration (S). The S/v vs. S transformation of the Michaelis - Menten equation was used to estimate km and v_{max}. For mixed populations of phytoplankton and macrophytes in Lake Balaton and a fish pond, km ranged between 5.54 to 17.97 μ mol NH^{+}_{4} - N L^{-1} and 0.52 to 2.63 μ mol NO^{-}_{3} - N L^{-1}, respectively. On this basis, enzyme kinetics would be a useful tool in constructing mathematical models for nitrogen cycle in freshwater ecosystems.

INTRODUCTION

The influence of ammonium and nitrate uptake on lake metabolism is largely based on indirect measurements. The inadequate evaluation of nitrogen reactions in lakes has prevented the direct elucidation of the role of nitrogen in eutrophication.

The present work deals with the uptake of some nitrogen compounds because of the main following interests: 1. Nitrogen is an important constituent of protoblasm, 2. Nitrogen frequently limits primary production in aquatic environments, and 3. It is a structural element of the cell and does not participate in cellular energetics like phosphorus or carbon. For these reasons, Dugdale and Goering (1967) suggested that algal productivity might be more fundamentally described in terms of nitrogen rather than phosphorus or carbon. Phytoplankton consumes inorganic nitrogen in the form of nitrate, nitrite and ammonia (Fogg, 1966) as well amino acids (Schull, 1974 & Wheeler et al., 1974) and urea (Carpenter et al., 1972 & McCarthy, 1972).

Estimation of the rates of ammonia and nitrate uptake was carried out in two different Hungarian ecosystems; Lake Balaton (Keszthely basin) and a fish pond in Hortobagy.

Generally, two techniques are used for measuring nitrogen assimilation by phytoplankton: 1. Direct chemical measurement (Eppley et al., 1969) by which the water sample is enriched with various amounts of NH^+ and NO_3^- and the decrease in their concentrations after a period of incubation is considered as the up take, and 2. ^{15}N-technique, which was introduced by Neess et al., (1962) and Dugdale and Goering (1967). This technique needs long consuming time and requires high concentrations up to 500 ug N L^{-1}(Prochazkova et al., 1970) and often necessitates the addition of high concentrations of ^{15}N-labeled to the incubation vessel to obtain suitable sensitivity. Unfortunately, the adddition of high concentrations of artificial substrate increases the uptake velocity. Consequently, many investigators have kept the concentration of labeled nutrients to about 10 % of the ambient level. For good results, this method requires long icubation time of 24 hours or longer. ^{15}N-NH^+ and ^{15}N-NO_3^- have been used extensively in marine studies, but occasionally in freshwater studies to characterize these nutrient fluxes (Toetz et al., 1973; Toetz et al., 1977 and Takashi & Saijo, 1981).

MATERIAL AND METHODS

a. Chemical Measurements

For ammonium and nitrate uptake measurements, the method of Eppley et al., (1969) was applied. Ten to fifteen liters of the lake water was collected form 0.5 m depth. Immediately after collection, the water samples were divided into a series of aliquots (one liter each). Five aliquots from that sreies were enriched with various amounts of NH_4 (5, 10, 20, 30 and 50 μ mol NH_4 L^{-1}) and the other five enriched with various amounts of NO^3 (2.5, 5, 10, 20 and 30 μ mol NO^3L^{-1}). A water sample from each aliquot was transferred to a corresponding incubating bottle of 300 ml capacity and then exposed directly in situ. Water samples without any enrichment were also exposed in situ to measure the uptake rate in the original concentration. The concentrations of dissolved NH_4 and NO^3 in the original water samples were determined immediately after collection. After 3-4 hours incubation in situ, the water in the icubating bottles were analysed for NH_4 and NO^3. Uptake was calculated as the differences between the concentrations of the series before and after the incubation period. Ammonia was determined by the indophenol blue procedure as described by Verdouw et al., (1978). Nitrate was determined as nitrite, after reduction by passing it through a column of copper-coated cadmium metal, (Strickland and Parson, 1968).

b. Theoretical Treatment of the Data

Eppley et al., (1969) showed that a hyperbola results when the uptake rate of a substrate (v) is plotted against its concentration (S) for marine phytoplankton. Many other workers have followed this method (Mac Isaac and Dugdale, 1969). The half-saturation constant Km (the concentration for half maximum uptake rate) can be calculated for its corresponding

Where, (v) is the uptake rate of a substrate, (S) is the concentration and V^{max} is the maximum velocity attained when all uptake are saturated with the substrate. The constant Km reflects the relative ability of phytoplankton to use low levels of nutrients which may be of ecological significance. The value of Km is measured by applying the fit between S/v and S. In the present transform, Km is the negative intercept on the S-axis and V^{max} in the slope inverse of the regression line:

$$Y = a + bx .$$

It is worthy to mention that, (S/v) vs. (S) plot is the best among other linear transformations because it gives expressive spread of the experimental points.

Eppley et al., (1969) used Michaelis-Menten's constant for NO³ uptake by marine phytoplankton in a culture and knowing the specific growth rates of each species at various levels of irridiance charaterizing the niches of certain marine algae. This approach could be used also in freshwater environment.

RESULTS AND DISCUSSION

I. Lake Balaton (Keszthely Basin)

Lake Balaton in Hungary is the largest body of freshwater in continental Europe. The Lake is about 75 Km long and 8 Km wide, its surface area is 600 Km^2. The average and maximum depths are 3 and 11 m, respectively. More details on the morphology of Lake Balaton was previously mentioned elsewhere (Abdelmoneim, 1983).

Since Lake Balaton is shallow, the collected samples were restricted to the surface water layer at 0.5 m depth. Toetz, (1976) found that the best estimates for Km would arise from the results recorded at 0.5 m depth where V^{max} is highest.

In July 1979 and May and June 1980, the rate of ammonium uptake increased with the increase of its concentration in accordance to Michaelis-Menten kinetics, while this was less pronounced in July, 1980, (Fig. 1).

For nitrate uptake, Michaelis-Menten kinetics was successfully applied in July 1979 and May and July 1980, while it failed in June, 1980, (Fig. 2). This may be attributed to the fact that the concentration of nitrate in the last mentioned month was high compared with those of the other dates and consequently it has no limiting effect.

II. Hortobagy Fish Pond

In the macrophyte covered fish pond of Hortobagy, ammonia and nitrate uptake of plankton and macrophytes were expressed by the Michaelis-Menten kinetic method.

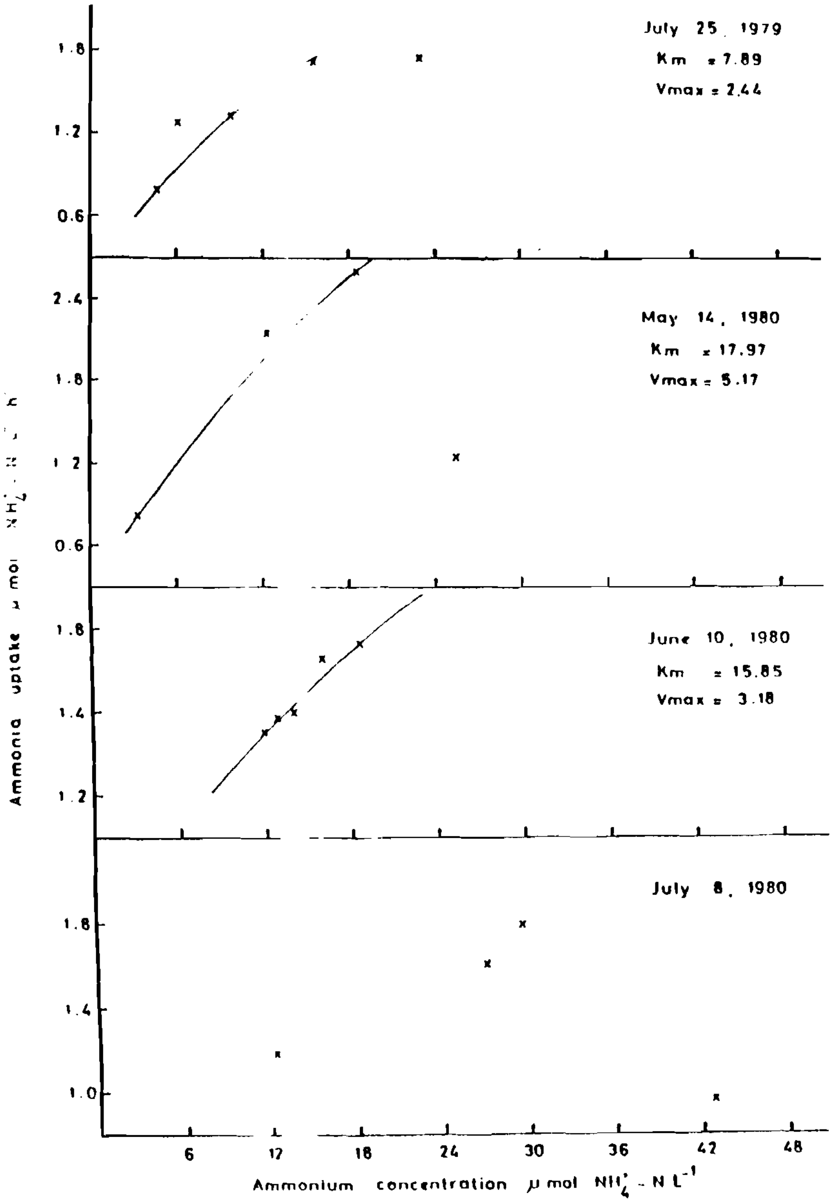

Fig. (1)
Ammonium uptake in Keszthely Basin, (Lake Balaton).

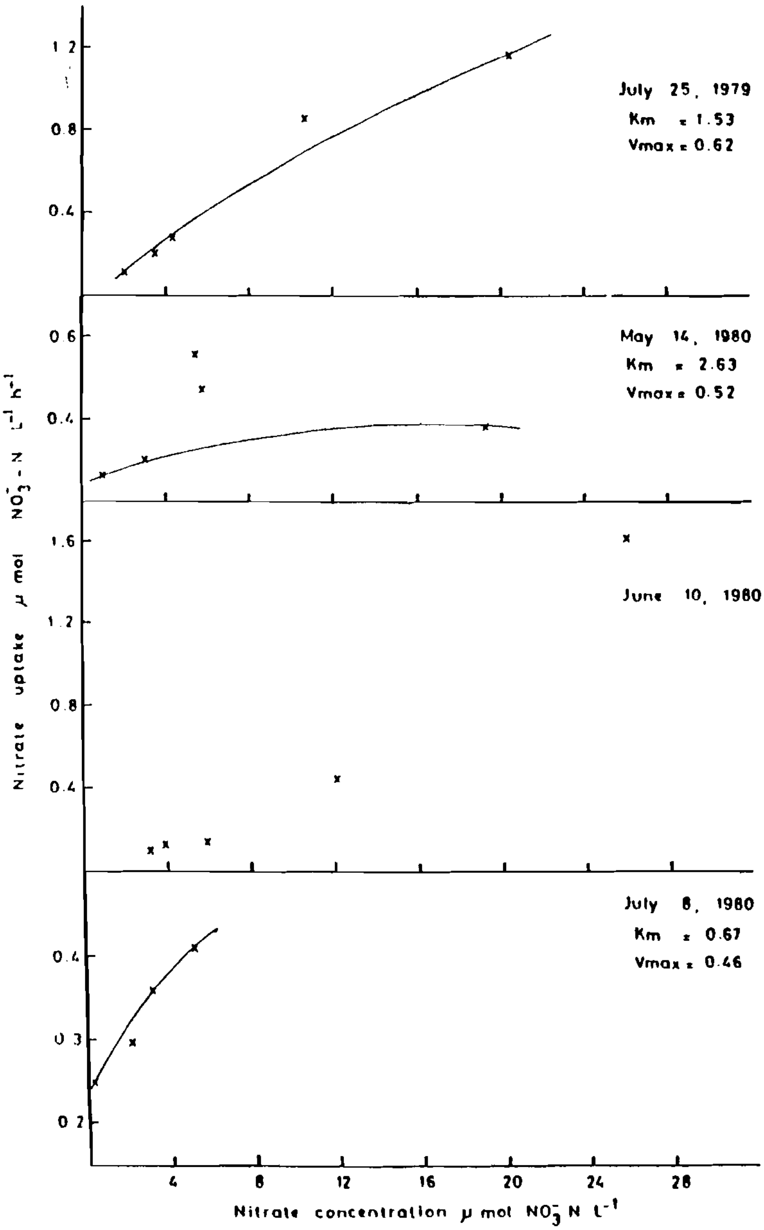

Fig. (2)
Nitrate uptake in Keszthely Basin, (Lake Balaton).

The ammonium uptake increased with increasing of ammonium concentration particularly at the beginning of the growing season but not in June and August 1979, (Fig. 3). Later on when ammonia deficiency ceased, the maximum uptake of photosynthesizing population was at the natural substrate level.

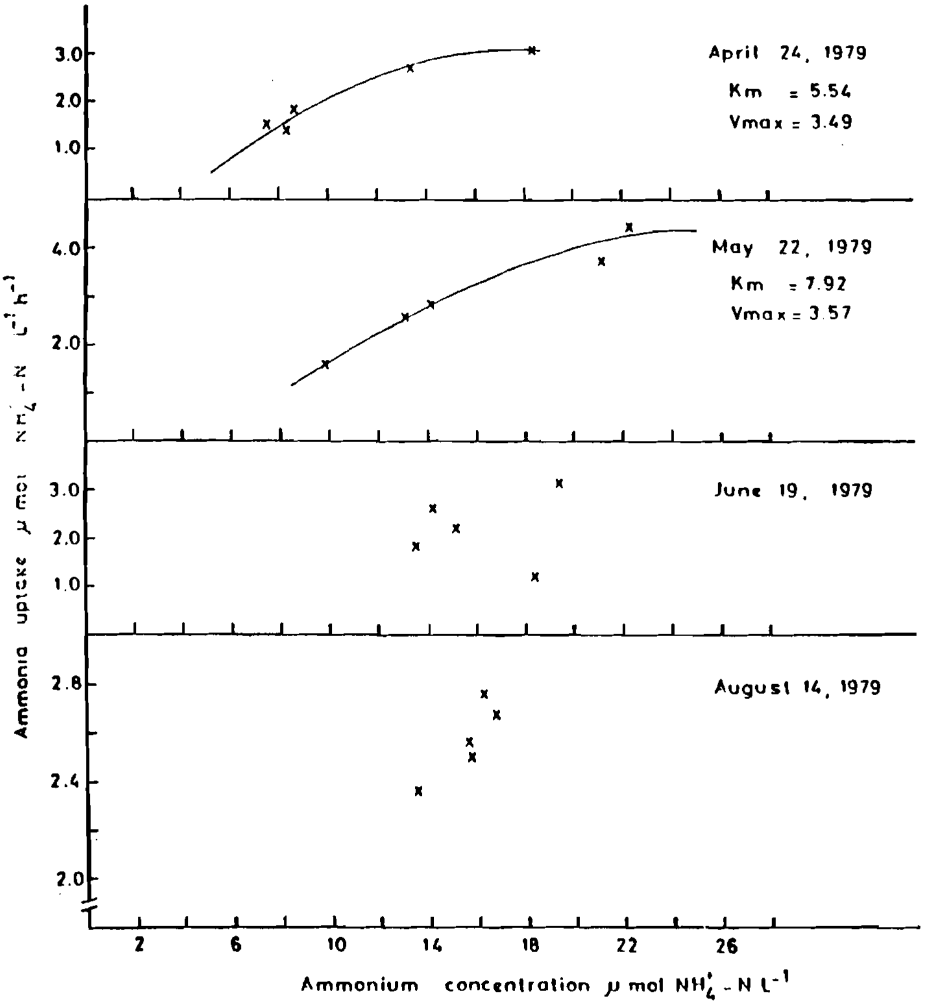

Fig. (3)

Ammonium uptake in macrophyte covered fish pond
(Hortobagy, Hungary)

Routine computer analysis shows that the Km values range between 5.54 and 7.92 μ mol NH_4 L^{-1} for a given phytoplankton community, while V^{max} equals to 3.49 and 3.57 μ mol NH_4 L^{-1} h^{-1} in April and May, respectively. Significant lower values of both Km and V^{max} are characteristics of poor nutient water.

However, in nitrate uptake, no umambiguous values were obtained, (Fig. 4). The uptake was growing even at high nitrate concentrations in May and JUne 1979, relative to nitrate enrichment. Meanwhile, there were no standard values obtained at natural low substrate concentrations. Saturation constants of nitrate were generally higher in natural water poor in nutrients.

In most cases, there was an increase of NO_3 uptake parallel to the increase of its concentration. In other words when NO_3 uptake does'nt obey Michaelis-Menten kinetics, one would expect that the ecosystem is not limited by NO_3 as its initial concentration is getting high. Similar results were reported in eutrophic waters, (MacIsaac and Dugdale, 1969; Toetz et al., 1973).

For comparison, Table (1) shows the range of Km for both NH_4 and NO_3 in some natural regions. It is obvious that Km for freshwater is much higher than that in marine habitats. Generally speaking, the confidence levels for Km are extremely wide. Toetz, et al., (1973) found that Km for nitrate uptake in Lake Keystone ranges between 14.6 and 173.9 μ mol L^{-1}, while in Lake Carl Blackwell it average 3.27 μ mol L^{-1}, (Toetz et al., 1977). In the present work Km for nitrate uptake in Lake Balaton ranged between 0.52 and 2.63 μ mol L^{-1}. No data for the fish pond were available as the nitrate uptake measurements were homogenous. This may be attributed to the fact that most plants prefer NH^+_4 than NO^-_3 as a source of nitrogen compounds, (MacIsaac and Dugdale, 1972).

For ammonium uptake, the Km values ranged between 5.54 to 17.97 μ mol L^{-1}. They are higher in Lake Balaton than that in the fish pond which reflect the higher trophic level in the lake. Beside the eutrophication problem, algal blooms may also be responsible for the high values of Km in this ecosystem.

Ammonium and Nitrate Uptake by Some Macrophytes and Algae

Some macrophytes and algae were obtained from the surface of the fish pond just prior to incubation. In all cases, a terminal part of a plant stem, 10 cm long was inserted in an incubating bottle of 300 ml capacity and filled with water sample. All the samples were enriched with 20 μ mol NH^+_4 - 20 μ mol NO^-_3 - 16 μ mol PO^{-3}_4 L. Ammonium and nitrate uptake were estimated as the decrease in their concentrations before and after the incubation period. Two parallel series on bottles were incubated separately; one series was exposed directly in situ and the other was kept in the dark. As shown in Table (2), the uptake rate of both ammonium and nitrate was generally higher for aquatic plants in the case of phytoplankton. There were no considerable differences between dark and light uptake of ammonium, while nitrate uptake was lower in the dark (light dependent) than in the light.

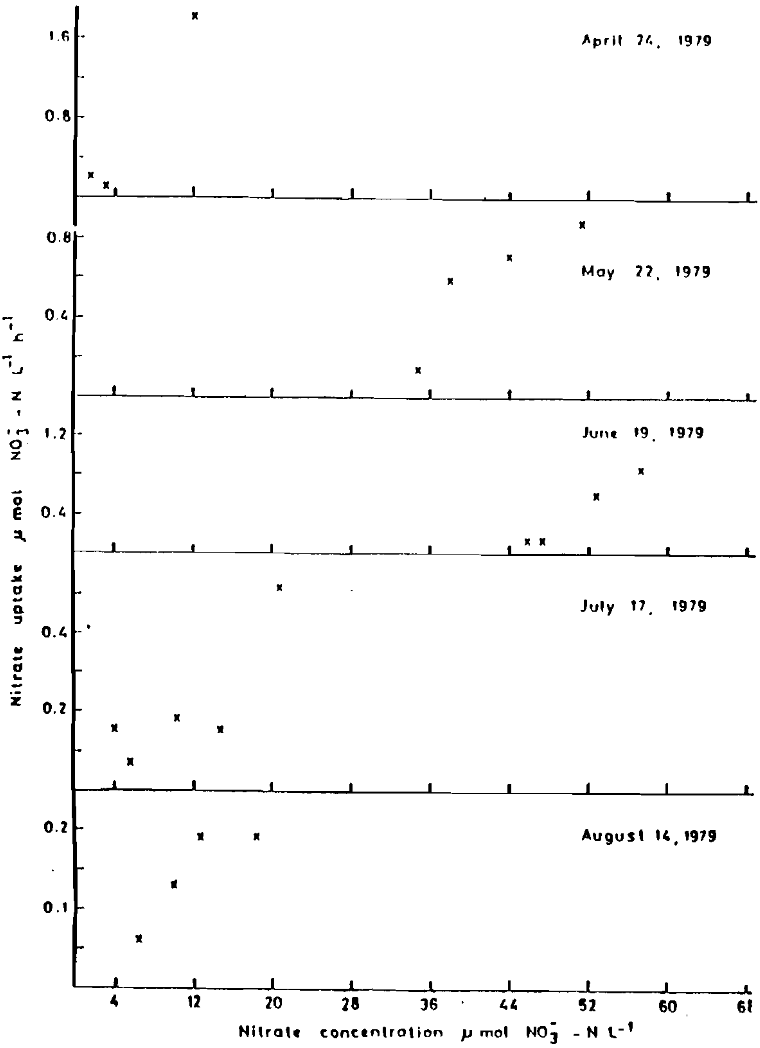

Fig. (4)
Nitrate uptake in macrophyte covered fish pond.
(Hortobagy, Hungary).

TABLE (1)

Values of Km for ammonia and nitrate uptake in different habitats.

Km for ammonia u mol L^{-1}	Km for nitrate u mol L^{-1}	Type of habitate	Author
0.1 - 0.5	0.1 - 0.7	Oceanic Phytoplankton	Eppley *et al*. 1969
---	1.07	Freshwater Phytoplankton	Pearson *et al*. 1969
0.1 - 0.6	0.2	Oligotrophic marine waters	MacIsaac & Dugdale, 1969
1.3	1.0	Eutrophic marine waters	MacIsaac & Dugdale, 1969
43.8 - 316.1	---	Ceratophyllum Plant belt.	Toetz, *et al*. 1973
2.8	14.6 - 173.9	Lake Keystone	Toez, *et al*. 1973
---	3.1	Lake Carl Black well	Toez, *et al*. 1973
6.74	---	Oceanic Phytoplankton	Carpenter & McCatthy,1975
---	14.9 - 148.0	Freshwater Phytoplankton	Wallen & Cartier, 1975
---	3.27	Lake Carl Blackwell	Toez *et al*. 1977
0.1 - 0.3	---	Freshwater algae	Healy, 1977
7.89 - 17.97	0.52 - 2.63	Lake Balaton	Present Study
5.54 - 7.92	---	Fish Pond	Present Study

TABLE (2)

Ammonium and nitrate uptake by some macrophytes and algae (u mol g^{-1} h^{-1}).

ORGANISM	Ammonium uptake		Nitrate uptake	
	light	dark	light	dark
Ceratophyllum spp.	5.41	5.57	7.05	5.57
Cladophora spp.	5.19	5.33	6.69	5.62
Myriophyllum spicatum	5.35	5.27	5.32	2.86
Potamogeton pectinatus	5.60	5.03	4.87	0.74
Vallisneria spp.	5.03	4.43	4.98	1.34
Phytoplankton	2.28	1.77	2.83	1.29

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