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Ecological Research Series

ALGAL NUTRIENT AVAILABILITY AND LIMITATION IN LAKE ONTARIO DURING IFYGL

Part II



**Environmental Research Laboratory
Office of Research and Development
U.S. Environmental Protection Agency
Duluth, Minnesota 55804**

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EPA-600/3-77-045
May 1977

ALGAL NUTRIENT AVAILABILITY AND LIMITATION
IN LAKE ONTARIO DURING IFYGL

Part II. Nitrogen Available in Lake Ontario Tributary
Water Samples and Urban Runoff from Madison, Wisconsin

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FOREWORD

Our nation's freshwaters are vital for all animals and plants, yet our diverse uses of water---for recreation, food, energy, transportation, and industry---physically and chemically alter lakes, rivers, and streams. Such alterations threaten terrestrial organisms, as well as those living in water. The Environmental Research Laboratory in Duluth, Minnesota develops methods, conducts laboratory and field studies, and extrapolates research findings

- to determine how physical and chemical pollution affects aquatic life
- to assess the effects of ecosystems on pollutants
- to predict effects of pollutants on large lakes through use of models
- to measure bioaccumulation of pollutants in aquatic organisms that are consumed by other animals, including man

This report provides information on the nitrogen availability in Lake Ontario tributary waters. The information is required in the understanding of algal nutrient growth in Lake Ontario.

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PREFACE

This project was conducted as part of the International Field Year for Great Lakes Research and consisted of three separate parts, all directed toward providing information needed to assess the factors limiting algal growth in Lake Ontario and the amounts of nitrogen and phosphorus in tributaries and drainage which would likely become available in the lake. Part I is concerned with a comprehensive study of the amounts of phosphorus entering Lake Ontario from U.S. tributaries which will likely become available in the lake. Particular attention is given to the particulate and organic forms of phosphorus in the major U.S. tributaries to the lake. Part II is concerned with a study of the amounts of available nitrogen entering Lake Ontario from the U.S. tributaries. Part III is concerned with the factors limiting algal growth in Lake Ontario and in the major U.S. tributaries. This report presents Part II of this study. Parts I and III are published as separate reports by the U.S. Environmental Protection Agency and entitled, Algal Nutrient Availability and Limitation In Lake Ontario During IFYGL, with the following subtitles:

Part I: Available Phosphorus in Urban Runoff and Lake Ontario
Tributary Waters

Part III: Algal Nutrient Limitation in Lake Ontario During IFYGL

ABSTRACT

Samples of water from the Niagara, Genesee, Oswego, and Black Rivers were collected from March to June, 1973. The samples were analyzed for nitrogen forms and were incubated in darkness under aerobic conditions to promote mineralization of soluble inorganic nitrogen from the organic nitrogen in the samples. The amounts of ammonia and nitrate were determined as a function of the time of incubation. Generally, over 50 percent of total nitrogen present in these river samples was immediately available for algal growth or potentially available after mineralization by bacteria. The results were highly variable from each tributary and no single value could be selected from the data obtained to describe the availability of total nitrogen in a given river.

This report was submitted in fulfillment of Contract No. R-800537-02 under the sponsorship of the Environmental Protection Agency. Work was completed as of June, 1975.

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SECTION I

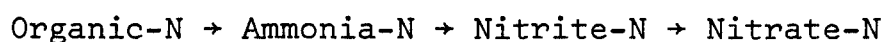
INTRODUCTION

Nitrogen is recognized as one of the most important constituents of living matter. It has been known to be a probable limiting algal nutrient in many waters. The increase in the loads of nitrogen into surface water in the presence of other nutrients, leads to the eutrophication process resulting in the excessive growth of algae and aquatic weeds and also depletion of oxygen in the hypolimnion of lakes that undergo thermal stratification. There are many sources of nutrients that get into receiving bodies of water, one of them being urban runoff. Current trend in the U.S. indicates that more and more of the population is being concentrated into urban regions. Although considerable amounts of time and money are being consumed to handle the sanitary wastes from the urban environment, little information is available on the significance of the stormwater runoff from urban areas.

Previous evidence indicates that the dominant form of nitrogen compound in urban runoff is organic nitrogen. These nitrogen compounds come from precipitation, dustfall, street litter and vegetation. They are generally not available to the algae for growth. However, after entering the lakes or rivers, the organic nitrogen compounds can be converted into ammonia by biochemical and physico-chemical activities. Under aerobic conditions, the ammonia form of nitrogen will be oxidized to nitrate. Since nitrogen as nitrate

and ammonia is readily available for algal growth, urban runoff represents an important source of nitrogen in the lakes and rivers.

Samples of water from the Niagara, Genesee, Oswego, and Black Rivers were collected from March to June, 1973. Also, samples in the Madison, Wisconsin urban runoff were collected. The samples were analyzed for nitrogen (N) forms, then were incubated in darkness under aerobic conditions to promote the regeneration of soluble, inorganic-N from the organic-N in the samples, by the following series of reactions:



The progress of the nutrient regeneration process was followed by means of ammonia and nitrate analyses made during the incubations. The amount of nitrogen in a form which would be available for algae was estimated by the sum of ammonia-N and nitrate-N and was expressed as a percent of the total N or of the organic-N in the samples.

A limited series of experiments was also performed to predict the nitrogen mineralization of particulate matter from river water in Lake Ontario. Particulate matter was suspended in Lake Ontario water and incubated under the same conditions used in the nutrient regeneration studies with whole river samples. The amount of algal-available nitrogen produced by the particles was estimated by the nitrate production and was expressed as a percent of the organic-N in the particulate matter. Algal bioassay tests were used to determine the fraction of organic nitrogen available for algal growth.

SECTION II

CONCLUSIONS

1. Samples of water from the Niagara, Genesee, Oswego, and Black Rivers generally showed over 50 percent of their total N to be either immediately available for algal growth or potentially available after mineralization by bacteria.

2. Because of the variation in total N availability and small number of samples tested from each tributary, no single value could be selected from the data to describe the availability of total N in a given river.

3. Efforts to estimate organic N availability showed a wide range of values. Low organic N availability values were found in samples with the high organic N concentrations.

4. Direct estimation of particulate organic-N availability by dark incubation of membrane-filterable particles yielded values which conflicted with the availability data from incubations of the unfiltered river waters.

5. Samples of urban stormwater drainage obtained from Madison, Wisconsin, showed that over 80 percent of the total nitrogen in the sample may be converted to an available form for algal growth in a period of approximately 100 days.

SECTION III

RECOMMENDATIONS

It is recommended that in those situations where nitrogen is thought to be the limiting element controlling algal growth, studies should be initiated which utilize the techniques developed in this investigation to determine what part of the total nitrogen entering the water body will likely become available to support algal growth in receiving waters. Additional large-scale studies are needed to determine the factors influencing the results of available nitrogen from various types of organic and particulate matter present in natural waters. Particular emphasis should be given to investigating such factors as the temperature of the solution, effects of mixing, trace element composition, and other factors which may influence the available nitrogen in a particular sample.

SECTION IV

LITERATURE REVIEW

Mineralization is the process of conversion of organic matter to inorganic form. This process can be brought about by changes in solution due to physico-chemical as well as bacterial action. Bacteria are the principal agents of conversion of nitrogen from one form to another in the nitrogen cycle, and the steps of the cycle of most concern to water quality are those involving water-soluble nitrogen, namely ammonification, nitrification, and denitrification. Decomposition of organic matter starts with autolysis, which leads to an increase of permeability, enabling several compounds already present in dissolved state, to leave the cell. Moreover, compounds in undissolved state may dissolve. The processes of liberation and mineralization of elements play an important part in the chemical cycle in fresh water because it makes nutrients available for use by aquatic micro-organisms. The main biological processes involving inorganic nitrogen are shown diagrammatically in Figure 1.

Nitrogen in urban runoff is in the forms of nitrite, nitrate, ammonium and organic nitrogen. Weibel et al. (1964) have studied urban land runoff in Cincinnati, Ohio as a factor in stream pollution and found that most of the nitrogen is in organic form in the range of 0.2 to 4.8 mgN/l. He also compared stormwater runoff loads and sanitary sewage

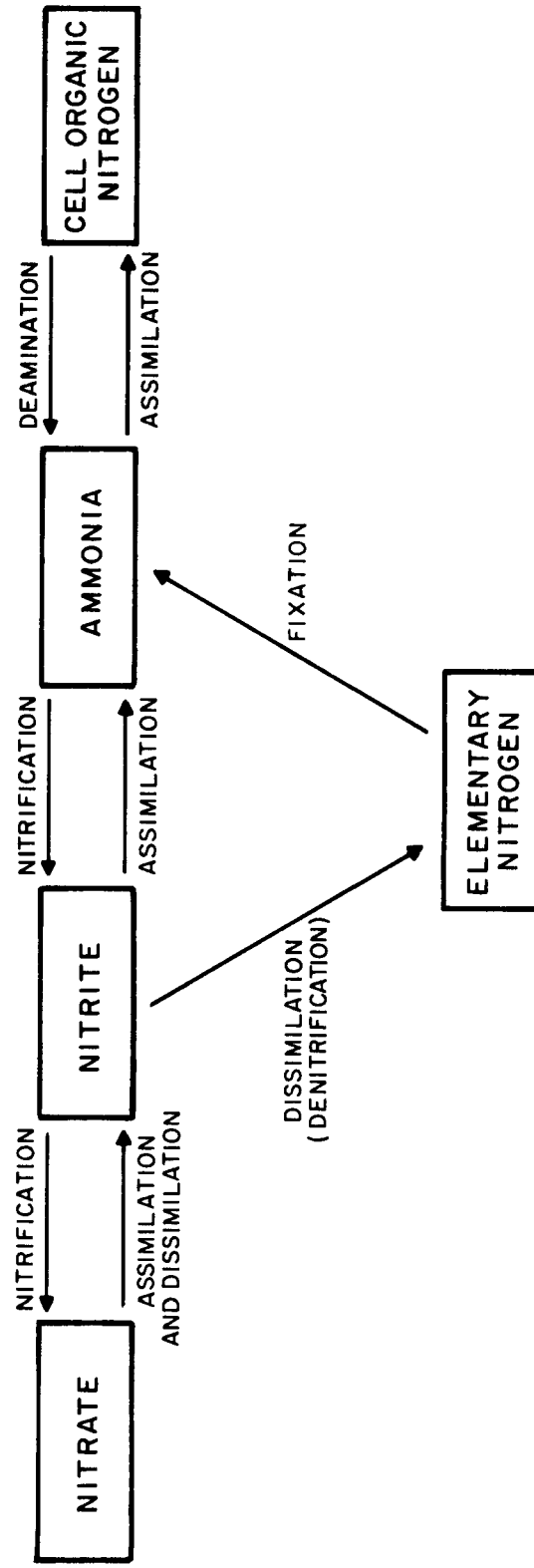


Figure 1. Main biological process involving nitrogen (Painter,1970)

loads and reported that in stormwater runoff the suspended loading was 1.4 times that of sewage where COD was 25 percent, BOD 6 percent, phosphorus 9 percent, and nitrogen 11 percent of raw sewage.

Lee et al. (1966) and Sonzogni and Lee (1972) had estimated the amount of nutrients entering Lake Mendota annually per acre of urban drainage. The results showed that most of nitrogen in urban runoff was in the organic form as was found in the Cincinnati study (Weibel et al., 1964). Sonzogni and Lee also noted that three times more nitrogen was entering Lake Mendota from urban runoff than the previous estimation by Lee. It is obvious from this information that the urban stormwater runoff cannot be neglected in considering waste loadings from urban areas.

Several recent reports have suggested that nitrogen more often might be the limiting factor in aquatic biomass production than heretofore believed. Gerloff and Skoog (1957) compared the growth of Microcystis aeruginosa in sterilized Lake Mendota water enriched with nitrogen, phosphorus and other essential elements singly and in various combinations. The addition of only phosphate or iron produced no increase in growth while the addition of nitrate alone resulted in approximately 64 percent of the growth obtained with all three elements added. They suggested nitrogen as the important algal growth-limiting nutrient in eutrophic Lake Mendota. However, conclusions from experiments of this type are limited in their application as the samples of water represent conditions only in very limited areas of the lake and at a specific time.

Lund (1965) stated that the low summer nitrate levels commonly observed in surface water of eutrophic lakes might suggest nitrogen limitation at that time. Recently, Lueschow et al. (1970) found indication that the mean monthly nitrogen

(organic or inorganic) and not phosphorus contents of surface waters of Southern Wisconsin lakes provides an index of their trophic status. This information would suggest that nitrogen might often be the limiting nutrient in eutrophic lakes, especially those high in available phosphorus.

Golterman (1960) studied the liberation and mineralization of nitrogen during sterile autolysis of Scenedesmus quadricauda. Autolysis was induced by U.V. irradiation or by chloroform treatment. Only 20 - 30 percent of nitrogen compound was liberated. The rest of nitrogen compounds were in the form of protein and nucleic acids, and remained as a slag. He tried to digest the nitrogen-slag-remaining by means of bacteria. When the slag was suspended in lake water, one half of the nitrogen was converted to ammonia in 5 days, while the other half was divided up into bacterial and Scenedesmus nitrogen, the mutual ratio of which could not be determined. This experiment showed that bacteria is the primary agent responsible for nutrients cycling in water.

Vaccaro (1965) reported on an experiment in which a mixed planktonic culture was allowed to decompose. In this experiment, phosphorus regeneration was more rapid than was nitrogen regeneration. Similar results have been obtained for nitrogen and phosphorus regeneration from aquatic macrophytes (Nichols and Keeney, 1973). Most of nitrogen is in ammonium form between day 7 to 30 and in nitrate between day 72 to 80. Temperature and oxygen were found to have influence in the regeneration process.

Chen, Keeney and Konrad (1972) investigated nitrification rates in lake sediment-water systems. Sediment samples from Wisconsin eutrophic and oligotrophic hard and soft-water lakes were incubated in deionized water at 10°C or 25°C in the dark in mixed and non-mixed systems, open to the

atmosphere. The amount of nitrate and ammonium nitrogen were determined routinely. Nitrate was not formed in the acid sediments from soft-water lakes. However, in calcareous sediment from hard-water lakes, nitrification proceeded readily when the system was stirred to increase oxygen diffusion. A one to three day lag phase occurred before nitrification commenced, and in all cases onset of nitrification was accompanied by a corresponding decline in soluble and exchangeable ammonium nitrogen, the latter value approaching zero by the end of the incubation period. Nitrate did not accumulate when the samples were not stirred. This study indicated that sediments did not add appreciable amounts of nitrate to water except in a well oxidized, mixed situation such as might be occurring in shallow areas or at lake turnover. Keeney (1973) stated that the organic material in sediments is more stable towards decomposition than that present in dead plant and animal remains, probably due to protection by reaction with organic compounds and clay minerals, formation of resistant heterocyclic material, and physical inaccessibility. The rapid nitrification rate (270 $\mu\text{gN/l}$ per hour at 25°C in Lake Mendota), however, indicated that this process could add appreciable nitrate to lake waters in turnover time. Temperature also had effect on nitrification rate, the higher the temperature, the more rapid nitrification.

Similar results were obtained by Austin (1970). She studied the release of nitrogenous compounds from lake sediments. Sediments from hard-water lake (Mendota) released large concentrations of dissolved inorganic nitrogen under aerobic conditions. The predominant form of inorganic nitrogen leached from the sediment was nitrate. In contrast, ammonium and soluble Kjeldahl nitrogen were the predominant species of nitrogen in the anaerobic studies. No detectable

amounts of ammonia or nitrite were released throughout the study. The release of nitrogen from hard-water lake sediment was greater than that of soft-water lake sediment. She also found that the release of nitrogen from lake sediment was greatly dependent upon currents and mixing but not the total nitrogen concentration of a lake sediment.

Lopez and Galvez (1958) studied the mineralization of the organic matter to determine the availability of ten Phillipine soils to release nitrogen in available form under submerged condition. They found that regardless of the nature of the soil organic matter and the microflora, the amount of mineralized nitrogen was significantly and positively correlated with their contents of organic matter, total nitrogen, and the nitrogen uptake by rice plants. Grain yields in greenhouse culture were significantly correlated with mineralized nitrogen.

While there have been numerous studies on the conversion of organic and particulate forms of nitrogen present in soils, lake sediments and algae, there have been no reported studies on this conversion in urban storm water drainage.

SECTION V

METHODS AND PROCEDURES

WATER COLLECTION

The Niagara, Genesee, Oswego, and Black Rivers were sampled during the spring of 1973, at points close to their discharge into Lake Ontario (Figure 2).

The samples were collected from the 0 to one meter depth in the rivers and were transported to Madison, Wisconsin, in plastic containers. No preservatives were added to the samples, which were refrigerated at 4°C upon reception at the University of Wisconsin Water Chemistry Laboratory at Madison. The average time between sampling and initial nitrogen analyses was approximately five days, of which one to two days were required for (unrefrigerated) transportation.

URBAN RUNOFF SAMPLES

The main classes of sites selected were: residential and exposed land under construction. The residential classes were further divided according to Madison zoning codes as follows:

R_1 - A single family residential district, with some low density multiple family dwellings.

R_2 - A single family residential district with some low density multiple family dwellings. This zone differed from R_1 in that less usable open space per dwelling unit was allowed in R_2 as compared to R_1 .

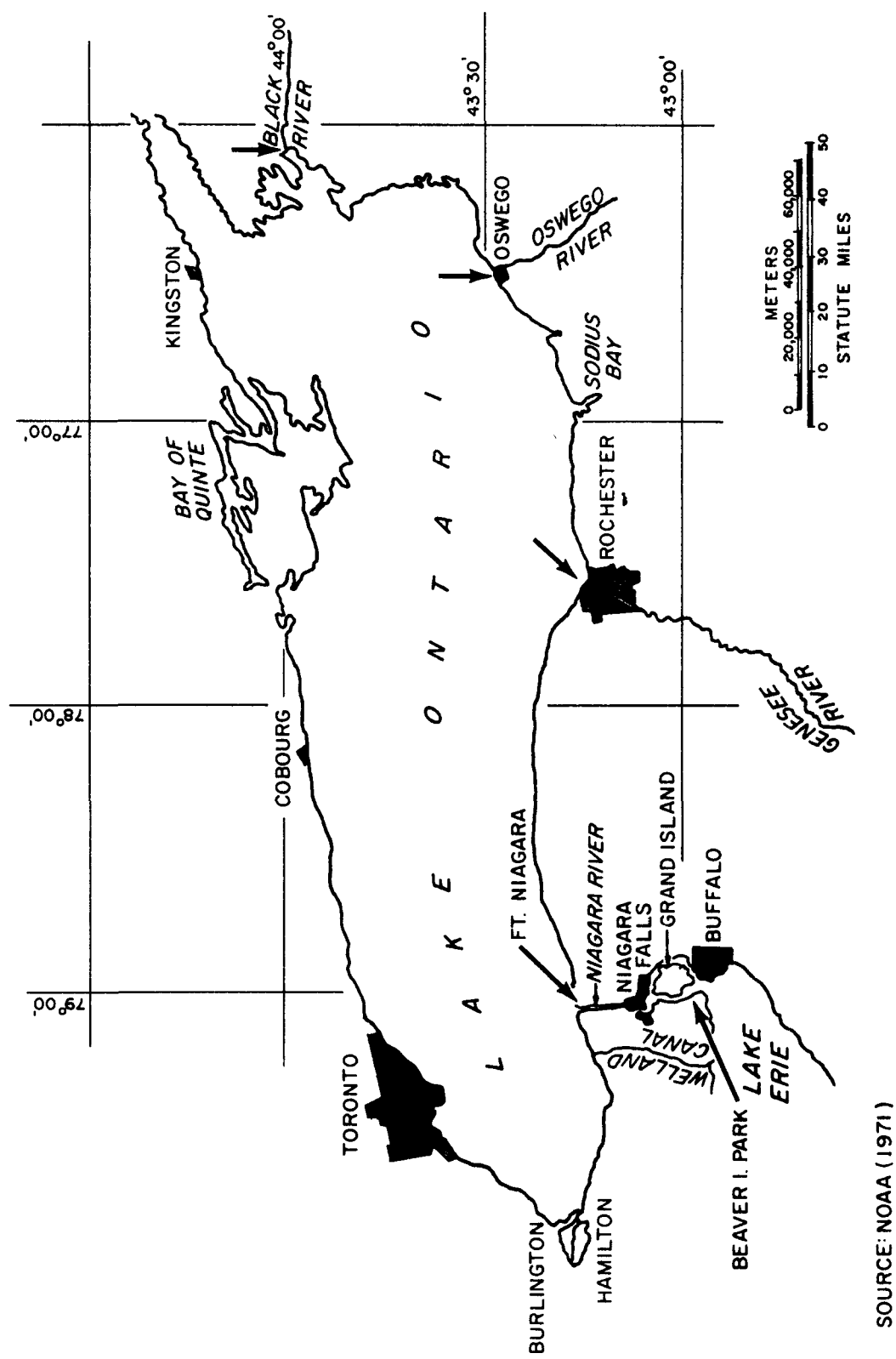


Figure 2. Lake Ontario and major tributaries

R₅ - A medium density residential area located in inlying urban parts of the city. The area being sampled was part of the University of Wisconsin campus.

The urban construction site was an area of Western Madison undergoing rapid development, with exposed terraces and severe erosion.

The specific location of each site is given in Table 1. All sites could be sampled within about 45 minutes - 1.5 hours, depending upon traffic conditions. Samples were collected during rainstorms which appeared to be intense enough for reasonable overland flows. Although samples taken at various times during a runoff could vary in concentration of organic nitrogen (Kluesener, 1972), for qualitative studies of availability, the concentration was not an important factor. Samples at each site were taken in one-gallon acid washed plastic cubitainers. All samples were brought to the laboratory and immediately stored at 4°C until analyzed.

All chemicals used in these studies were reagent grade, with the following exceptions: Sodium hypochlorite was purchased commercially as Clorox (5.25 percent NaClO), Selenium oxychloride (SeOCl₂) was USP.

EXPERIMENTAL METHODS

Nitrogen in runoff or rivers was considered as soluble or particles, based on filtration through 0.45 micron pore-size membrane filters. In this study, available nitrogen is defined as the nitrogen measured chemically as nitrate or ammonia or which can be shown to be used by algae in a growth bioassay. The various nitrogen forms to be analyzed are:

Table 1. URBAN RUNOFF SAMPLING STATIONS
IN MADISON, WISCONSIN

<hr/> <hr/>		
Drainage Area		
<u>Zoning Code</u>	<u>Station</u>	<u>Location</u>
R1	A	Inlet of the large storm sewer in the median strip of Whitney, near the Montauk place intersection.
R2	B	Inlet of the large storm sewer in the median strip of Manitau Way, near the Tumalo Trail intersection.
R5	D	Outlet of storm sewer pipe, near the U.W. Water Chemistry Laboratory, which carries drainage from the Bascom Hill campus area.
Urban Construction	F	Street gutter flow from the construction of apartment houses near the corner of Island Drive and Masthead Streets, one block from the Mineral Point Road-Island Drive intersection.
<hr/> <hr/>		

Ammonium Nitrogen ($\text{NH}_4^+\text{-N}$)

Analysis of a filtered sample, using alkaline phenol procedure adapted for the Auto Analyzer (Kluesener, 1969).

Total Kjeldahl Nitrogen

Sulfuric acid digestion of a sample, followed by measurement of ammonium salt formed using alkaline phenol procedure or Orion ammonia electrode procedure (Model 95-10) (Orion, 1971).

Soluble Kjeldahl Nitrogen

Sulfuric acid digestion of a filtered sample, followed by measurement of ammonium salt formed using alkaline phenol procedure or Orion ammonia electrode procedure.

Nitrate Nitrogen

Direct analysis of a filtered sample, using the modified Brucine Method (Jenkins and Medsker, 1964) either manually or on the Technicon Auto-Analyzer.

Nitrite-N was not determined, as the relative contribution of this species to the total initial N of the river waters was assumed to be negligible. Hence, the concentration of Total N in the samples was computed from the sum of the initial nitrate-N plus the initial TKN. Detailed procedures have been described by Sirisinha (1973).

In tests of nutrient regeneration from river water particles, the river water was filtered through a membrane filter, and the particles retained on the filters were scraped into Lake Ontario water, using a metal spatula. The initial particulate organic-N concentration due to

the river water particles was found by TKN analysis of the lake water-particle suspension, minus the TKN found in lake water controls with no river water particles.

NUTRIENT REGENERATION INCUBATIONS

Triplicate 400 ml volumes of whole river water samples or suspensions of river water particles in lake water were placed in glass one-liter bottles stoppered with foam or cotton plugs. Triplicate bottles of lake water served as controls for the incubations of river water particles in lake water. All test bottles were incubated at $20 \pm 1^{\circ}\text{C}$ under black plastic sheets in a walk-in algal culture incubator room. The bottles were shaken daily and whenever sampled. After various periods of incubation, aliquots were removed from the bottles for analyses of ammonia-N and nitrate-N. In some cases, the ammonia determinations were omitted if the preceding ammonia concentration was negligible. In the experiments with suspensions of particles, only the nitrate-N concentrations were determined in the sample particle suspensions and in the controls. The total incubation period used in the nutrient regeneration experiments ranged from 35 to 100 days.

A BIOASSAY TEST FOR NITROGEN AVAILABILITY FROM PARTICLES

This algal assay is based on Liebig's law of the minimum which states that "growth is limited by the substance that is present in minimal quantity in respect to the needs of the organism." Increase in absorbance as a measure of the growth of algae was not used in the present experiment due to the interference of particles scattering light. Instead, the growth of algae was measured by direct algal counting method. Selenastrum capricornutum was chosen as test organism because it has

several superior qualities as a laboratory organism, it is solitary and easy to identify, it grows easily in culture with little variation in form when nutrient conditions are varied, it seems to have low temperature requirements, it can tolerate both strongly acidic and alkaline waters (Forsberg, 1972), and it has a rapid growth (National Eutrophication Research Program, 1971).

According to Murray (1971), light intensity of 500 ft-c reduced the growth of S. capricornutum. There was also evidence of a slight growth rate limitation of Selenastrum at 200 ft-c in the present experiment. Continuous "cool-white" fluorescent lighting 400 ft-c \pm 10 percent was used. Intensity was measured adjacent to the flask at the liquid level.

Reagents

Stock and standard nitration solutions: the same as described in nitrate determination except the range of standards were from 13.5 gN/ml to 81 μ gN/ml.

Synthetic Algal Nutrient Medium (National Eutrophication Research Program, 1971).

Final concentration of nutrients.

Macronutrients - The following salts, reagent grade, in milligrams per liter of ammonia-free water.

Compound	Concentration (mg/l)	Element	Concentration (mg/l)
K ₂ HPO ₄	1.044	P	0.186
MgCl ₂	5.700	Mg	2.904
MgSO ₄ ·7H ₂ O	14.700	S	1.911
CaCl ₂ ·2H ₂ O	4.410	Ca	2.143
NaHCO ₃	15.000	C	1.202
NaNO ₃	25.500	N	4.200
		Na	11.001
		K	0.469

Micronutrients - The following salts, reagent grade, in micrograms per liter of ammonia-free water.

	($\mu\text{g/l}$)		($\mu\text{g/l}$)
H_3BO_3	185.520	B	32.460
MnCl_2	264.264	Mn	115.374
ZnCl_2	32.709	Zn	15.691
CoCl_2	0.780	Co	0.354
CuCl_2	0.009	Cu	0.004
$\text{NaMoO}_4 \cdot 2\text{H}_2\text{O}$	7.260	Mo	2.878
FeCl_3	96.000	Fe	33.051
$\text{Na}_2\text{EDTA} \cdot 2\text{H}_2\text{O}$	300.000		

Stock solutions.

Macronutrients - Stock solutions of individual salts might be made up in 1000 times the final concentration.

Micronutrients - The trace metals, FeCl_3 and EDTA were combined in a single stock mix at 1000 times final concentration.

Preparation of medium.

Combination of stock solutions - 1ml of each of the stock solutions (macronutrients and micronutrients were added to ammonia-free water to give a final volume of 1 liter. The trace metal - FeCl_3 EDTA mixture (Micronutrients) was added after filtration.

All media should be stored in dark to avoid any photochemical changes.

Cell suspension - A 50 ml of fresh culture of S. capricornutum in AAP medium with 3x N and P concentration was used. The tube was covered with parafilm and centrifuged at 1200 rpm for 20 minutes. The packed cells were then washed twice with 10 ml of fresh AAP (-N) medium in order to get rid of excess nitrogen materials in the

medium. These cells were then suspended in 30 ml of new AAP (-N) medium and counted with a haemocytometer. The following formula was used to make enough inoculum for 100 flasks, with 10^4 cells/flask.

$$\frac{2700 \times 10^4 \text{ cells}}{\text{cell count (cells/ml) of washed culture}} = \begin{array}{l} \text{ml of washed cul-} \\ \text{ture diluted to} \\ \text{100 ml with AAP-N} \\ \text{medium} \end{array}$$

The concentration of cells after dilution of washed culture to 100 ml should be 27×10^4 cells/ml or $27 \times$ the desired initial population level of 1×10^4 cells/ml. Use 1 ml of 27×10^4 cells/ml suspension per bioassay flask.

Bioassay Test

A 200 ml sample from urban runoff or Lake Ontario tributaries were filtered through 0.45 micron Millipore filter, and particles were scraped off into 200 ml of AAP (-N) medium. A 10 or 20 ml of suspension was removed for total Kjeldahl-N determination. Volume corrections were made to get the results of organic-N concentration in each bioassay flask.

Three sets of five replicates of the following were performed and incubated at $24 \pm 1^\circ\text{C}$, 400 ft-c light intensity.

<u>Set 1</u>	Blank flasks contained	25 ml AAP (-N) medium
		1 ml ammonia-free water
		1 ml cell suspension
<u>Set 2</u>	Standard flasks contained	25 ml AAP (-N) medium
		1 ml NO_3^- -N spike
		1 ml cell suspension

Set 3 Sample flasks contained 25 ml particulate suspension in AAP (-N) medium
1 ml ammonia-free water
1 ml cell suspension

The shape of the growth curve was found by plotting the absorbance at 750 nm for standard culture vs time. The growth plateaus of all standards were obtained from the above plots. The cells in standards and samples were counted on the day the plateau was reached. A haemocytometer was used for cell counting. The concentration of nitrogen vs average cell counts for the standard was plotted, and the available-N content of the samples was then calculated.

The initial experiments using the above bioassay method failed to give satisfactory results, even though the experiments were repeated many times. This failure was due to contamination by Anabaena, which is the nitrogen-fixing organism, from the studied particles. Hence, a more refined bioassay technique was used which gave satisfactory results. The procedure is described below.

The samples of both urban runoff and Lake Ontario tributaries after 100 days of incubation were filtered through a 0.45 micron Millipore filter. Then 20 ml of the filtrate was used as the nitrogen source instead of the particles. The experiments were set up as follows:

Set 1 Blank flasks contained 21 ml of deionized water
5 ml of 5 x AAP (-N) medium
1 ml of cell suspension

<u>Set 2</u>	Standard flasks contained	20 ml of deionized water
		5 ml of 5 x AAP (-N) medium
		1 ml of nitrate stand- ards
		1 ml of cell suspension
<u>Set 3</u>	Sample flasks contained	20 ml of filtrate
		5 ml of 5 x AAP (-N) medium
		1 ml of deionized water
		1 ml of cell suspension

Filtration made the sample sterile; neither Anabaena nor bacteria were present in the filtrate. After ten days of incubation, the samples were measured at 750 nm. The growth of algae are shown by comparing with the standard curves.

SECTION VI

RESULTS

INITIAL NITROGEN AVAILABILITY

The analyses of New York rivers (Table A.1, Appendix A) for initial nitrogen forms showed the following ranges for "algal-available" N (ammonia-N + nitrate-N) and organic-N, expressed as a percent of the total N in the samples.

Table 2. INITIAL N AVAILABILITY

Percent of Total N as:			
<u>River</u>	<u>Available N</u>	<u>Organic-N</u>	<u>No. of Samples</u>
Niagara	30-67	33-70	3
Genesee	51-55	45-49	4
Oswego	44-69	31-56	8
Black	27-58	42-73	4

Thus, in some of the samples, the extent of nitrogen mineralization was relatively low, 30 percent or less, while in other samples, over 60 percent of the total N was already in an algal-available form by the time of the initial analyses.

The fraction of total N not present as ammonia-N or nitrate-N was assumed to be organic-N. Table 3 shows the initial organic-N concentrations in the samples, as well as a breakdown of the organic-N into soluble and particulate fractions, computed by the equations:

Soluble organic-N = SKN - Ammonia-N

Particulate organic-N = TKN - SKN

The concentrations of organic N in samples of a given river were quite variable, as were the relative amounts of soluble or particulate organic-N. In the Oswego R. samples, for example, the particulate fraction of organic-N accounted for 13 to 78 percent of the organic-N.

INCUBATIONS OF RIVER WATERS

The data collected from the individual bottles of river water during dark incubations are given in Table A.1 of Appendix A. The mean values for ammonia-N and nitrate-N from each triplicate set of bottles are listed in Table A.2, Appendix A. The sum of these mean values estimated the algal-available N as a function of time during the incubations; these sums are given in Table A.2, expressed in concentration units and as percents of the total N in the sample.

Ammonia-N levels generally dropped to the analytically detectable limit of 0.05 mgN/l in 25 to 50 days. Values of ammonia-N of less than 0.05 mgN/l were considered as equal to zero in all calculations of percentage availability. Formation of nitrate from the ammonia appeared to be completed by 25 to 50 days in most samples.

Table 4 presents the results of the dark incubations in terms of the maximum algal available N values observed after 35 to 50 days of incubation.

The Niagara R. samples showed 54 to 91 percent of their total N available as ammonia plus nitrate after the 35 or 50 days of dark incubation. Figure 3 illustrates the changes in available N as a function of time. Samples No. 41 and 50 showed very similar behavior, while Sample No. 56

Table 3. DISTRIBUTION OF ORGANIC NITROGEN FORMS IN RIVER WATER SAMPLES

Sample No.	Organic-N	Soluble Organic-N		Particulate Organic-N	
	(mgN/l)	(mgN/l)	(%Organic-N)	(mgN/l)	(%Organic-N)
<u>Niagara R.</u>					
41	0.15	0.06	40	0.09	60
50	0.31	0.18	58	0.13	42
56	0.79	0.59	75	0.20	25
<u>Genesee R.</u>					
34	1.08	0.37	34	0.71	66
42	0.68	0.17	25	0.51	75
51	0.59	0.15	25	0.44	75
58	1.02	0.44	43	0.58	57
<u>Oswego R.</u>					
28	0.55	0.45	82	0.10	18
31	0.39	0.34	87	0.05	13
35	0.39	0.33	85	0.06	15
43	0.68	0.15	22	0.53	78
47	0.45	0.30	67	0.15	33
48	0.47	0.20	43	0.27	57
52	0.83	0.24	29	0.59	71
59	1.04	0.59	57	0.45	43
<u>Black R.</u>					
36	0.42	0.29	69	0.13	31
44	0.25	0.10	40	0.15	60
53	0.48	0.30	62	0.18	38
60	0.91	0.56	62	0.35	38

appeared to be significantly lower than the other two samples with regard to its nitrogen mineralization, and relatively higher with regard to total N concentration (Table 4).

The Genesee R. samples had maximum available N values of 60 to 75 percent of total N. The samples with the lowest values were Nos. 34 and 58, both of which had relatively high total N values compared to Samples No. 42 and 51 (Table 4). Figure

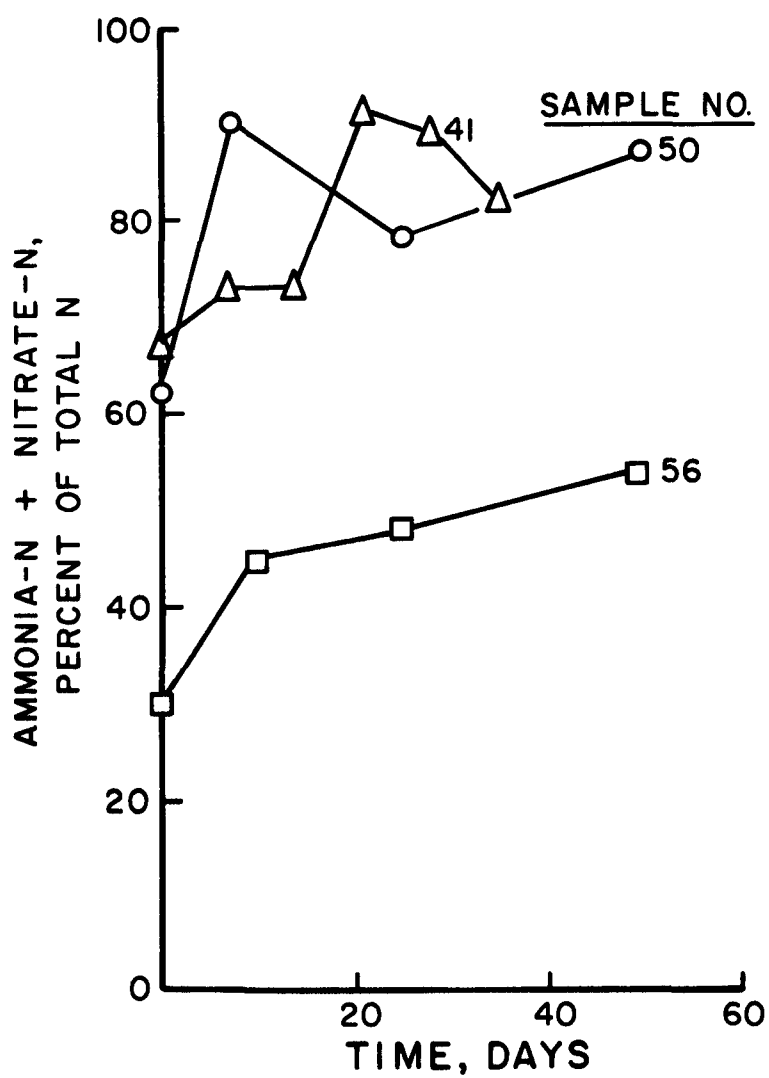


Figure 3. Percent of total N as ammonia-N + nitrate-N during dark incubation of Niagara R. samples

4 shows the close agreement between Samples No. 42 and 51 and between Nos. 34 and 58.

Oswego R. waters (Figures 5 and 6) showed maximum availability values of 58 to 91 percent of total N, although most of the samples had values between 58 and 82 percent. The lowest availability was seen in Sample No. 59 which also had the highest total N concentration (Table 4). The rather sharp increases in available N seen after 64 days in Samples No. 31 and 35 (Figure 6) were probably analytical errors. The curves had essentially reached a plateau by day 64, after which no further changes would be expected.

The Black R. samples (Figure 7) demonstrated consistent maximum availability values of 67 to 75 percent of total N in three samples, while in the third sample (No. 60), the availability was low, only 36 percent at its maximum value. This sample also had the highest total N content of the sample set (Table 4).

In an attempt to determine whether a constant fraction of the organic forms from a given river were being mineralized in the dark incubations, the maximum values of ammonia-N plus nitrate-N given in Table 4 were expressed as a percent of the organic-N in the sample and tabulated in Table 5. The most outstanding feature of the data in this table is the relationship of percent availability to the initial concentration of organic-N. Those samples high in total N concentrations and low relative total N availability appeared to have high initial organic-N concentrations and low organic-N availability. With the exception of Sample No. 34, all of the samples with high organic-N concentrations were collected on the June 16-17 sampling trip.

As a check on the nitrogen mass balance of the samples during the incubations, the initial total N values of some samples were compared to the total N value in those samples

Table 4. MINERALIZATION OF TOTAL N TO AMMONIA
AND NITRATE IN 35-50 DAYS

Sample No.	Initial mgN/l of total N	Maximum observed NH ₃ -N + NO ₃ ⁻ -N mgN/l	% total N	Total time of incubation, days
<u>Niagara R.</u>				
41	0.45	0.41	91	35
50	0.82	0.74	90	50
56	1.13	0.61	54	50
<u>Genesee R.</u>				
34	2.21	1.33	60	50
42	1.52	1.11	73	35
51	1.26	0.95	75	50
58	2.26	1.35	60	50
<u>Oswego R.</u>				
28	1.34	1.04	78	50
31	1.27	1.00	79	50
35	1.16	1.06	91	50
43	1.42	0.98	69	35
47	1.15	0.87	76	50
48	1.01	0.83	82	50
52	1.49	1.04	70	50
59	2.30	1.32	58	50
<u>Black R.</u>				
36	0.89	0.62	70	50
44	0.59	0.44	75	35
53	0.75	0.50	67	50
60	1.25	0.45	36	50

Table 5. MINERALIZATION OF ORGANIC-N TO
AMMONIA AND NITRATE IN 35-50 DAYS

Sample No.	Initial mgN/l as:		Maximum	Increase in	
	Organic-N	NH ₃ -N+ NO ₃ -N	NH ₃ -N + NO ₃ -N	mgN/l	% of Organic-N
<u>Niagara R.</u>					
41	0.15	0.30	0.41	0.11	73
50	0.31	0.51	0.74	0.23	74
56	0.79	0.34	0.61	0.27	34
<u>Genesee R.</u>					
34	1.08	1.13	1.33	0.20	18
42	0.68	0.84	1.11	0.27	40
51	0.59	0.67	0.95	0.28	48
58	1.02	1.24	1.35	0.11	11
<u>Oswego R.</u>					
28	0.55	0.79	1.04	0.25	46
31	0.39	0.88	1.00	0.12	31
35	0.39	0.77	1.06	0.29	74
43	0.68	0.74	0.98	0.24	35
47	0.45	0.70	0.87	0.17	38
48	0.47	0.54	0.83	0.29	62
52	0.83	0.66	1.04	0.38	46
59	1.04	1.26	1.34	0.08	8
<u>Black R.</u>					
36	0.42	0.47	0.62	0.15	36
44	0.25	0.34	0.44	0.10	40
53	0.48	0.27	0.50	0.23	48
60	0.91	0.34	0.45	0.11	12

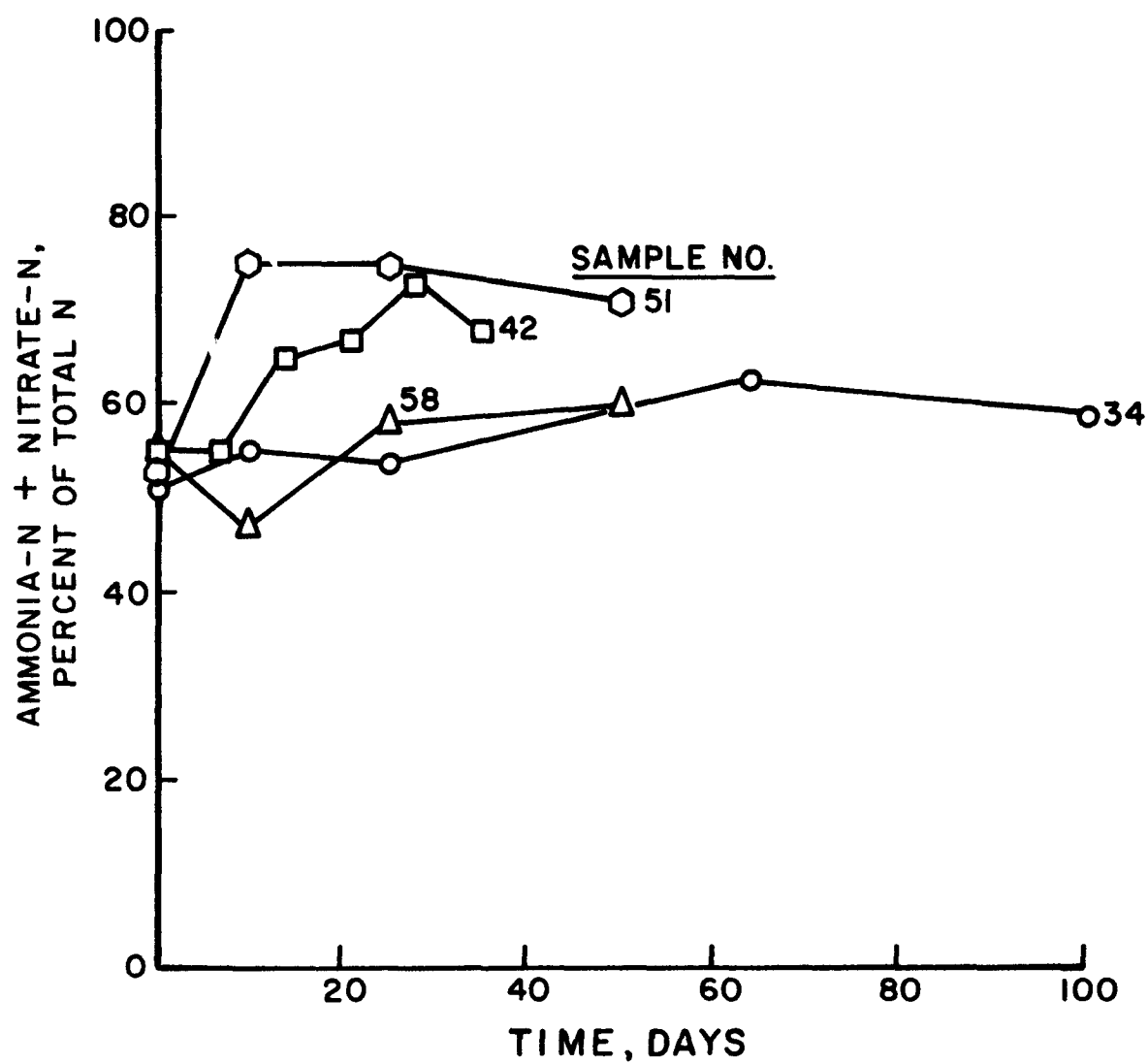


Figure 4. Percent of total N as ammonia-N + nitrate-N during dark incubation of Genesee R. samples

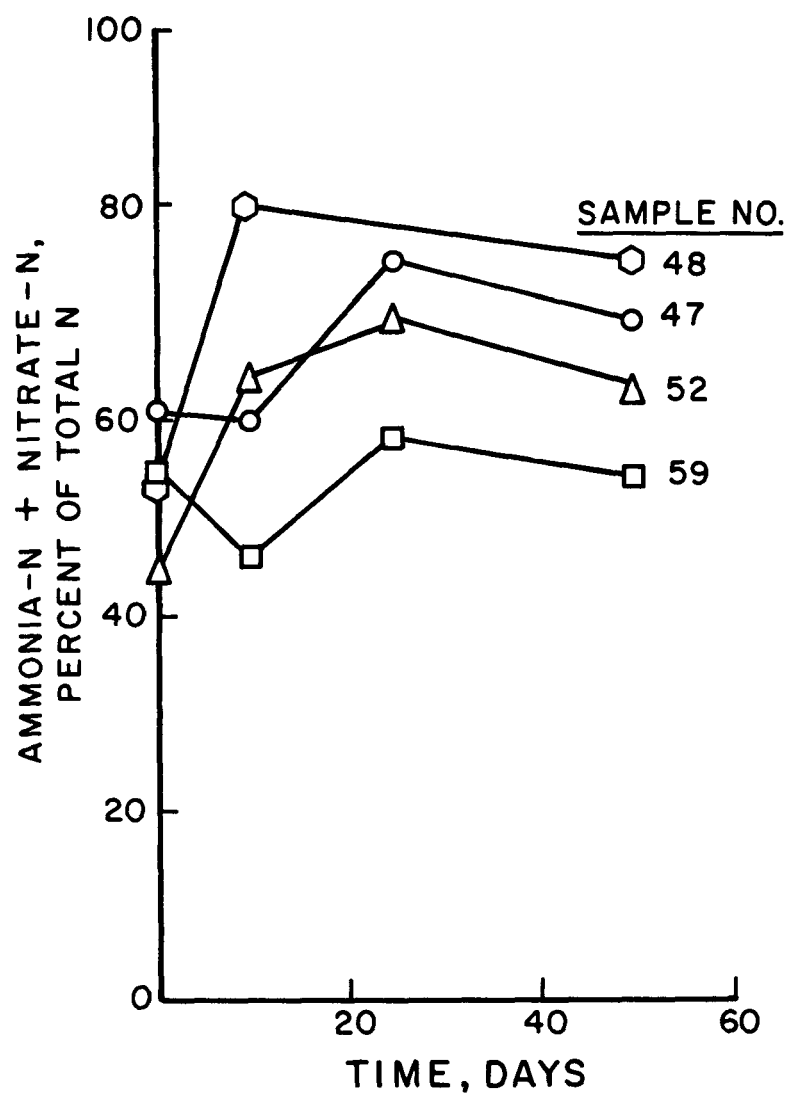


Figure 5. Percent of total N as ammonia-N + nitrate-N during dark incubation of Oswego R. samples

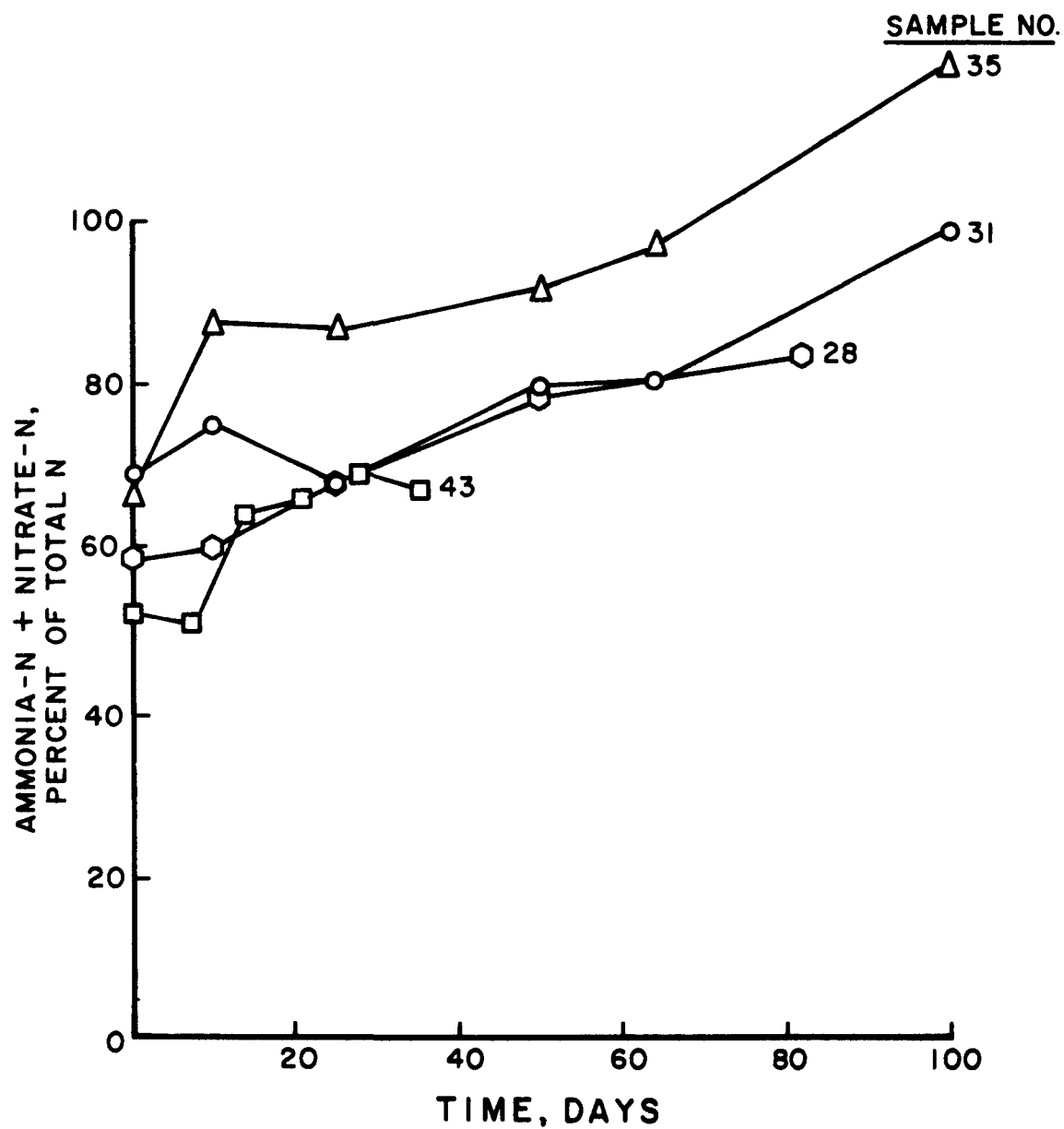


Figure 6. Percent of total N as ammonia-N + nitrate-N during dark incubation of Oswego R. samples

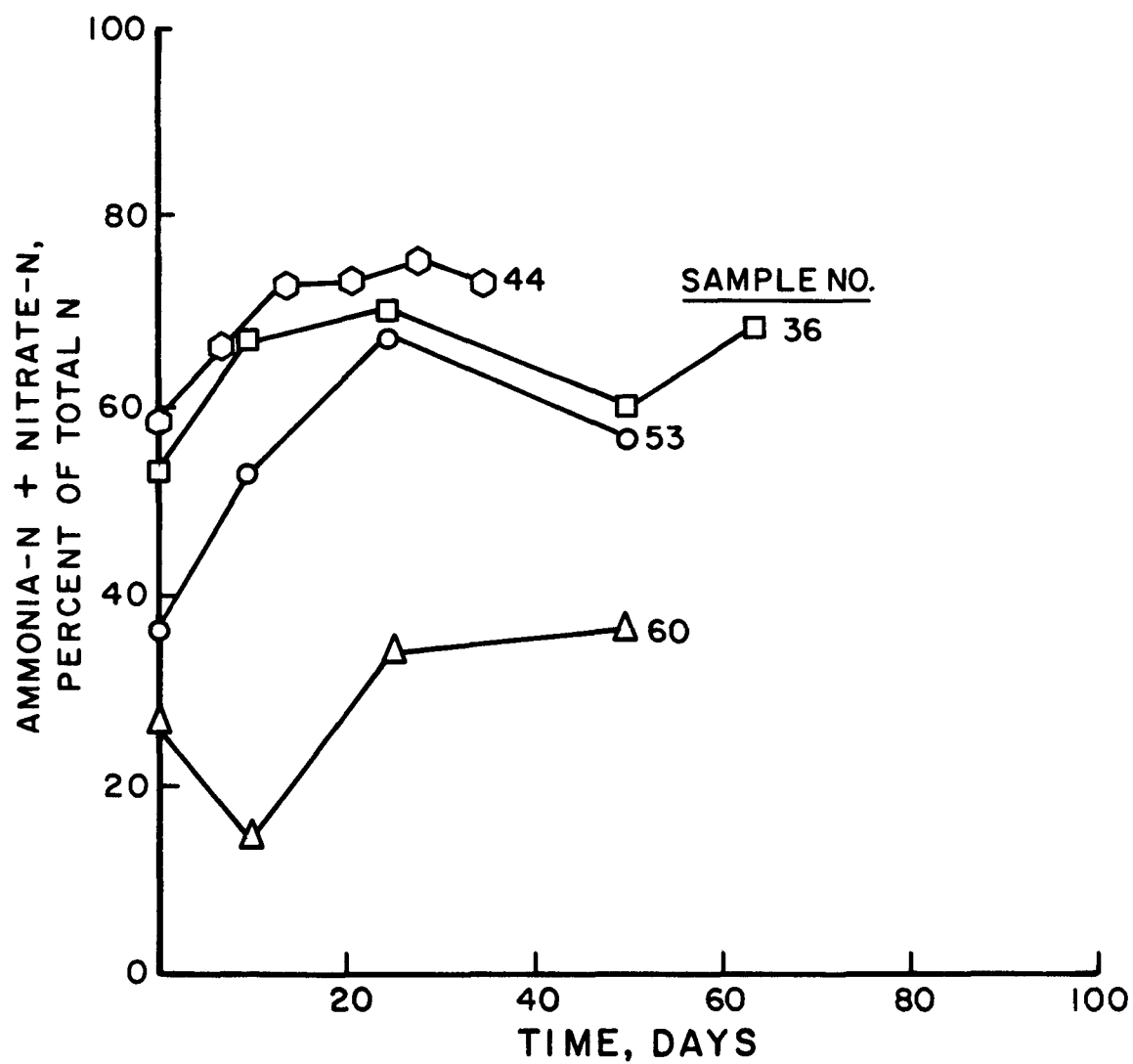


Figure 7. Percent of total N as ammonia-N + nitrate-N during dark incubation of Black R. samples

after incubation. In both cases, the total N was computed from the sum of TKN plus nitrate-N, neglecting Nitrite-N.

Table 6 shows the results of the comparisons. Except for Samples No. 31 and 35, the final and initial values appeared to agree as closely as could be expected from analytical considerations. The final values from Samples No. 31 and 35 appeared to be higher than the initial values by a significant amount.

Table 6. NITROGEN MASS BALANCES

Sample	Initial total N (mgN/l)	Final total N (mgN/l)	Incubation (days)
28	1.34	1.28	82
31	1.27	1.49	64
35	1.16	1.59	64
43	1.42	1.33	35
42	1.52	1.44	35
41	0.45	0.52	35
44	0.59	0.65	35

MINERALIZATION OF PARTICULATE ORGANIC N

Tables A.3 through A.4 of Appendix A give the detailed data from the individual incubations of river water particles in lake water. Figure 8 shows the average production of nitrate-N from particles of Genesee R. (No. 42) water incubated in lake water from Station No. 10. Only 26 percent of the organic-N in the particles was released to solution as nitrate-N. The mineralization appeared to be complete after about 14 days. A similar test with Oswego R. (No. 43) particles in the same lake water showed about 75 percent conversion of organic-N to nitrate-N after about 21 days (Figure 9).

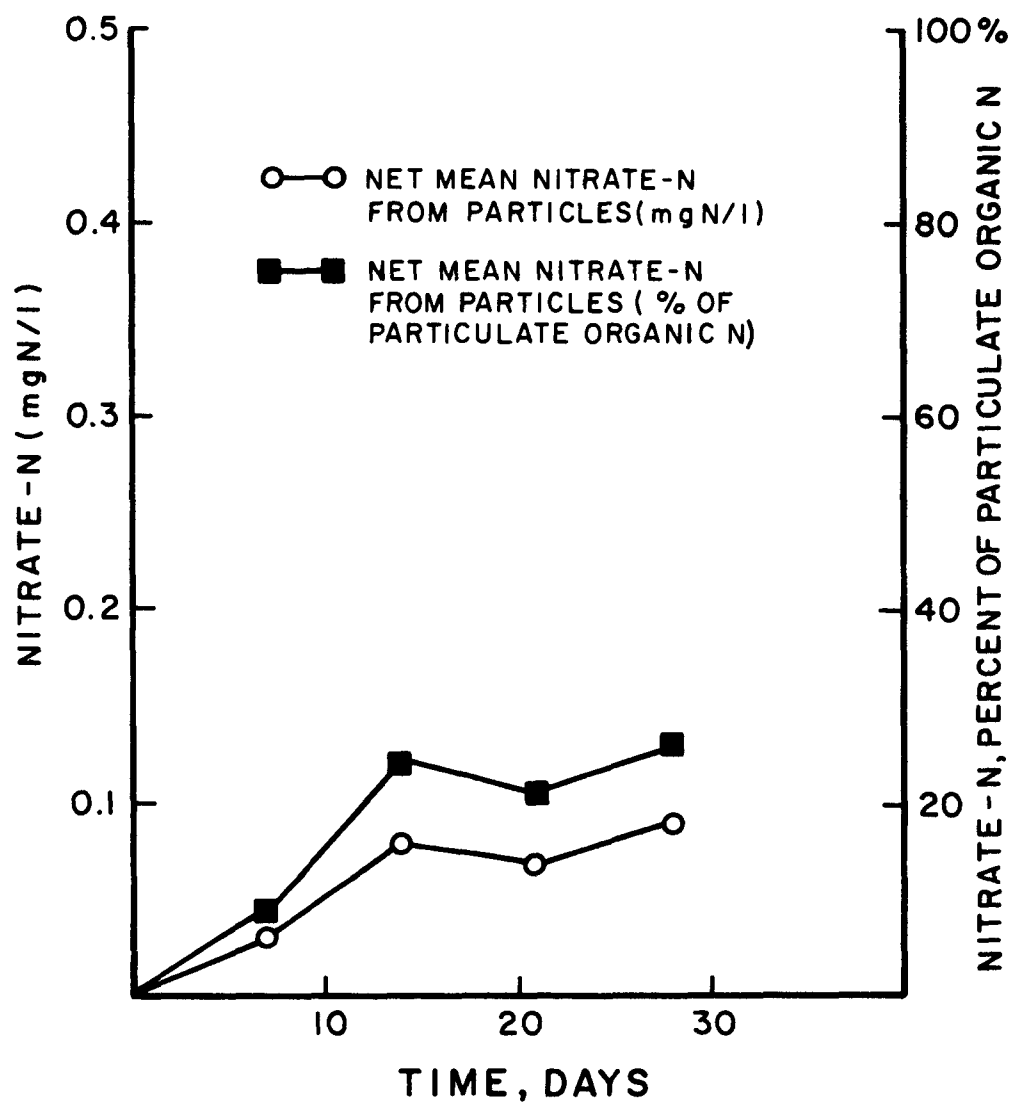


Figure 8. Nitrate-N production from Genesee R. (42) particles in Lake Ontario (10) water

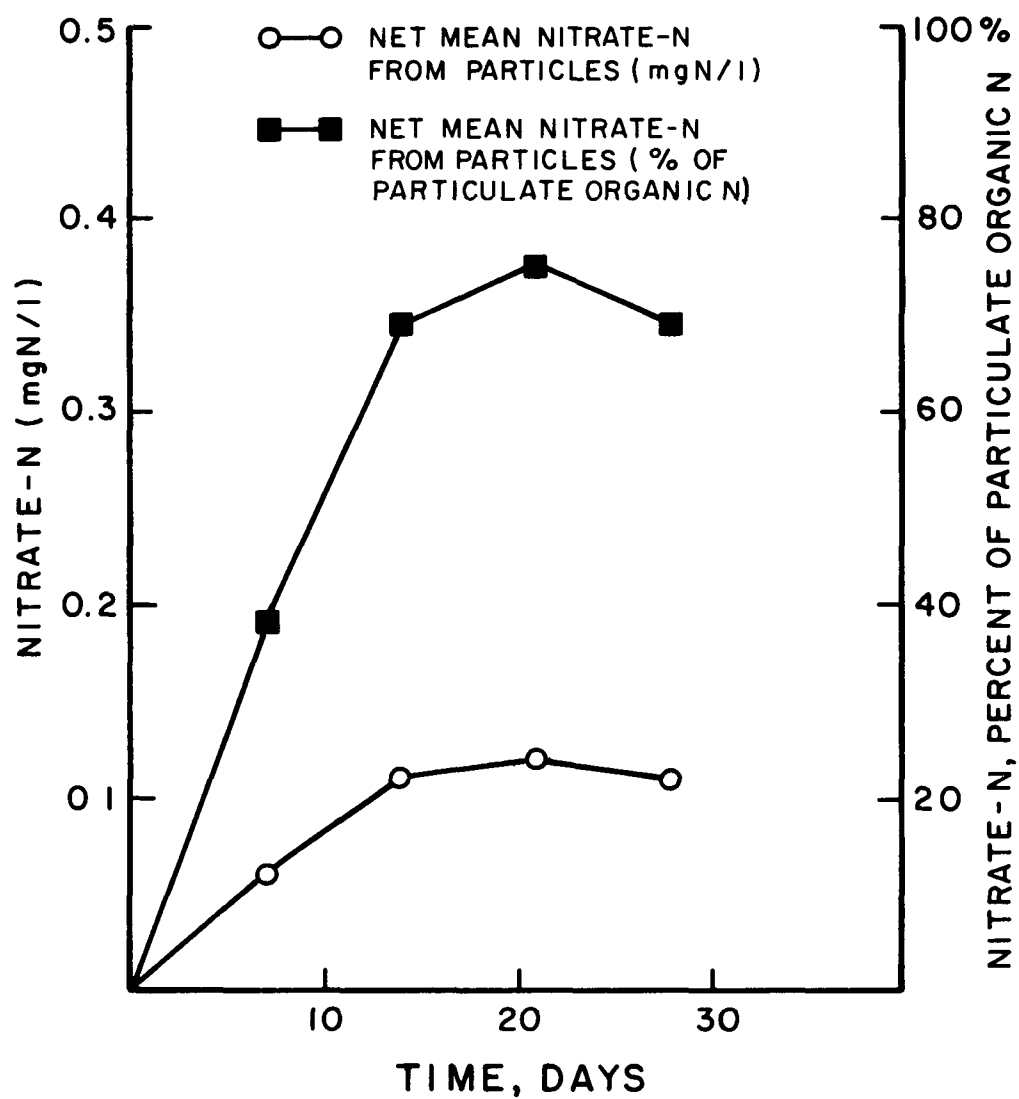


Figure 9. Nitrate-N production from Oswego R. (43) particles in Lake Ontario (10) water

In lake water from Station No. 96 samples of Genesee River (No. 58) and Oswego River (No. 59) particles released 67 and 108 percent, respectively, of their organic-N as nitrate-N (Figures 10 and 11) after 25 days.

MINERALIZATION OF URBAN RUNOFF SAMPLES

The chemical characteristics for nitrogen species analyses are given in Table 7. Thirteen urban runoff samples collected from Madison, Wisconsin areas were performed for the long-term study of mineralization. A period of 82-100 days was selected for this study. All of the samples for mineralization study were run in triplicate and were performed at $21 \pm 1^\circ\text{C}$ under aerobic conditions in the dark. The range obtained in the analysis of incubated triplicate bottles were shown in the figures. Assuming no occurrence of photosynthesis and bacterial nitrogen fixation, the total Kjeldahl nitrogen value from day zero was used to calculate total-N. Percent availability was calculated from NH_4^+ -N and NO_3^- -N each day that a sample was taken. Figures 12 to 15 demonstrated the mineralization of total Kjeldahl-N to NH_4^+ -N and NO_3^- -N of runoff water from the Whitney Way station at different days of rainfall. Although different concentrations of nitrogen forms were found at day zero, all the curves showed the same trend of nitrogen forms changing. In Figures 12, 14, and 15 there was an immediate increase in the NH_4^+ -N concentration at day 10 of incubation while in Figure 13 this happened at day 25. After that, the NH_4^+ -N in solution decreased to 0.05 mgN/l by the end of the study. The NO_3^- -N increased steadily in all samples. However, a sharp increase in concentration was observed at day 25 in Figures 12 and 14, at day 50 in Figure 13 and at day 82 in Figure 15. The concentration of both soluble and

Table 7. INITIAL CONCENTRATIONS OF SELECTED NITROGEN SPECIES IN THE SAMPLES USED IN THIS INVESTIGATION

Samples	Date Collected	NH ₄ ⁺ -N (mgN/l)	NO ₃ ⁻ -N (mgN/l)	Total Kjeldahl-N (mgN/l)	pH
<u>Madison Urban Runoff Samples</u>					
A-6	10/20/72	0.13	0.02	1.85	
A-8	12/30/72	0.59	0.45	1.60	
A-9	1/17/73	0.80	0.21	2.40	
A-12	3/5/73	1.12	0.71	4.28	
B-6	10/20/72	0.14	0.005	3.60	
B-8	12/30/72	0.75	0.62	1.50	
B-9	1/17/73	0.82	0.31	2.30	
B-12	3/5/73	1.32	0.30	4.24	
D-6	10/20/72	0.08	0.24	2.45	
D-8	12/30/72	0.64	0.80	1.70	
D-10	1/18/73	0.51	1.56	2.70	
D-12	3/5/73	1.07	0.335	4.60	
F-9	1/17/73	0.85	0.63	1.60	

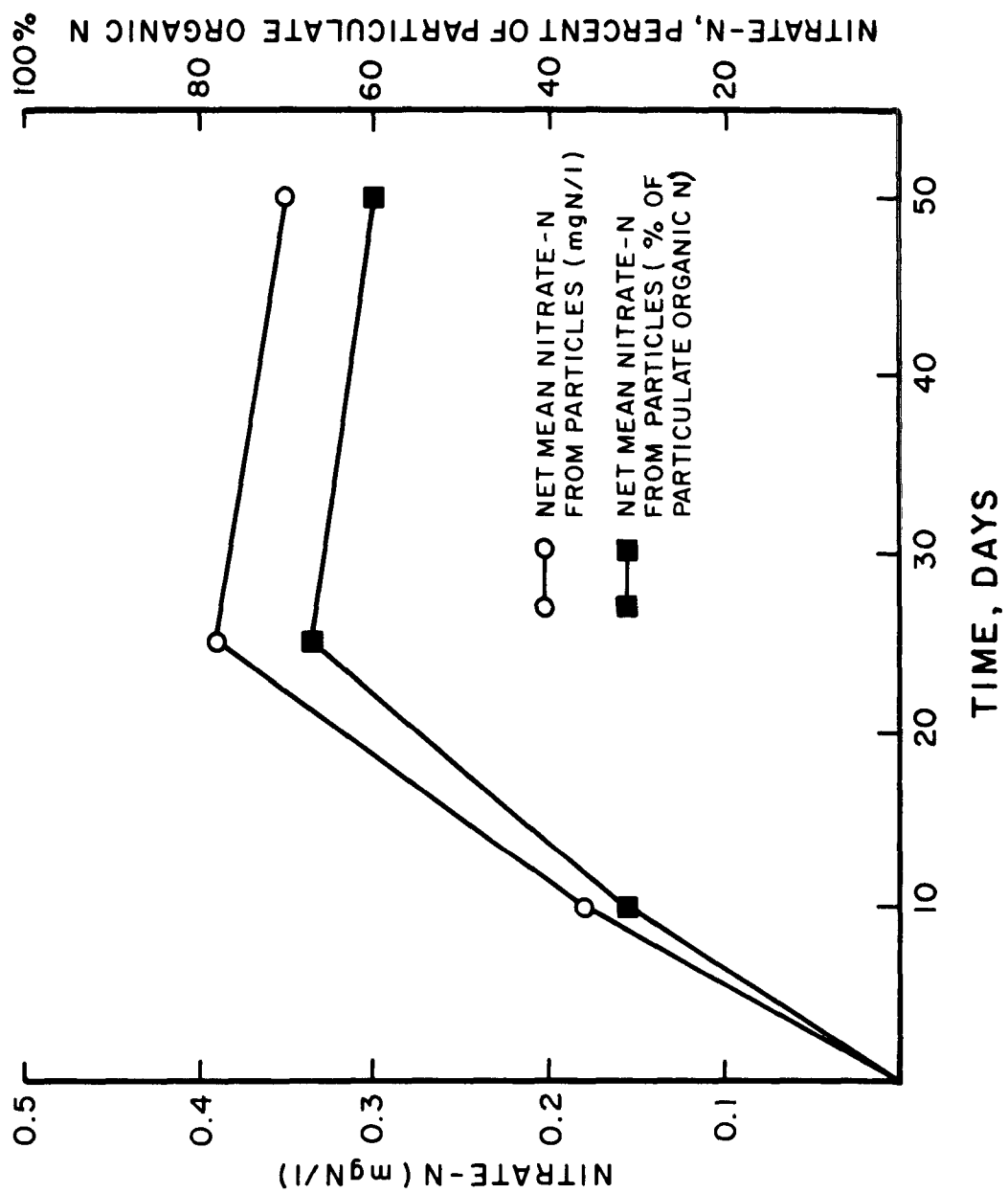


Figure 10. Nitrate-N production from Genesee R. (58) particles in Lake Ontario (96) water

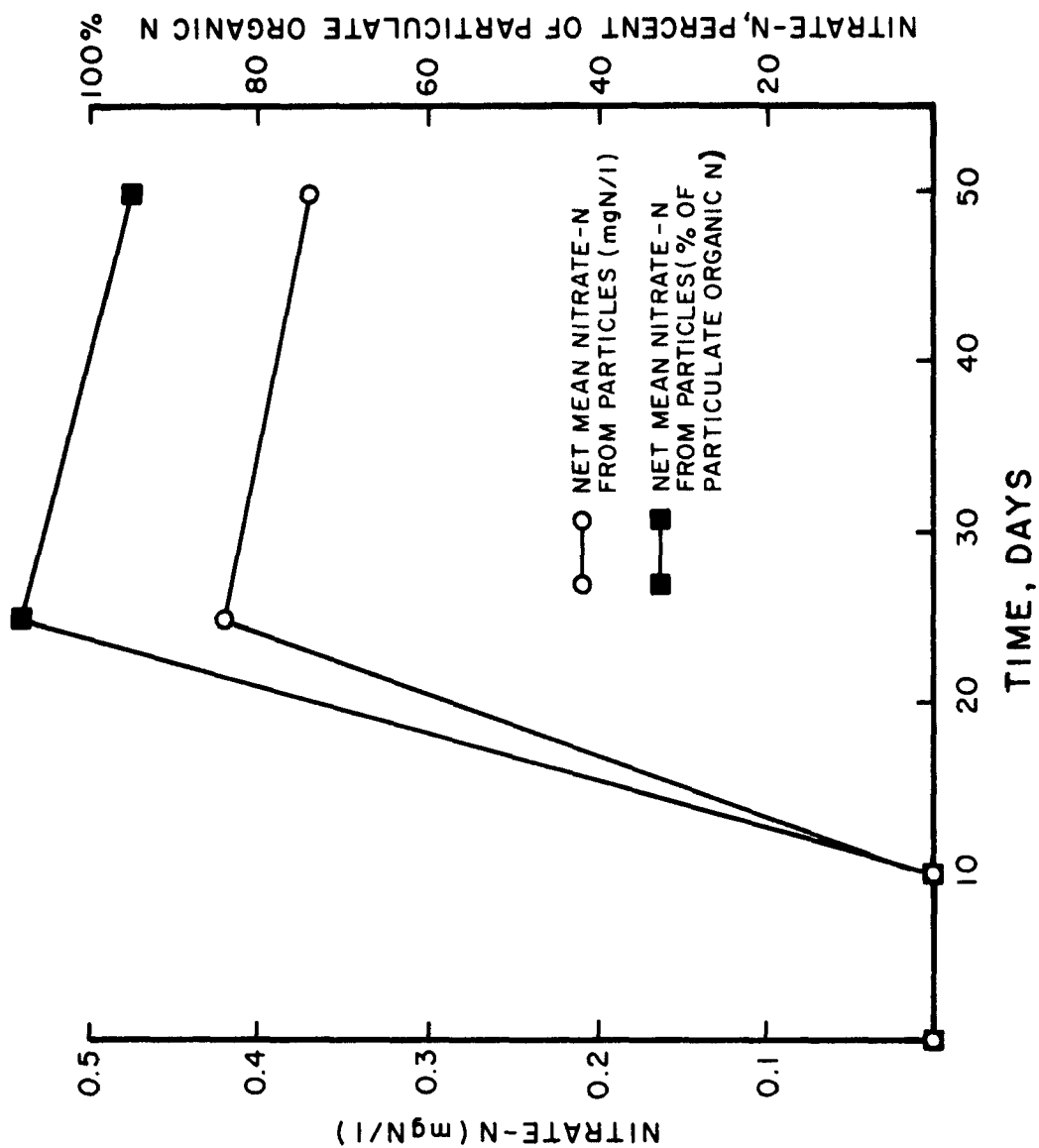


Figure 11. Nitrate-N production from Oswego R.(59) particles in Lake Ontario (96) water

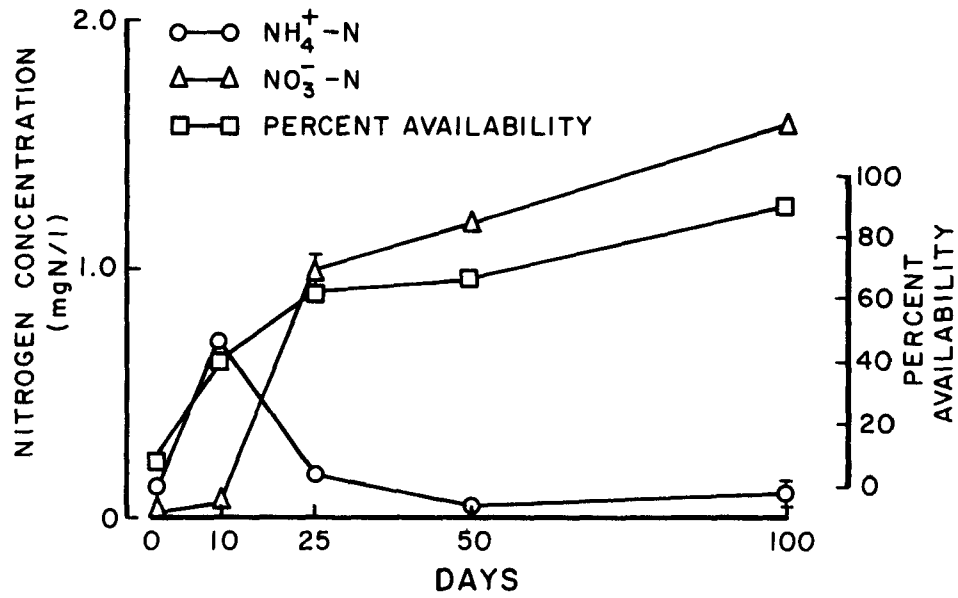


Figure 12. The mineralization of runoff water from Whitney Way station collected on October 20, 1972 incubated under aerobic conditions

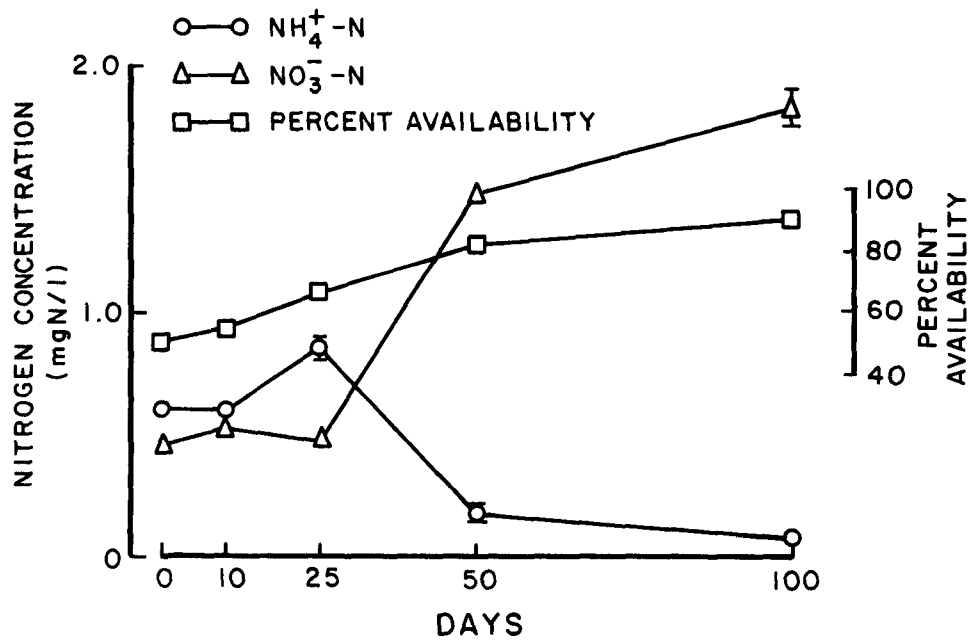


Figure 13. The mineralization of runoff water from Whitney Way station collected on December 30, 1972 incubated under aerobic conditions

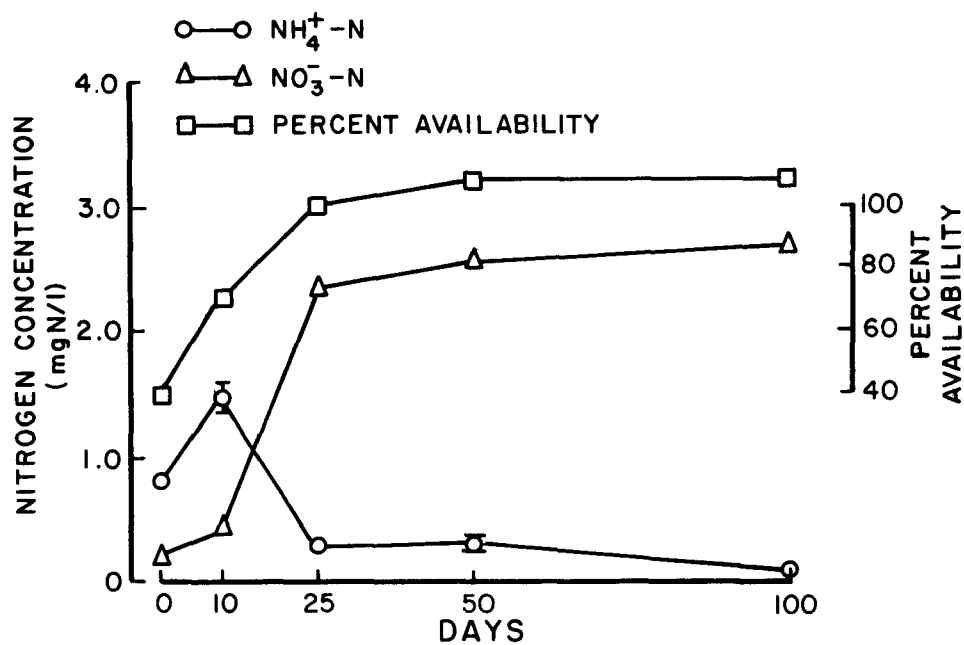


Figure 14. The mineralization of runoff water from Whitney Way station collected on January 17, 1973 incubated under aerobic conditions

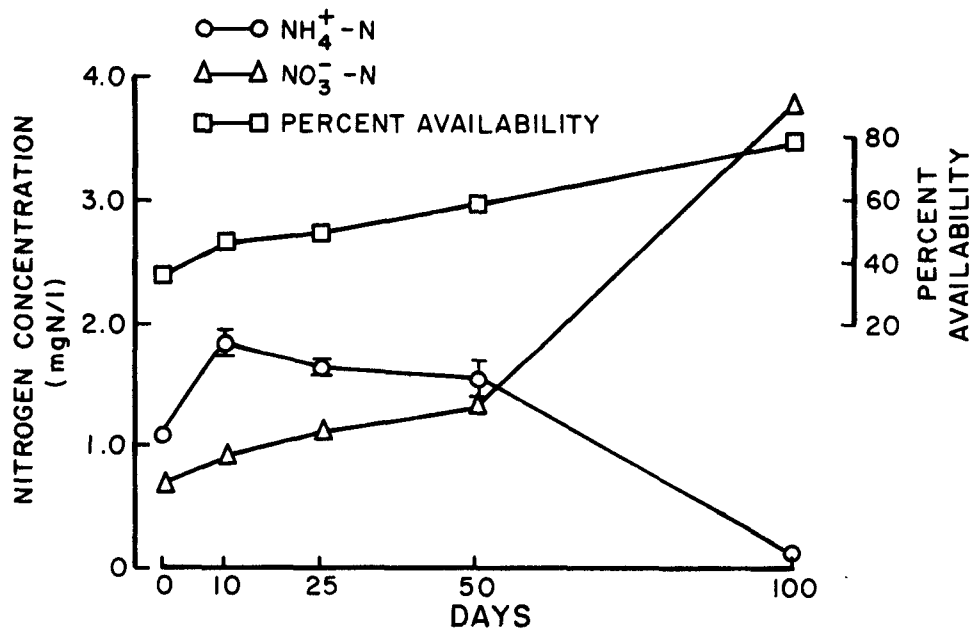


Figure 15. The mineralization of runoff water from Whitney Way station collected on March 5, 1973 incubated under aerobic conditions

total Kjeldahl-N decreased at the end of the experiment. The percent availability of all figures were found to increase steadily throughout the incubation period.

Figures 16 to 19 showed the mineralization of total Kjeldahl-N to NH_4^+ -N and NO_3^- -N of runoff water from the Manitau Way station on different days of rainfall. The soluble and total Kjeldahl-N decreased at the end of the study. A sharp increase in NH_4^+ -N was observed in Figures 16 and 19 at day 10. It then decreased to 1.0 mgN/l at day 10 and declined to 0.10 mgN/l at the end of the study. The NH_4^+ -N in Figures 17 and 18 gradually declined over the period of 100 days. There was a slow increase in NO_3^- -N of all samples throughout the experiment. The percent availability increased until the end of the experiment.

The results of the study of runoff water from the Water Chemistry Station are shown in Figures 20 and 23. There was an increase in NH_4^+ -N at day 10 in Figure 20 and 23; after that there was a decrease throughout the experiment. No change occurred in NO_3^- -N in Figure 20 at day 10 while in Figure 23, a sharp increase was observed. However, after day 10 both figures showed the increase in NO_3^- -N until the end. There was a decrease in NH_4^+ -N in Figure 21 and 22 from the beginning to the end of the experiment. An increase in NO_3^- -N in Figure 22 was observed from day 0 to day 100 while in Figure 21 a decrease was found at day 25. The soluble and total Kjeldahl-N also decreased at the end of the study. The percent availability increased throughout the incubation time.

The results obtained from the Stone Ridge Apartment station were demonstrated in Figure 24. The NH_4^+ -N increased at day 10 and after that decreased. The NO_3^- -N had

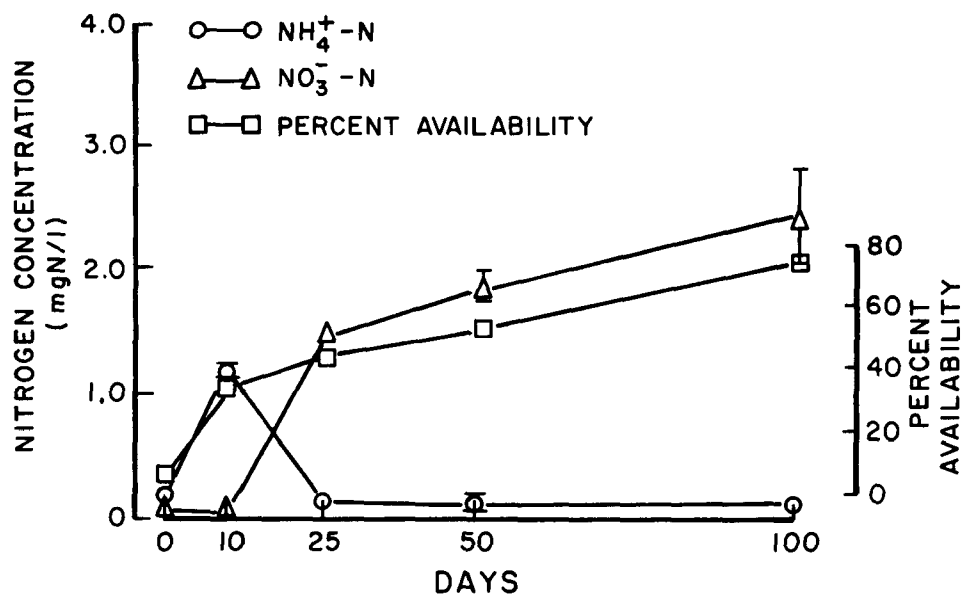


Figure 16. The mineralization of runoff water from Manitau Way station collected on October 20, 1972 incubated under aerobic conditions

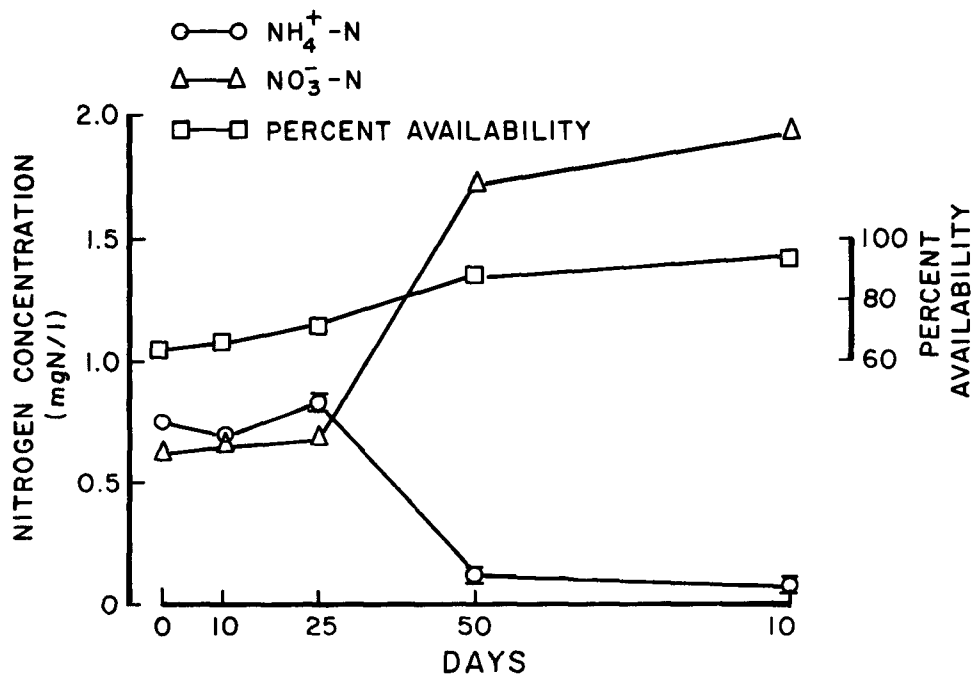


Figure 17. The mineralization of runoff water from Manitau Way station collected on December 30, 1972 incubated under aerobic conditions

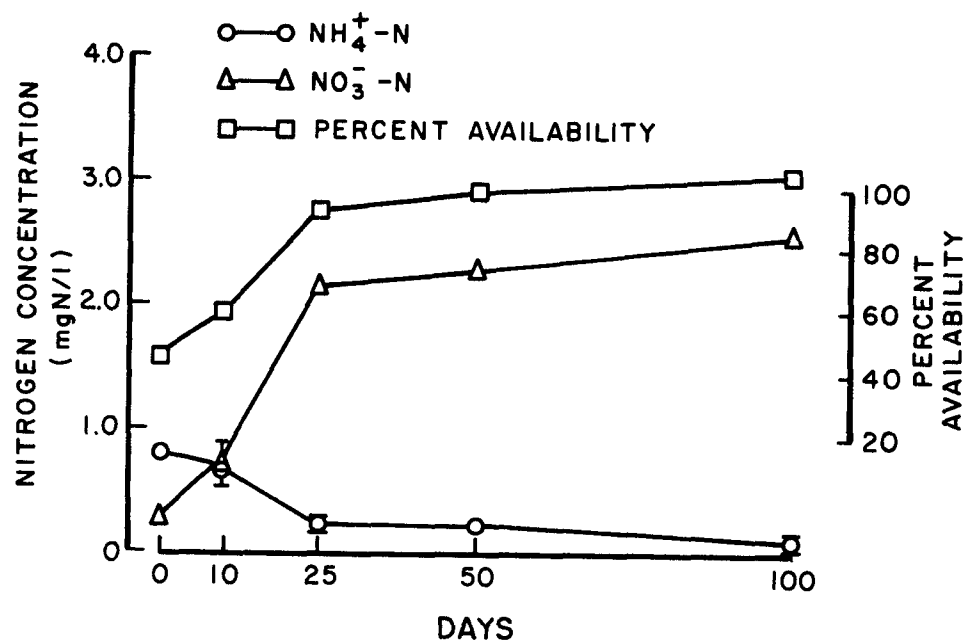


Figure 18. The mineralization of runoff water from Manitou Way station collected on January 17, 1973 incubated under aerobic conditions

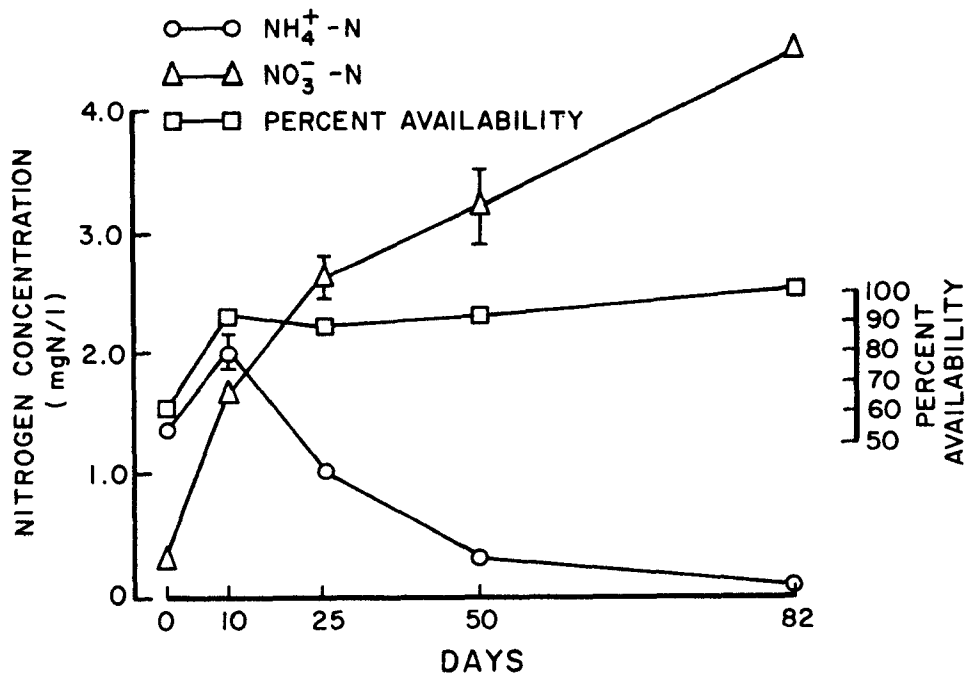


Figure 19. The mineralization of runoff water from Manitou Way station collected on March 5, 1973 incubated under aerobic conditions

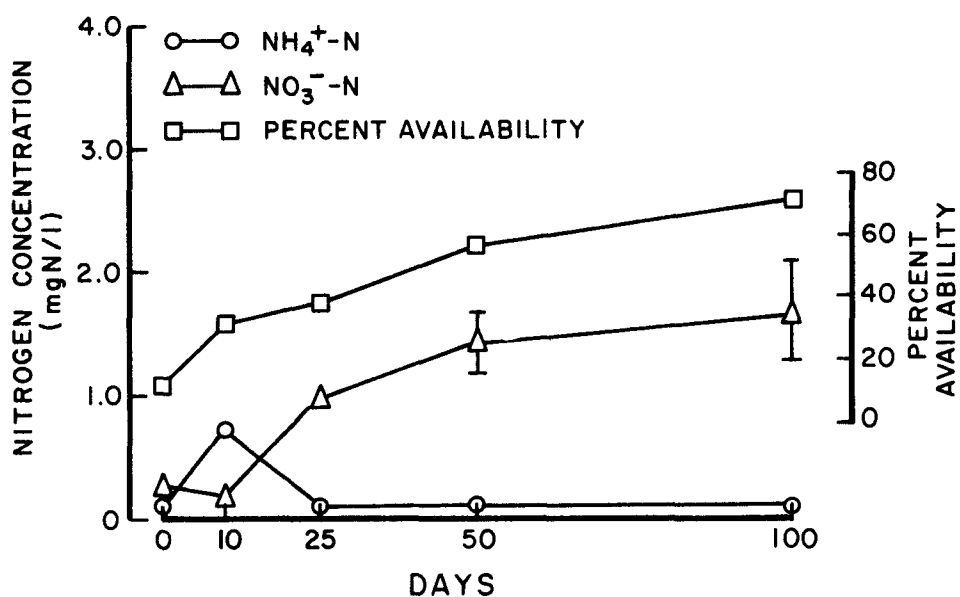


Figure 20. The mineralization of runoff water from Water Chemistry lab station collected on October 20, 1972 incubated under aerobic conditions

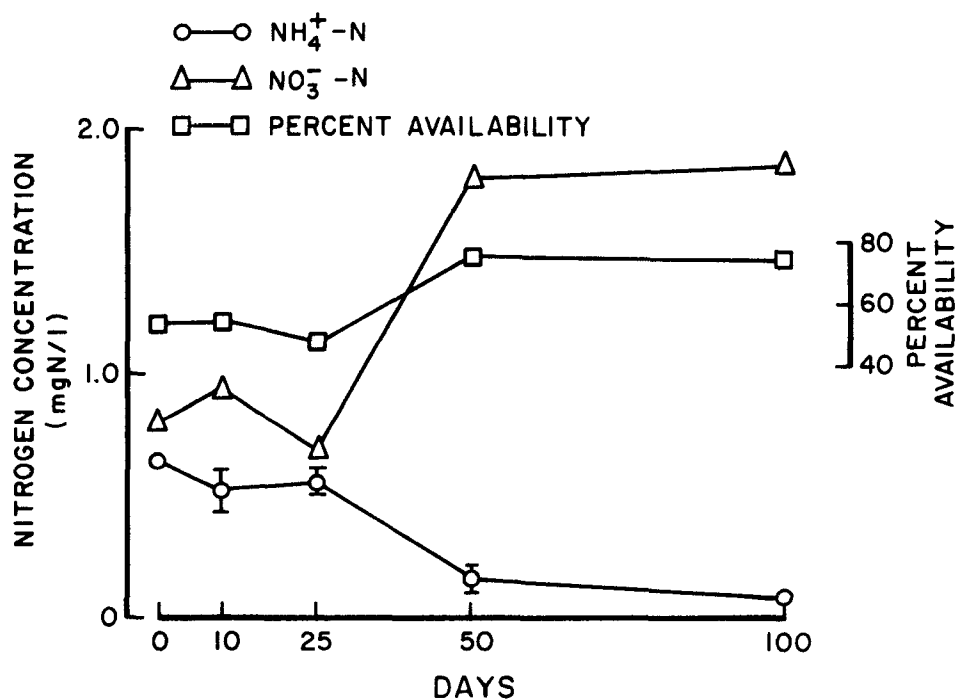


Figure 21. The mineralization of runoff water from Water Chemistry lab station collected on December 30, 1972 incubated under aerobic conditions

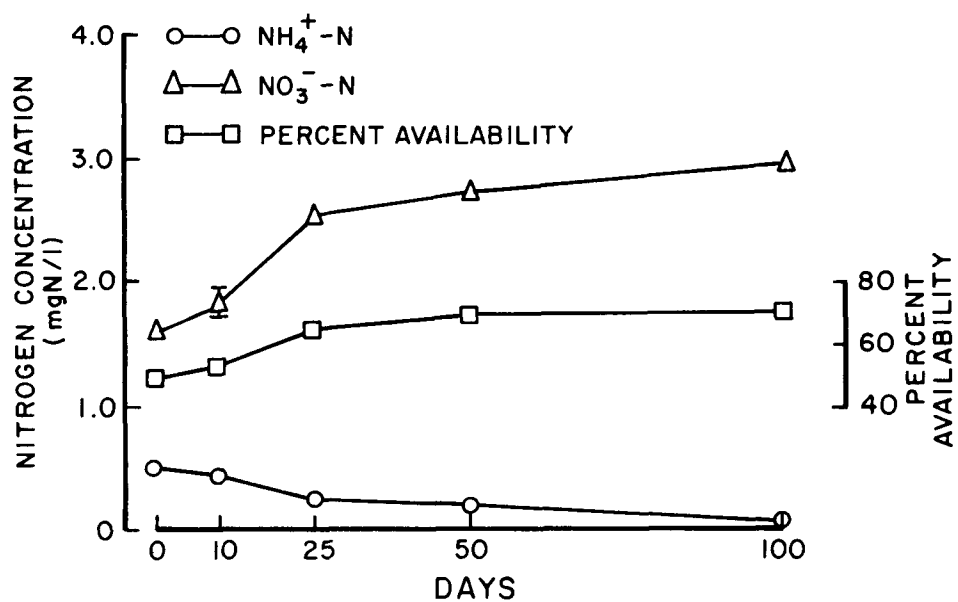


Figure 22. The mineralization of runoff water from Water Chemistry lab station collected on March 5, 1973 incubated under aerobic conditions

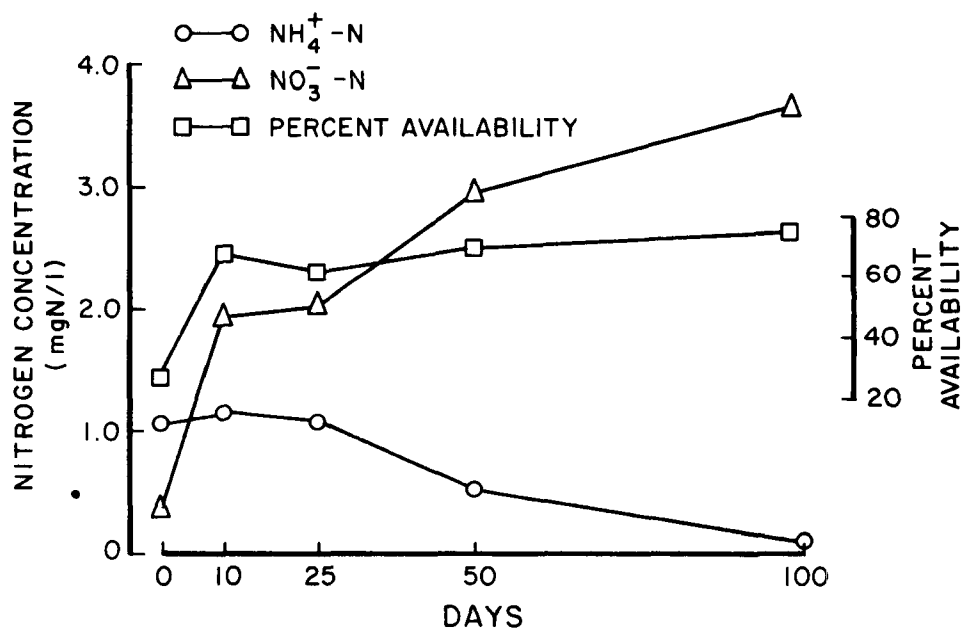


Figure 23. The mineralization of runoff water from Water Chemistry lab station collected on March 5, 1973 incubated under aerobic conditions.

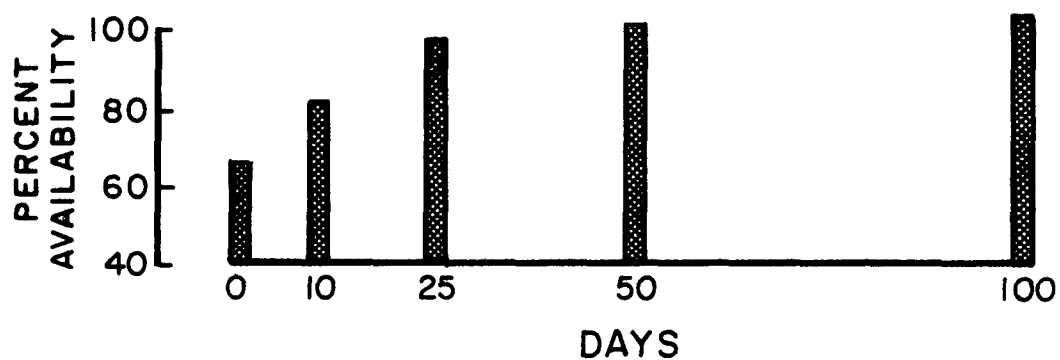
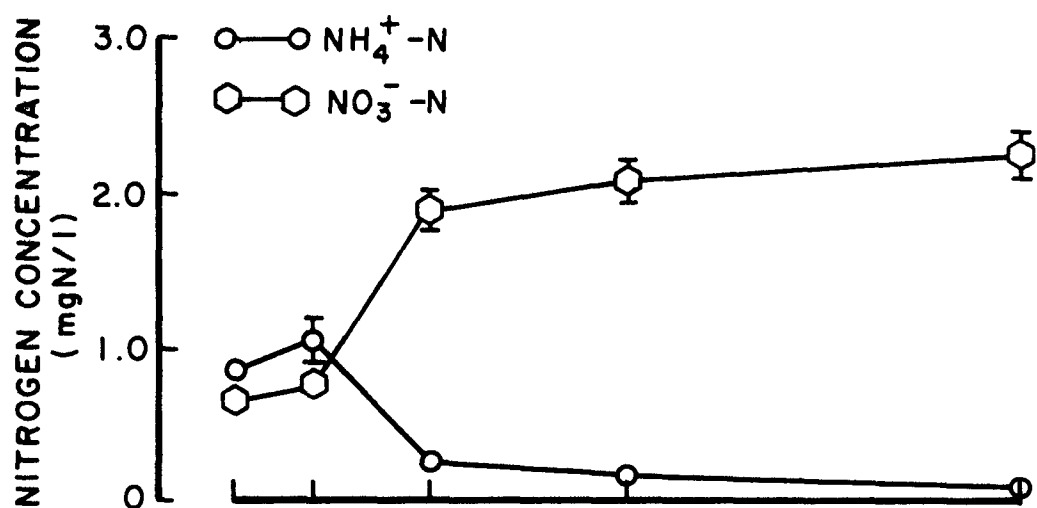


Figure 24. The mineralization of runoff water from Stone Ridge apartment station collected on January 17, 1973 incubated under aerobic conditions

a continuous increase until the end. The soluble and total Kjeldahl-N decreased as the percent availability increased.

The results of the triplicate run of each sample were very similar in their mineralization pattern.

BIOASSAY TEST FOR AVAILABILITY TO ALGAL GROWTH FROM PARTICLES

The algal bioassay test was set up to directly determine the fraction of organic-N available for algal growth. The particles from both urban runoff and Lake Ontario tributaries were suspended in AAP (-N) medium and incubated at $24 \pm 1^{\circ}\text{C}$, 400 ft-c light intensity. Selenastrum capricornutum was used as the test organism. Standards and blanks were also run. Figure 25 shows the growth curve of Selenastrum using various nitrate concentrations as the source of nitrogen. The growth of algae was measured at 750 nm and was plotted versus day of incubation. The cells in both the samples and the standards were counted on day 21. There was not much growth of cells in sample flasks; however, the nitrogen fixing blue-green algae, Anabaena, were found in large amounts compared to Selenastrum. The experiments were repeated. The incubation time was changed to 7 days hoping that the Selenastrum would grow faster than the Anabaena. On day 7, the samples were counted, no Anabaena were present but there was also no growth of Selenastrum. The samples were counted every three days. Finally Anabaena was found to be present in the samples on day 14 and no growth of Selenastrum was observed. Another experiment was then performed to solve the problem of Anabaena contamination from particles. The filtrate from 100 days incubation samples was used as a nitrogen source instead of the particles. The results of this study are presented in Table 8. The increase of absorbance which is related to the growth of Selenastrum was observed in all samples.

Table 8. THE NITROGEN AVAILABILITY FROM PARTICLES
USING BIOASSAY TEST AT 10 DAYS

Samples	Absorbance at 750 nm	Concentration mgN/l
A-12-1	0.25	2.15
2	0.25	2.15
3	0.26	2.20
B-12-1	0.33	>4.00
2	0.32	>4.00
3	0.33	>4.00
D-12-1	0.32	>4.00
2	0.33	>4.00
3	0.32	>4.00
28-1	0.10	0.75
2	0.11	0.85
3	0.11	0.85
41-1	0.045	0.30
2	0.045	0.30
3	0.045	0.30
42-1	0.11	0.85
2	0.11	0.85
3	0.11	0.85
43-1	0.10	0.75
2	0.095	0.73
3	0.10	0.75
44-1	0.05	0.35
2	0.05	0.35
3	0.05	0.35

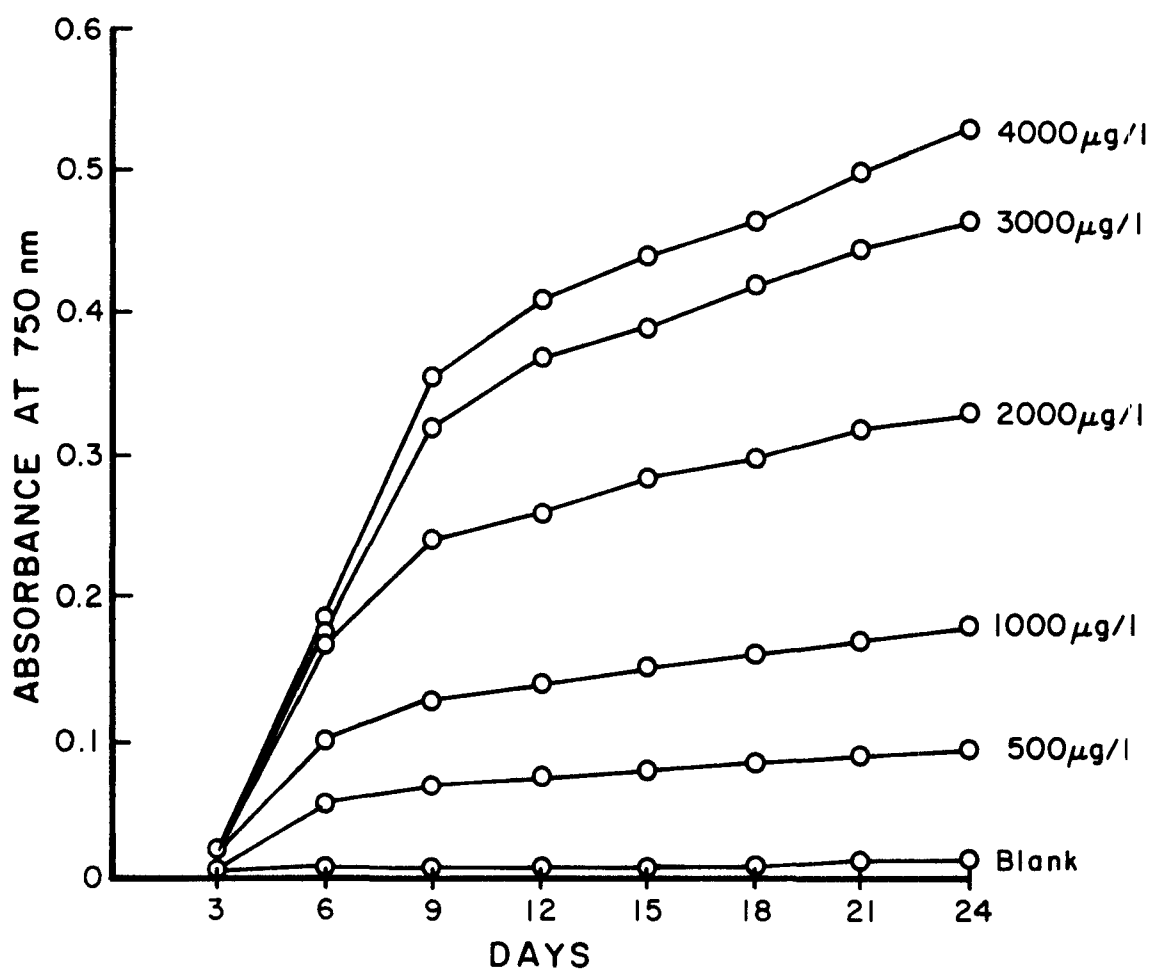


Figure 25. The growth curve of Selenastrum capricornutum using nitrate as nitrogen source

SECTION VII

DISCUSSION

LAKE ONTARIO SAMPLES

River water samples containing organic N forms from runoff, domestic sewage, industrial wastes, and aquatic organisms were incubated in darkness to promote nitrogen mineralization by the bacteria in the samples. Under such conditions, bacterial populations have been shown to rise in two to three days to levels 10^3 to 10^5 times the populations in natural waters (Renn, 1937). Consequently, the rate of inorganic N production from organic N sources in such tests was related to the rate of bacterial growth, the length of the viable period, and the rate of autolysis after death. (Renn, 1937). This rate may not be the same as the rate in natural waters because of the different bacterial population densities. However, the objective of the tests was to estimate the long-term susceptibility of the organic N in the rivers to mineralization, with the assumption that any organic N which was mineralized in the dark incubation could eventually produce available N for algal growth in Lake Ontario. The results of these tests are intended to be the upper bounds on the nitrogen mineralization in each sample.

Although the nitrite-N was assumed negligible in computation of the final reported results, the error from this assumption was probably small. Nitrate values

generally showed little change after 25 to 50 days, indicating that all nitrite had been converted to nitrate. Since only the maximum value of the sum of nitrate plus ammonia was selected for the final reported result of an incubation, the error from neglecting nitrite-N was minimized. The reported values of (ammonia-N + nitrate-N) at incubation times of less than 25 days (Figures 3-7) may have been lower than the true "available -N", however, because of nitrite production from ammonia during the initial stages of the incubations.

The apparent increases in total N during the incubations of Samples 31 and 35 for 64 days (Table 6) were probably the result of neglecting nitrite-N in the calculation of initial total N. Since any nitrite initially present would be converted to nitrate during the incubation, the final total N value ($\text{NO}_3\text{-N} + \text{TKN}$) would include the contribution from nitrite and be larger than the initial total N value. The values given in Table 4 should probably be based on the final total N values, in the case of Samples 31 and 35. Recalculation of the available N percentages yields the value of 67 percent for both samples instead of the values of 79 and 91 percent shown in Table 4.

Other workers have noted an increase in total N during regeneration studies of nitrogenous organic N (Von Brand et al., 1937, Sawyer et al., 1944). Such an increase could be the result of nitrogen fixation by bacteria. A sample of Oswego R. water (59) which had been incubated for 33 days was tested for N-fixation by an acetylene reduction procedure and found to have a fixation rate of 2.6 $\mu\text{gN/l}$ per week (Loneragan, 1973), or 0.024 mgN/l for a 64 day incubation period. This amount of nitrogen is much less than the increases shown by

Samples 31 and 35 in Table 6 (0.22 to 0.43 mgN/l). A similar test on Sample 58 from the Genesee R. indicated N-fixation of 0.039 mgN/l, predicted for a 64-day incubation. Black R. Sample 60 showed no N-fixation.

The incubations of unfiltered river water demonstrated that a large percentage of the total N in the rivers should be considered eventually available for algal growth in Lake Ontario. In many samples the high availability was related to the high concentrations of ammonia and nitrate initially present. These "readily available" N forms were the result of (1) direct inputs from runoff and wastewaters, (2) mineralization of organic N between the point of discharge to the river and the sampling station near Lake Ontario, or (3) mineralization in the samples prior to their initial analysis in Madison. With the exception of Sample 60 (Black R.), all samples showed over 50 percent of their total N to be subject to mineralization or already mineralized. The wide range of values and the small number of samples tested from each river preclude any one estimate of nitrogen availability for a given river. Rather, the data (Table 4), should be considered as temporary upper and lower bounds on N availability until more detailed data are available. Thus, the data from Table 4 can be summarized as follows:

<u>River</u>	<u>Range of Availability (% of Total N)</u>
Niagara	54-91
Genesee	60-75
Oswego	58-91
Black	36-75

Use of these percentages in conjunction with a nutrient budget based on total N loadings from each river would provide estimates of the available N loadings from the rivers.

Another approach to the correction of total N loading data to an available N basis would use the following equation:

$$\text{Available N} = \text{Ammonia-N} + \text{Nitrite-N} + \text{Nitrate-N} + (\text{Org.-N}) \times (\% \text{ Org.-N Avail.})$$

where the nitrogen forms are measured in samples collected near the mouth of the river. The data in Table 5 indicated, however, that the percent of organic-N which could be considered available (% Org.-N Avail.) may be extremely variable and related to the concentration of organic matter in the sample. Those samples with the highest organic-N in each set of samples appeared to have the lowest percent of organic N available. As boundary conditions on organic-N availability, the data from Table 5 can be summarized as follows:

<u>River</u>	<u>Range of Availability (% of Org. -N)</u>
Niagara	34-74
Genesee	11-48
Oswego	8-74
Black	12-48

It is important to note that these data were collected during the spring flow period only, and not during other seasons of the year, when the quantity and quality of the organic-N inputs may be different, particularly with respect to agricultural runoff sources. Consequently, these estimates are only valid for the spring flow period and the N loadings carried by the rivers to Lake Ontario during this period.

As much as 78 percent of the organic-N in some of the samples was present as particulate organic-N. This operationally defined class may have also included some ammonia bound to the surface of soil particles. The eventual fate of particulate N forms in Lake Ontario is difficult to predict, since it depends upon physical

factors such as particle settling rate and rate of subsequent deposition of covering layers of sediment. The tests with unfiltered water attempted to reduce these physical factors to negligible levels by providing frequent swirling of the test bottles. Biological factors were investigated directly by incubating membrane-filterable particles in lake water. The two Genesee R. samples tested showed different particulate organic-N availability than was shown by the total organic-N (soluble plus particulate) in each sample (from Table 5):

Percent availability of:

<u>Sample No.</u>	<u>Org.-N</u>	<u>Part. Org.-N</u>
42	40	26
58	11	67

The same analysis of the Oswego R. samples showed:

Percent availability of:

<u>Sample No.</u>	<u>Org.-N</u>	<u>Part. Org.-N</u>
43	35	75
59	8	108

If it is assumed that soluble organic compounds are more readily decomposed than are particulate forms, then the availability of organic-N should always be greater than the availability of particulate organic-N, since organic-N includes both soluble and particulate forms. The data above show the opposite for all but Sample 42. In the case of Sample 59, if 100 percent of the particulate organic N in the sample (0.45 mgN/l) were available, as indicated above, the available percent of organic-N would be at least $0.45/1.04 \times 100 = 43$ percent. The value determined from incubation of unfiltered water was only eight percent, however. The same type of disparity was seen with Samples 58 and 43.

Since some of the particulate organic-N in these samples may have been in the form of planktonic organisms, some comparisons are possible with the data from other workers who have investigated the decomposition of aquatic organisms. Waksman et al. (1931) found that marine zooplankton residues yielded over 50 percent of their total N as ammonia during bacterial decomposition. Golterman (1960) performed sterile lysis of Scenedesmus quadricauda by ultraviolet radiation or chloroform treatment and reported only 20-30 percent release of the N compounds. The remaining N forms, present as insoluble nucleic acids and proteins, were suspended in lake water and allowed to decompose. About half of the N in the residue was converted to ammonia in five days, for an overall mineralization percentage of 55-70 percent. These data are similar to the percentages of organic N availability found for Samples 58 and 43, of 67 and 75 percent, respectively. Somewhat lower figures were reported by Foree and Barrow (1970), who found about 54 percent of the initial particulate N remaining in mixed cultures of algae, indigenous bacteria, and zooplankton after 200 days of aerobic decomposition at 20°C. This would represent a percent availability of 46 percent of the particulate N.

URBAN RUNOFF SAMPLES

The average concentration of nitrogen species from the triplicate bottles of urban runoff from different stations in Madison areas were summarized in Table 9.

The highest increase in NH_4^+ -N on day 10 of Samples A-6, A-9, B-6, B-12, D-6, and D-12 and on day 25 of Samples A-8, A-12 and B-8 indicated the gradual conversion of the organic-N to NH_4^+ -N. After that under aerobic

Table 9. THE AVERAGE CONCENTRATION OF NH_4^+ -N, NO_3^- -N AND TOTAL KJELDAHL-N IN URBAN RUNOFF OF MADISON AREAS

Day	A-6			A-8			A-9			A-12			Average of A station		
	NH_4^+ -N	NO_3^- -N	TKN	NH_4^+ -N	NO_3^- -N	TKN	NH_4^+ -N	NO_3^- -N	TKN	NH_4^+ -N	NO_3^- -N	TKN	NH_4^+ -N	NO_3^- -N	TKN
0	0.13	0.02	1.85	0.59	0.45	1.60	0.80	0.21	2.40	1.12	0.71	4.28	0.66	0.34	2.44
10	0.70	0.06		0.596	0.51		1.42	0.41		1.39	0.95		1.01	0.48	
25	0.18	0.99		0.86	0.49		0.28	2.34		1.68	1.10		0.75	1.23	
50	0.05	1.18		0.19	1.49		0.30	2.56		1.63	1.13		0.54	1.59	
100	0.12	1.58	0.73	0.08	1.80	1.36	0.06	2.78	1.14	0.10	3.75	1.24	0.09	2.47	0.87
0	0.14	0.005	3.60	0.75	0.62	1.50	0.82	0.31	2.30	1.32	0.30	4.24	0.75	0.30	2.91
10	1.14	0.026		0.68	0.72		0.78	0.72		2.00	1.65		1.15	0.77	
25	0.14	1.45		0.84	0.69		0.25	2.10		1.00	2.58		0.55	1.70	
50	0.10	1.85		0.12	1.73		0.29	2.20		0.28	3.26		0.19	2.26	
100	0.18	2.60	1.45	0.08	1.91	0.93	0.12	2.60	1.03	0.10	4.43	1.60	0.12	2.88	1.25
0	0.08	0.24	2.45	0.64	0.80	1.70	0.51	1.56	2.70	1.07	0.33	4.60	0.57	0.73	2.88
10	0.69	0.18		0.50	0.94		0.43	1.78		1.43	1.91		0.76	1.20	
25	0.09	0.97		0.62	0.65		0.25	2.50		1.06	2.04		0.50	1.54	
50	0.10	1.43		0.172	1.76		0.24	2.70		0.51	2.96		0.25	2.21	
100	0.13	1.78	1.58	0.06	1.80	1.15	0.08	2.90	2.28	0.09	3.63	2.05	0.09	2.52	1.76

condition NH_4^+ -N will be oxidized to NO_2^- -N and NO_3^- -N, respectively. This agreed with the increase in NO_3^- -N while NH_4^+ -N decreased after day 10 in the samples. As expected, NO_3^- -N accumulated in all the experiments and reached the highest value on the final day of incubation while NH_4^+ -N approached zero by the end of the study. At day 100, the decrease in total Kjeldahl-N in all runoff samples incubated at 21°C under aerobic condition supported the evidence that organic-N mineralization was occurring in the studied system.

The same results were also obtained from the experiment on the sediments of Wisconsin lakes (Chen *et al.*, 1972). Nitrification occurred after a 3-day lag phase in Chen's experiment instead of 10 days in this experiment. However, in all cases, onset of nitrification was accompanied by a corresponding decline in soluble and exchangeable NH_4^+ -N. He also observed an effect of temperature on nitrification rate. The rate of nitrification was increasing 2.7 times on a 15°C temperature increase. Thus, an even higher rate of nitrification would be expected to occur in shallow water such as the rivers and the epilimnion of the lakes than in deep water where higher water temperatures exist.

No change in NH_4^+ -N on Samples B-9, D-8, and D-10 was observed on day 10 or day 25. This may be due to the equal rate of mineralization and nitrification in these samples. The increase in NO_3^- -N in these three samples also agreed with this explanation.

The average values of NH_4^+ -N, NO_3^- -N and total Kjeldahl-N at each station were also calculated and are presented in Table 9.

The average NH_4^+ -N of all three stations increased at day 10 and decreased after that. The NO_3^- -N gradually

increased until the end of incubation. The total Kjeldahl-N of all three stations (A,B, and D) decreased at the end. The patterns of mineralization at different stations (Figures 26 through 28) were the same and indicated no effect of land usage on mineralization patterns.

No difference of mineralization patterns between the urban runoff samples and Lake Ontario tributary samples were noticed in this study. However, the total Kjeldahl-N in Lake Ontario tributary waters were very low compared to the urban runoff values. This showed that the initial total Kjeldahl-N in each sample had no effect on mineralization patterns.

The mineralization patterns of particles were observed to be the same as of water from urban runoff and Lake Ontario tributaries. This indicated that both soluble organic-N and particulate organic-N could be mineralized to NH_4^+ -N and followed by nitrification under aerobic conditions.

The concentration of NO_3^- -N, NH_4^+ -N and organic-N in each sample at day zero and day 100 were summed up to get the total-N. The increase in total nitrogen at day 100 was observed in most of the samples. Theoretically, in the closed system study, the total-N content should be constant. Only transformation of nitrogen occurred. The increase in total-N in the studied system could be explained by the fact that some bacteria (green and purple bacteria, the azotobacter group, and some anaerobic spore formers) could convert dissolved gaseous nitrogen to NH_3 (Kluesener, 1972). This agreed with Sawyer et al. (1944) who found a increase of 11.2 mg

Release of ammonia and nitrate in urban runoff in Madison, Wisconsin

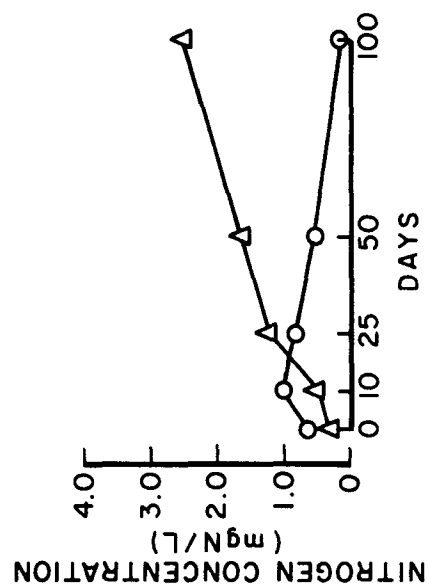


Figure 26. Station A (Whitney Way)

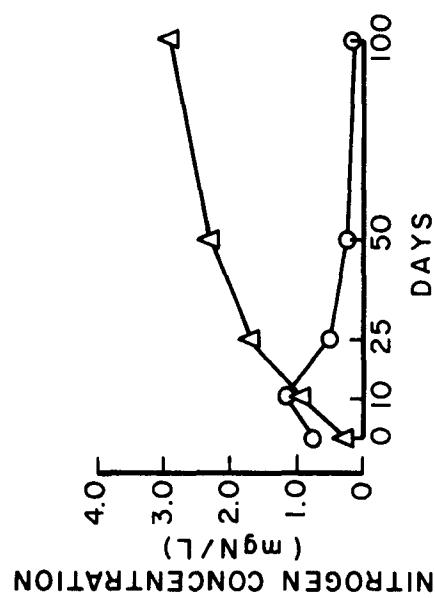


Figure 27. Station B (Manitoway Way)

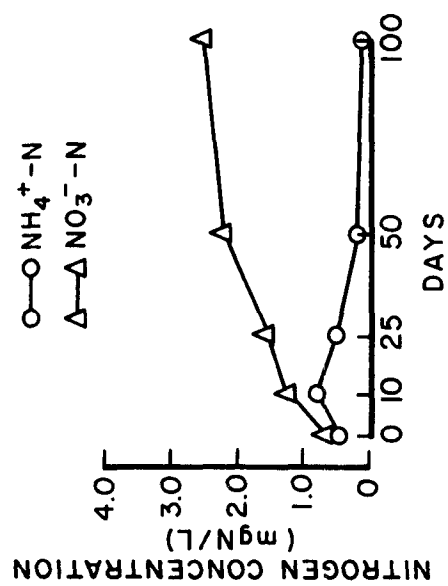


Figure 28. Station C (Water Chemistry Laboratory)

total-N in 188 days in an incubated sample of 90 percent Lake Mendota water mixed with 10 percent sewage effluent. This amount yielded a production of 0.06 mgN/day. The wide distribution of N-fixing organisms in Lake Mendota was also found by Tew (1959).

Von Brand et al. (1937) investigated the regeneration of nitrogenous organic matter in sea water at room temperature in the dark. From measurements of the disappearance of nitrogen in the decomposing plankton material and the appearance of nitrogen in the form of NH_4^+ -N, NO_2^- -N and NO_3^- -N in the water, he also found a continuous increase in total-N throughout the experiment.

BIOASSAY TEST FOR AVAILABILITY

The particles from Lake Ontario tributaries and urban runoff were incubated with Selenastrum in AAP(-N) medium to study the availability of nitrogen. The negative results with the growth of Selenastrum in this experiment were observed. However, it cannot be concluded that the tested particles did not serve as sources of nitrogen because it was a short-term study. The failure might be due to the fact that the rate of mineralization was not rapid enough to supply nitrogen to the tested algae in a medium with no nitrogen. Anabaena, N-fixing blue green algae, was observed in the culture after day 14 of incubation. This blue-green algae was thought to be originally present in the particles. Under the conditioned study, every necessary nutrient for algae growth was present except nitrogen; however, these blue-green algae could fix nitrogen from the atmosphere resulting in the massive growth of them in the tested sample while the Selenastrum could not grow.

The later experiment performed on the filtrate after the end of mineralization gave the successful results in the growth

of tested algae as shown in Table 8. This showed that the NH_4^+ -N and NO_3^- -N resulting from the mineralization and nitrification could be available for algal growth. The average concentration of nitrogen in the filtrate per studied flask was shown in Table 10. The growth of algae which related with the absorbance was correlated well with the nitrogen concentration in the filtrate. However, the average availability of nitrogen measure by chemical test was shown to be less than the result obtained from bioassay test. It is likely that the algae is able to use soluble organic-N besides NH_4^+ -N and NO_3^- -N in the filtrate for growth.

Table 10. COMPARISON OF NITROGEN AVAILABILITY FROM CHEMICAL AND BIOASSAY METHOD

Samples	Chemical N-availability (mgN/l) per flask	Absorbance	Bioassay N-avail- ability (mgN/l) per flask
A-12	2.85	0.25	2.15
B-12	3.33	0.33	> 4.00
D-12	2.73	0.33	> 4.00
28	0.81	0.11	0.85
41	0.27	0.045	0.30
42	0.76	0.11	0.85
43	0.69	0.10	0.75
44	0.31	0.05	0.35

AVAILABILITY STUDIES

The short-term availability was considered to be more important than the long-term availability in the lakes. In this study, short-term availability was defined as the amount of NH_4^+ -N and NO_3^- -N that was found to be present in the interstitial water of samples in zero to 10 days of incubation. The long-term availability referred to the incubation time of 50-100 days.

In Table 11, the average percent nitrogen availability in short-term (10 days) and long-term (100 days) studies of urban runoff from different stations in the Madison areas are summarized. The average percent availability from residential area station A, B, and D was found to be very close together in the range of 47-55 percent for short-term study and in the range of 72-97 percent for long-term study. If the estimated annual amount of nutrients entering Lake Mendota from urban area (4.6 lbs/acre/yr) (Sonzogni and Lee, 1972) was applied, the amount of nitrogen to be available for plants after entering the lake in 10 days would be in the range of 2.2-2.5 lbs/acre/yr.

TABLE 11. SUMMARIZATION OF THE PERCENT N-AVAILABILITY IN SHORT-TERM AND LONG-TERM STUDIES OF URBAN RUNOFF

Samples	Zoning Code	Short-term		Long-term	
		Percent Availability	Average	Percent Availability	Average
A-6-1	R1	40		95	
2		42	41	92	91
3		40		86	
A-8-1	R1	54		90	
2		55	54	89	92
3		54		97	
A-9-1	R1	67		110	
2		76	71	110	109
3		69		107	
<u>Overall Average of Station A</u>			55		97
B-6-1	R2	30		80	
2		31	31	82	74
3		33		61	
B-8-1	R2	67		95	
2		66	66	93	94
3		67		95	
B-9-1	R2	70		105	
2		60	58	105	104
3		44		103	
<u>Overall Average of Station B</u>			52		91
D-6-1	R5	37		83	
2		31	32	80	71
3		29		51	
D-8-1	R5	61		76	
2		54	58	74	75
3		58		74	
D-10-1	R5	51			
2		55	52	72	72
3		50		73	
<u>Overall Average of Station D</u>			47	70	72

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APPENDIX A

Table A.1. NITROGEN MINERALIZATION OF NEW YORK RIVER WATERS

Sample No.	Incubation Days	mgN/l as:		<u>TKN</u>	<u>SKN</u>
		<u>Ammonia-N</u>	<u>Nitrate-N</u>		
28	0	0.18	0.61	0.73	0.63
Oswego R.					
March 2, 1973	10	0.05	0.74		
		0.05	0.76		
		0.05	0.74		
	25	0.06	0.86		
		0.06	0.84		
		0.06	0.84		
	50	0.13	0.92		
		0.13	0.92		
		0.12	0.90		
	82	0.05	1.07		
		0.05	1.07		
		0.05	1.05		
31	0	0.20	0.68	0.59	0.54
Oswego R.					
March 28, 1973	10	0.25	0.68		
		0.24	0.68		
		0.32	0.68		
	25	0.08	0.90		
		0.13	0.67		
		0.11	0.70		
	50	0.05	0.95		
		0.05	0.95		
		0.05	0.95		
	64	< 0.05	1.00	0.45	
		< 0.05	0.99	0.50	
		< 0.05	1.05	0.50	
	100	0.05	1.23		
		< 0.05	1.23		
		< 0.05	1.26		

<u>Sample No.</u>	<u>Incubation Days</u>	mgN/l as:		<u>TKN</u>	<u>SKN</u>
		<u>Ammonia-N</u>	<u>Nitrate-N</u>		
35	0	0.21	0.56	0.60	0.54
Oswego R. April 7, 1973	10	0.35	0.68		
		0.32	0.68		
		0.32	0.68		
	25	0.10	0.92		
		0.10	0.92		
		0.10	0.85		
	50	0.05	0.92		
		0.05	1.05		
		0.05	1.05		
	64	0.05	1.05	0.47	
		0.06	1.05	0.60	
		0.06	1.05	0.55	
	100	< 0.05	1.20		
		< 0.05	1.65		
		0.06	1.26		
43	0	0.28	0.46	0.96	0.43
Oswego R. May 1, 1973	7	0.24	0.46	0.85	
		0.25	0.46	0.71	
		0.28	0.46	1.13	
	14	0.10	0.80	0.57	
		0.11	0.80	0.65	
		0.10	0.82	--	
	21	0.10	0.82	0.61	
		0.12	0.82	0.61	
		0.11	0.82	0.48	
	28	0.06	0.95	0.36	
		0.07	0.90	0.36	
		0.06	0.92	0.45	
	35	0.08	0.87	0.50	0.41
		0.07	0.90	0.35	0.41
		0.07	0.87	0.50	0.45
47	0	0.10	0.60	0.55	0.40
Oswego R. May 14, 1973	10	0.05	0.60		
		0.05	0.68		
		0.07	0.60		

<u>Sample No.</u>	<u>Incubation Days</u>	mgN/l as:		<u>TKN</u>	<u>SKN</u>
		<u>Ammonia-N</u>	<u>Nitrate-N</u>		
47	25	0.09	0.88		
(continued)		0.06	0.72		
		0.06	--		
	50	--	0.82		
		--	0.79		
		--	0.80		
48	0	0.29	0.25	0.76	0.49
Oswego R.					
May 18, 1973	10	0.23	0.68		
		0.19	0.62		
		0.21	0.57		
	25	0.20	--		
		0.17	--		
		0.20	--		
	50	< 0.05	0.74		
		< 0.05	0.77		
		0.05	0.79		
52	0	0.31	0.35	1.14	0.55
Oswego R.					
May 28, 1973	10	0.37	0.62		
		0.22	0.68		
		0.30	0.66		
	25	0.22	0.88		
		0.17	0.88		
		0.09	0.88		
	50	< 0.05	0.90		
		0.06	1.01		
		< 0.05	0.92		
59	0	0.58	0.68	1.62	1.17
Oswego R.					
June 17, 1973	10	0.34	0.75		
		0.31	0.74		
		0.25	0.82		
	25	< 0.05	1.37		
		< 0.05	1.29		
		< 0.05	1.36		
	50	--	1.25		
		--	1.22		
		--	1.24		

<u>Sample No.</u>	<u>Incubation Days</u>	<u>mgN/l as:</u>		<u>TKN</u>	<u>SKN</u>
		<u>Ammonia-N</u>	<u>Nitrate-N</u>		
34	0	0.15	0.98	1.23	0.52
Genesee R.					
April 7, 1973	10	0.25	0.95		
		0.14	1.00		
		0.29	0.95		
	25	0.07	1.15		
		< 0.05	1.25		
		< 0.05	1.20		
	50	0.05	1.27		
		0.05	1.30		
		0.05	1.27		
	64	0.07	1.27	1.10	
		0.05	1.35	1.20	
		0.05	1.35	1.20	
	100	0.05	1.32		
		0.06	1.32		
		0.05	1.08		
42	0	0.28	0.56	0.96	0.45
Genesee R.					
May 1, 1973	7	0.24	0.59	1.27	
		0.24	0.61	1.13	
		0.22	0.61	1.12	
	14	0.12	0.86	0.65	
		0.12	0.88	0.65	
		0.11	0.88	0.65	
	21	0.11	0.90	0.80	
		0.10	0.92	0.68	
		0.12	0.90	0.69	
	28	0.07	1.05	0.55	
		0.06	1.05	0.60	
		0.06	1.05	0.60	
	35	0.07	0.95	0.47	0.43
		0.08	0.95	0.47	0.36
		0.07	0.97	0.50	0.34
51	0	0.32	0.35	0.91	0.47
Genesee R.					
May 28, 1973	10	0.22	0.74		
		0.22	0.72		
		0.27	0.66		

<u>Sample No.</u>	<u>Incubation Days</u>	<u>mgN/l as:</u>		<u>TKN</u>	<u>SKN</u>
		<u>Ammonia-N</u>	<u>Nitrate-N</u>		
51 (continued)	25	0.20	0.86		
		0.05	0.85		
		< 0.05	--		
	50	0.06	0.92		
		< 0.05	0.88		
		< 0.05	0.91		
58 Genesee R. June 17, 1973	0	0.60	0.64	1.62	1.04
	10	< 0.05	0.98		
		< 0.05	0.99		
		< 0.05	1.20		
	25	< 0.05	1.29		
		< 0.05	1.34		
		< 0.05	--		
	50	--	1.40		
		--	1.24		
		--	1.42		
41 Niagara R. April 30, 1973	0	0.16	0.14	0.31	0.22
	7	0.15	0.17	0.43	
		0.18	0.17	0.39	
		0.14	0.17	0.45	
	14	0.12	0.19	0.25	
		0.14	0.21	0.34	
		0.14	0.21	0.34	
	21	0.13	0.28	0.35	
		0.11	0.29	0.35	
		0.11	0.29	0.35	
	28	0.08	0.32	0.65	
		0.09	0.32	0.26	
		0.07	0.32	0.26	
	35	0.07	0.30	0.26	0.40
		0.07	0.30	0.20	0.32
		0.07	0.30	0.20	0.32
50 Niagara R. May 27, 1973	0	0.15	0.36	0.46	0.33
	10	0.12	0.70		
		< 0.05	0.76		
		0.05	0.76		

<u>Sample No.</u>	<u>Incubation Days</u>	mgN/l as:		<u>TKN</u>	<u>SKN</u>
		<u>Ammonia-N</u>	<u>Nitrate-N</u>		
50 (continued)	25	< 0.05	0.56		
		< 0.05	0.72		
		< 0.05	--		
	50	--	0.72		
		--	0.66		
		--	0.76		
56 Niagara R. June 16, 1973	0	0.07	0.27	0.86	0.66
	10	0.15	0.35		
		0.18	0.28		
		0.20	0.36		
	25	< 0.05	0.64		
		< 0.05	0.34		
		< 0.05	0.64		
	50	--	0.61		
		--	0.60		
		--	0.61		
	0	< 0.05	0.47	0.42	0.29
	10	0.13	0.50		
		0.08	0.50		
		0.08	0.50		
36 Black R. April 7, 1973	25	0.10	0.50		
		0.13	0.48		
		0.16	0.50		
	50	< 0.05	0.54		
		< 0.05	0.53		
		< 0.05	0.53		
	64	0.05	0.56	0.30	
		0.05	0.56	0.30	
		0.05	0.56	0.30	
	100	0.06	0.52		
		0.06	0.48		
		0.05	0.62		
44 Black R. May 1, 1973	0	0.10	0.24	0.35	0.20
	7	0.13	0.27	0.53	
		0.14	0.26	0.47	
		0.12	0.26	0.39	

<u>Sample No.</u>	<u>Incubation Days</u>	<u>mgN/l as:</u>		<u>TKN</u>	<u>SKN</u>
		<u>Ammonia-N</u>	<u>Nitrate-N</u>		
44 (continued)	14	0.16	0.29	0.51	
		0.15	0.28	0.43	
		0.13	0.28	0.43	
	21	0.16	0.29	0.49	
		0.18	0.25	0.55	
		0.12	0.29	0.45	
	28	0.07	0.38	0.36	
		0.06	0.36	0.31	
		0.07	0.36	0.31	
	35	0.07	0.37	0.30	
		0.07	0.36	0.30	
		0.07	0.36	0.28	
53	0	0.08	0.19	0.56	0.38
Black R. May 28, 1973	10	< 0.05	0.28		
		< 0.05	0.64		
		0.07	0.28		
	25	0.05	0.56		
		< 0.05	0.39		
		0.06	0.56		
	50	0.05	0.40		
		< 0.05	0.35		
		< 0.05	0.35		
60	0	0.07	0.27	0.98	0.63
Black R. June 17, 1973	10	< 0.05	0.22		
		< 0.05	0.16		
		< 0.05	0.16		
	25	< 0.05	0.42		
		< 0.05	0.45		
		< 0.05	0.38		
	50	--	0.36		
		--	0.49		
		--	0.50		

Table A.2. SUMMARY DATA TABLE
NITROGEN MINERALIZATION OF NEW YORK RIVER WATERS

Sample No.	Incubation Days	All Data: mgN/l					(A)	(B)	(A)/(B)
		NH ₃ -N	NO ₃ -N	TKN	SKN	NH ₃ -N + NO ₃ -N	Total N	%	
28	0	0.18	0.61	0.73	0.63	0.79	1.34	59	
Oswego	10	0.05	0.75			0.80		60	
River	25	0.06	0.85			0.91		68	
	50	0.13	0.91			1.04		78	
	82	0.05	1.06	0.22	0.15	1.11		83	
31	0	0.20	0.68	0.59	0.54	0.88	1.27	69	
Oswego	10	0.27	0.68			0.95		75	
River	25	0.11	0.76			0.87		68	
	50	0.05	0.95			1.00		79	
	64	< 0.05	1.01	0.48		1.01		80	
	100	< 0.05	1.24			1.24		98	
35	0	0.21	0.56	0.60	0.54	0.77	1.16	66	
Oswego	10	0.33	0.68			1.01		87	
River	25	0.10	0.90			1.00		86	
	50	0.05	1.01			1.06		91	
	64	0.06	1.05	0.54		1.11		96	
	100	< 0.05	1.37			1.37		118	
43	0	0.28	0.46	0.96	0.43	0.74	1.42	52	
Oswego	7	0.26	0.46	0.90		0.72		51	
River	14	0.10	0.81	0.61		0.91		64	
	21	0.11	0.82	0.57		0.93		66	
	28	0.06	0.92	0.39		0.98		69	
	35	0.07	0.88	0.45	0.42	0.95		67	
47	0	0.10	0.60	0.55	0.40	0.70	1.15	61	
Oswego	10	0.06	0.63			0.69		60	
River	25	0.07	0.80			0.87		76	
	50	--*	0.80			0.80		70	
48	0	0.29	0.25	0.76	0.49	0.54	1.01	53	
Oswego	10	0.21	0.62			0.83		82	
River	25	0.19	----			----		--	
	50	< 0.05	0.77			0.77		76	

* Not determined; assumed equal to zero for sum of (nitrate+ammonia)-N

Sample No.	Incubation Days	NH ₃ -N	NO ₃ -N	TKN	SKN	(A) NH ₃ -N + NO ₃ -N	(B) Total N	(A)/(B) %
52	0	0.31	0.35	1.14	0.55	0.66	1.49	44
Oswego	10	0.30	0.65			0.95		64
River	25	0.16	0.88			1.04		70
	50	< 0.05	0.94			0.94		63
59	0	0.58	0.68	1.62	1.17	1.26	2.30	55
Oswego	10	0.30	0.77			1.07		46
River	25	< 0.05	1.34			1.34		58
	50	--*	1.24			1.24		54
34	0	0.15	0.98	1.23	0.52	1.13	2.21	51
Genesee	10	0.23	0.97			1.20		55
River	25	< 0.05	1.20			1.20		54
	50	0.05	1.28			1.33		60
	64	0.06	1.32	1.17		1.38		63
	100	0.05	1.24			1.29		59
42	0	0.28	0.56	0.96	0.45	0.84	1.52	55
Genesee	7	0.23	0.60	1.17		0.83		55
River	14	0.12	0.87	0.65		0.99		65
	21	0.11	0.91	0.72		1.02		67
	28	0.06	1.05	0.58		1.11		73
	35	0.07	0.96	0.48	0.38	1.03		68
51	0	0.32	0.35	0.91	0.47	0.67	1.26	53
Genesee	10	0.24	0.71			0.95		75
River	25	0.08	0.86			0.94		75
	50	< 0.05	0.90			0.90		71
58	0	0.60	0.64	1.62	1.04	1.24	2.26	55
Genesee	10	< 0.05	1.06			1.06		47
River	25	< 0.05	1.32			1.32		58
	50	--*	1.35			1.35		60
41	0	0.16	0.14	0.31	0.22	0.30	0.45	67
Niagara	7	0.16	0.17	0.42		0.33		73
River	14	0.13	0.20	0.31		0.33		73
	21	0.12	0.29	0.35		0.41		91
	28	0.08	0.32	0.26		0.40		89
	35	0.07	0.30	0.22	0.35	0.37		82
50	0	0.15	0.36	0.46	0.33	0.51	0.82	62
Niagara	10	< 0.05	0.74			0.74		90
River	25	< 0.05	0.64			0.64		78
	50	--*	0.71			0.71		87

Sample No.	Incubation Days	NH ₃ -N	NO ₃ -N	TKN	SKN	(A) NH ₃ -N + NO ₃ -N	(B) Total N	(A)/(B) %
56	0	0.07	0.27	0.86	0.66	0.34	1.13	30
Niagara	10	0.18	0.33			0.51		45
River	25	< 0.05	0.54			0.54		48
	50	--*	0.61			0.61		54
36	0	< 0.05	0.47	0.42	0.29	0.47	0.89	53
Black	10	0.10	0.50			0.60		67
River	25	0.13	0.49			0.62		70
	50	< 0.05	0.53			0.53		60
	64	0.05	0.56	0.30		0.61		68
	100	0.06	0.54			0.60		67
44	0	0.10	0.24	0.35	0.20	0.34	0.59	58
Black	7	0.13	0.26	0.46		0.39		66
River	14	0.15	0.28	0.46		0.43		73
	21	0.15	0.28	0.50		0.43		73
	28	0.07	0.37	0.33		0.44		75
	35	0.07	0.36	0.29		0.43		73
53	0	0.08	0.19	0.56	0.38	0.27	0.75	36
Black	10	< 0.05	0.40			0.40		53
River	25	< 0.05	0.50			0.50		67
	50	< 0.05	0.37			0.37		56
60	0	0.07	0.27	0.98	0.63	0.34	1.25	27
Black	10	< 0.05	0.18			0.18		14
River	25	< 0.05	0.42			0.42		34
	50	--*	0.45			0.45		36

Table A.3. MINERALIZATION BEGUN MAY 7, 1973
 GENESEE R. (42) AND OSWEGO R. (43) PARTICLES IN L. ONTARIO
 WATER FROM STATION 10

A. Initial Data						
<u>Sample No.</u>	<u>TKN (mgN/l)</u>	<u>NO₃⁻-N (mgN/l)</u>				
L. Ontario	0.24	0.26				
water (10)	0.24	0.26				
	0.24	0.26				
mean values	0.25	0.26				
400 ml (10) +	0.54					
Particles from	0.54					
400 ml (42)	0.68					
mean values	0.59	0.26				
400 ml (10) +	0.36					
Particles from	0.36					
400 ml (43)	0.50					
mean values	0.41	0.26				
B. Dark Incubation (all values mgN/l as NO ₃ ⁻ -N)*						
<u>Sample</u>	<u>Time (days)</u>	<u>0</u>	<u>7</u>	<u>14</u>	<u>21</u>	<u>28</u>
L. Ontario		0.26	0.26	0.27	0.27	0.25
water (10)		0.26	0.26	0.27	0.27	0.27
		0.26	0.26	0.27	0.27	0.26
mean values		0.26	0.26	0.27	0.27	0.26
400 ml (10) +		--	0.29	0.35	0.34	0.35
Particles from		--	0.29	0.34	0.34	0.35
400 ml (42)		--	0.29	0.35	0.34	0.35
mean values		0.26	0.29	0.35	0.34	0.35
400 ml (10) +		--	0.32	0.38	0.40	0.36
Particles from		--	0.32	0.38	0.38	0.36
400 ml (43)		--	0.33	0.38	0.40	0.38
mean values		0.26	0.32	0.38	0.39	0.37

* Each value shown represents the nitrate concentration of one test bottle.

Table A.4. SUMMARY OF NET MEAN NITRATE-N RELEASED FROM PARTICLES INTO NO. 10 LAKE WATER

Sample No.	Particulate org. N from river mgN/l	Net Mean NO_3^- -N from Particles (mgN/l)			Net Mean NO_3^- -N from Particles (% of particulate org. N)		
		7	14	21	7	14	21
42							
Particles	0.34	0.03	0.08	0.07	0.09	24	21
							26
43							
Particles	0.16	0.06	0.11	0.12	0.11	38	69
						69	75
							69

Table A.5 MINERALIZATION BEGUN JUNE 21, 1973
GENESEE R. (58) AND OSWEGO R. (59) PARTICLES IN LAKE ONTARIO
WATER FROM STATION 96

A. Initial Data

<u>Sample No.</u>	<u>TKN (mgN/l)</u>	<u>NO₃⁻-N (mgN/l)</u>
L. Ontario	0.49	0.0
water (96)	0.49	0.0
	0.54	0.0
mean values	0.51	0.0
400 ml (96) +	1.10	
Particles from	1.10	
800 ml (58)	1.08	
mean values	1.09	0.0
400 ml (96) +	0.96	
Particles from	0.88	
800 ml (59)	0.87	
mean values	0.90	0.0

B. Dark Incubation (all values mgN/l as NO₃⁻-N)*

	Time (days)	<u>0</u>	<u>10</u>	<u>25</u>	<u>50</u>
<u>Sample No.</u>					
L. Ontario		0.0	0.0	0.0	0.18
water (96)		0.0	0.0	0.14	0.17
		0.0	0.0	0.0	0.12
mean values		0.0	0.0	0.0	0.16
400 ml (96) +		--	0.22	0.43	0.55
Particles from		--	0.16	0.45	0.47
800 ml (58)		--	0.16	0.40	0.51
mean values		0.0	0.18	0.43	0.51
400 ml (96) +		--	0.0	0.43	0.52
Particles from		--	0.0	0.45	0.52
800 ml (59)		--	0.0	0.51	0.56
mean values		0.0	0.0	0.46	0.53

* Each value shown represents the nitrate concentration of one test bottle.

Table A.6. SUMMARY OF NET MEAN NITRATE-N RELEASED FROM PARTICLES INTO NO. 96 LAKE WATER

Sample No.	Particulate org. N from river mgN/l	Net Mean NO_3^- -N from Particles			Net Mean NO_3^- -N from Particles (% of particulate org. N)		
		10	25	50 days	10	25	50 days
58 Particles	0.58	0.18	0.39	0.35	31	67	60
59 Particles	0.39	0.00	0.42	0.37	0	108	95

APPENDIX B

NITROGEN MINERALIZATION FROM MADISON, WISCONSIN, URBAN RUNOFF SAMPLES

Sample No.	Incubation Day	$\text{NH}_4^+\text{-N}$ (mgN/l)	$\text{NO}_3^-\text{-N}$ (mgN/l)	Total Kjeldahl-N (mgN/l)	Soluble Kjeldahl-N (mgN/l)
A-6	0	0.13	0.02	1.85	1.00
		0.67	0.08		
		0.74	0.05		
		0.69	0.05		
	25	0.15	0.93		
		0.20	1.06		
		0.20	0.98		
	50	0.05	1.15		
		0.05	1.20		
		0.05	1.20		
	100	0.16	1.62	0.71	0.32
		0.16	1.56	0.75	0.32
		0.04	1.56	0.75	0.32
A-8	0	0.59	0.45	1.60	0.67
	10	0.59	0.52		
		0.59	0.53		
		0.61	0.50		
	25	0.91	0.48		
		0.80	0.50		
		0.87	0.49		
	50	0.21	1.50		
		0.21	1.50		
		0.16	1.47		
	100	0.08	1.75	0.98	0.45
		0.08	1.75	1.08	0.45
		0.08	1.90	1.04	0.38
A-9	0	0.80	0.21	2.40	1.70
	10	1.35	0.40		
		1.58	0.40		
		1.35	0.45		

Sample No.	Incubation Day	NH ₄ ⁺ -N (mgN/l)	NO ₃ ⁻ -N (mgN/l)	Total Kjeldahl-N (mgN/l)	Soluble Kjeldahl-N (mgN/l)
A-9 (cont'd)	25	0.26	2.40		
		0.30	2.30		
		0.29	2.32		
	50	0.30	2.60		
		0.34	2.60		
		0.26	2.50		
	100	0.07	2.80	1.02	0.66
		0.08	2.80	1.12	0.66
		0.05	2.75	1.30	0.66
A-12	0	1.12	0.71	4.28	2.40
	10	0.43	0.93		
		1.78	0.97		
		1.98	0.97		
	25	1.71	1.10		
		1.62	1.12		
		1.71	1.10		
	50	1.74	1.32		
		1.44	1.37		
		1.72	1.32		
	82	0.10	3.75	1.70	0.52
		0.12	3.75	1.02	0.52
		0.10	3.75	1.02	0.20
B-6	0	0.14	0.005	3.60	1.20
	10	1.09	0.02		
		1.14	0.03		
		1.21	0.02		
	25	0.21	1.48		
		0.10	1.48		
		0.13	1.41		
	50	0.05	1.95		
		0.05	1.75		
		0.22	1.85		
	100	0.14	2.85	1.40	0.63
		0.23	2.85	1.35	0.63
		0.19	2.10	1.60	0.55
B-8	0	0.75	0.62	1.50	0.75

Sample No.	Incubation Day	NH ₄ ⁺ -N (mgN/l)	NO ₃ ⁻ -N (mgN/l)	Total Kjeldahl-N (mgN/l)	Soluble Kjeldahl-N (mgN/l)
B-8 (cont'd)	10	0.70	0.72		
		0.67	0.72		
		0.68	0.73		
	25	0.87	0.71		
		0.85	0.74		
		0.80	0.63		
	50	0.16	1.70		
		0.11	1.75		
		0.11	1.75		
	100	0.11	1.90	0.98	0.38
		0.08	1.90	0.84	0.35
		0.06	1.95	0.98	0.32
B-9	0	0.82	0.31	2.30	1.50
	10	0.92	0.90		
		0.89	0.67		
		0.54	0.60		
	25	0.21	2.10		
		0.26	2.10		
		0.30	2.20		
	50	0.26	2.25		
		0.32	2.27		
		0.30	2.35		
	100	0.14	2.60	1.14	0.50
		0.14	2.60	0.90	0.60
		0.10	2.60	1.06	0.60
B-12	0	1.32	0.30	4.24	2.52
	10	1.83	1.67		
		2.15	1.67		
		2.04	1.62		
	25	1.08	2.50		
		0.93	2.80		
		0.99	2.45		
	50	0.28	3.40		
		0.29	3.50		
		-	2.90		

Sample No.	Incubation Day	NH ₄ ⁺ -N (mgN/l)	NO ₃ ⁻ -N (mgN/l)	Total Kjeldahl-N (mgN/l)	Soluble Kjeldahl-N (mgN/l)
B-12 (cont'd)	82	0.10	4.40	1.40	0.20
		0.11	4.50	1.60	0.30
		0.10	4.40	1.80	0.20
D-6	0	0.08	0.24	2.45	0.77
	10	0.78	0.20		
		0.65	0.19		
		0.64	0.15		
	25	0.05	1.00		
		0.18	1.03		
		0.05	0.88		
	50	0.05	1.50		
		0.12	1.65		
		0.15	1.15		
	100	0.16	2.07	1.70	0.34
		0.12	2.02	1.55	0.34
		0.12	1.25	1.50	0.34
D-8	0	0.64	0.80	1.70	1.50
	10	0.59	0.94		
		0.43	0.92		
		0.49	0.96		
	25	0.76	0.63		
		0.60	0.68		
		0.51	0.64		
	50	0.11	1.80		
		0.21	1.75		
		0.21	1.75		
	100	0.06	1.85	1.08	0.32
		0.06	1.78	1.22	0.35
		0.06	1.78	1.16	0.32
D-10	0	0.51	1.56	2.70	0.89
	10	0.46	1.70		
		0.46	1.90		
		0.39	1.75		
	25	0.21	2.50		
		0.30	2.50		
		0.26	2.50		

Sample No.	Incubation Day	NH_4^+-N (mgN/l)	NO_3^--N (mgN/l)	Total Kjeldahl-N (mgN/l)	Soluble Kjeldahl-N (mgN/l)
D-10 (cont'd)	50	0.22	2.75		
		0.26	2.68		
		0.24	2.68		
	100	0.08	3.00	2.24	0.50
		0.10	3.00	2.30	0.56
		0.08	2.90	2.30	0.56
D-12	0	1.07	0.33	4.60	2.52
	10	2.00	1.90		
		1.25	1.95		
		1.04	1.90		
	25	1.05	2.05		
		-	2.05		
		1.10	2.02		
	50	0.54	2.90		
		0.50	3.00		
		0.49	3.00		
	100	0.08	3.60	2.00	0.20
		0.10	3.60	2.08	0.40
		0.10	3.70	2.08	0.20
F-9	0	0.85	0.63	1.60	1.20
	10	0.92	0.80		
		1.16	0.75		
		1.20	0.70		
	25	0.21	2.00		
		0.30	1.98		
		0.32	1.80		
	50	0.15	2.22		
		0.17	2.22		
		0.20	1.95		
	100	0.10	2.10	0.62	0.56
		0.08	2.35	0.56	0.50
		0.13	2.40	0.74	0.50

TECHNICAL REPORT DATA <i>(Please read Instructions on the reverse before completing)</i>		
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16. ABSTRACT <p>Samples of water from the Niagara, Genesee, Oswego and Black Rivers were collected from March to June 1973. The samples were analyzed for nitrogen forms and were incubated in darkness under aerobic conditions to promote mineralization of soluble inorganic nitrogen from the organic nitrogen in the samples. The amounts of ammonia and nitrate were determined as a function of the time of incubation. Generally over 50 percent of total nitrogen present in these river samples was immediately available for algal growth or potentially available after mineralization by bacteria. The results were highly variable from each tributary, and no single value could be selected from the data obtained to describe the availability of total nitrogen in a given river.</p> <p style="text-align: right; margin-right: 100px;">U.S. Environmental Protection Agency Region 5, Library (5PL-16) 230 S. Dearborn Street, Room 1670 Chicago, IL 60604</p>		
17. KEY WORDS AND DOCUMENT ANALYSIS		
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Nitrogen Ammonia Bacteria Nutrients	Lake Ontario	06C, 06F 08H
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