

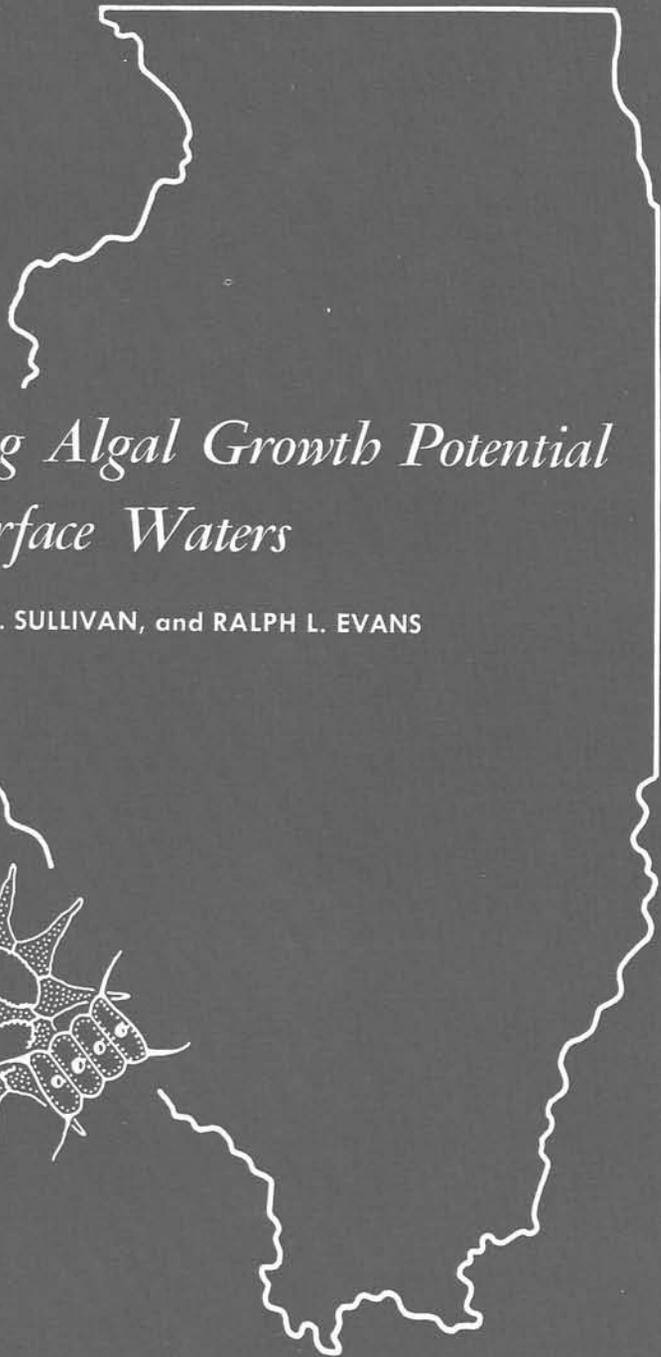
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STATE OF ILLINOIS

DEPARTMENT OF REGISTRATION AND EDUCATION



*A Technique for Evaluating Algal Growth Potential
in Illinois Surface Waters*

by WUN-CHENG WANG, WILLIAM T. SULLIVAN, and RALPH L. EVANS

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Potential in Illinois Surface Waters*

by WUN-CHENG WANG, WILLIAM T. SULLIVAN, and RALPH L. EVANS

Title: A Technique for Evaluating Algal Growth Potential in Illinois Surface Waters.

Abstract: Various techniques were used to measure the algal growth potential (AGP) of Illinois surface waters, and correlation analyses showed the filterable organic mass to be the most reliable criterion. The dry weight of organic mass produced by algal growth during a 7-day incubation in natural water substrate was designated as the AGP of the water tested. The biomass produced was usually dependent on the nutrient level in the water. Mean AGPs ranged from 12 to 82 mg/1 for lake and stream sources and from 120 to 135 mg/1 for sewage sources. The procedures developed provide a diagnostic tool for planning and managing Illinois water resources.

Reference: Wang, Wun-Cheng, William T. Sullivan, and Ralph L. Evans. A Technique for Evaluating Algal Growth Potential in Illinois Surface Waters. Illinois State Water Survey, Urbana, Report of Investigation 72, 1973.

Indexing Terms: algae, algal growth potential, eutrophication, fluorescence, nutrients, light absorption, organic particulate matter, sewage, surface water, water quality.

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A Technique for Evaluating Algal Growth Potential in Illinois Surface Waters

by **Wun-Cheng Wang, William T. Sullivan, and Ralph L. Evans**

SUMMARY

During a period of 19 months water samples collected from a number of lakes, streams, and sewage sources were examined by a variety of techniques to develop a predictive procedure for assaying the algal growth potential of Illinois surface waters. The period of study encompassed seasonal changes in the natural waters. Twenty-seven incubation runs, composed of 155 samples from 3 lakes, 4 streams, and 2 sewage sources, were evaluated.

Algal growth, in filtered samples inoculated with an Illinois River culture, was measured by changes in light absorption, fluorescence of algal pigments, filterable organic and inorganic mass, total alkalinity, and hardness. From correlation analyses, filterable organic mass was found to be the most reliable criterion for quantifying algal growth. Experimental error, expressed as the coefficient of variation, was less than 5 percent. Inconsistencies attributable to the use of a variable natural inoculum were found to be minimal.

The analysis of nitrogen levels throughout the period of maximum algal growth suggests that ammonium nitrogen was the source of nitrogen preferred by the organisms. Nitrate nitrogen was used only after the ammonium was depleted. Changes in alkalinity and hardness were found to be parallel; the biological utilization of carbon per atom of carbon precipitated as carbonate ranged from 1.1:1 in Lake Bloomington to 4.7:1 in Lake Michigan.

The algal growth potential (AGP) is here defined as the algal or organic mass resulting from a 7-day incubation of a culture grown on a natural water substrate under standardized laboratory conditions and expressed as milligrams of dry organic mass per liter of sample. For the natural waters examined, the mean AGP ranged from 12 mg/l in Lake Eureka to 82 mg/l in the Illinois River; for the sewage sources, the range was 120-135 mg/l. Since the trophic level of a natural water source is, to some degree, characterized by its AGP, this technique has promise as another diagnostic tool for defining the water quality of streams and lakes in Illinois.

INTRODUCTION

Alterations in water quality noted throughout the nation have also been seen in Illinois lakes and streams. Such changes, especially during the past 20 years, have been well documented by many researchers. Beeton¹ reported concentrations of conservative substances to have increased with time in Lake Michigan; Gerstein² expressed concern over the upward trend of ammonium nitrogen at the intake of the South District Filtration Plant serving the city of Chicago. Harneson and Larson³ have presented convincing evidence of increasing nitrate concentrations in 8 Illinois streams within a span of 5 years. Ackermann et al.⁴ have pointed out that chloride concentrations in the Illinois River have increased 130 percent during a 70-year period. Mills et al.⁵ have documented man's cultural effects upon the micro and macro biota of the Illinois River; and more recently Starrett's⁶ treatise on the mussels of that river has shown the loss of some of its desirable functional aquatic balance. Changes in the species composition of zooplankton in Lake Michigan, reported by Bartsch,⁷ also suggest water quality deterioration.

One major consequence of water quality change is eu-

trophication. Eutrophication is, of itself, both causative and symptomatic of the true problem since the end product is superabundant algal growth. It is these organisms and the consequences of their proliferation that produce, by mankind's standard, gross water quality deterioration.

The most direct evaluation of eutrophication is the study of living algal organisms, particularly from the standpoint of their growth potential. As the primary participants in the aquatic food chain, algae are the most immediate biotic reflection of the nutrient status of their environment. Their short life cycle and faculty for rapid adaptation make them ideal organisms for laboratory examination. Their causative role in water chemistry changes could suggest possible growth limiting factors, which would provide a means by which nuisance blooms might be anticipated or averted.

Scope of Study

The study was conducted on waters of the Illinois River basin including Lake Michigan. The lake and the river have an unusual relationship in that the lake is, in a sense,

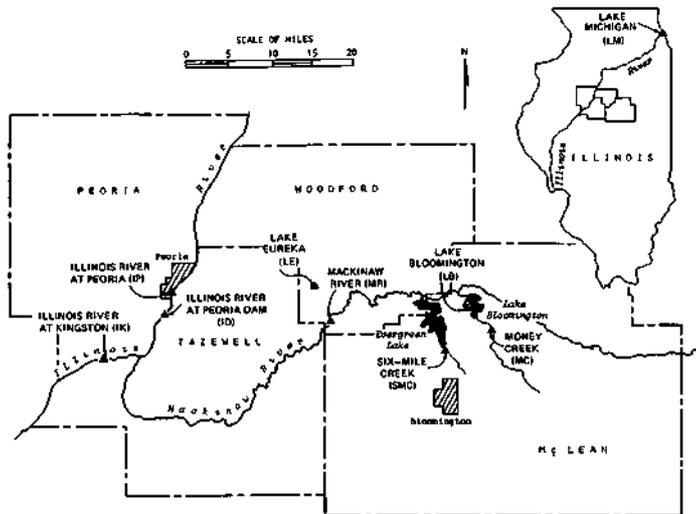


Figure 1. Location of sampling stations

a tributary of the river. Carefully regulated volumes of lake water are diverted to the Illinois River through a network of waterways in the vicinity of Chicago. This direction of flow is in marked contrast to that of other rivers in the Lake Michigan basin.

Altogether 27 incubation runs at biweekly intervals were performed from January 1970 through July 1971. Samples for the first seven runs were obtained from four sources: Lake Eureka (LE), the Illinois River at Peoria (IP), a tertiary sewage pond (TSP) operated by the Greater Peoria Sanitary District, and raw sewage (RS) from a sewage treatment works owned by the city of Washington, Illinois.

Three runs (8, 9, and 10) were experiments designed to test the effect of various inocula on algal growth. The natural water substrate was obtained from the Illinois River at Peoria, and the inocula used originated from Lake Eureka, the Illinois River, a tertiary sewage pond, and raw sewage.

Seventeen runs (11 to 27) covered a 1-year period. Water samples subjected to algal growth potential evaluation during this period were obtained from Lake Bloomington (LB), Lake Michigan (LM), Money Creek (MC), Mackinaw River (MR), Six-Mile Creek (SMC), and the Illinois River at 3 locations, i.e., near Peoria at milepoint 161.1 (IP), at the Peoria Lock and Dam near milepoint 157.8 (ID), and

near the Kingston Terminal at milepoint 146.8 (IK). These sampling locations are shown in figure 1.

Objectives and Report Plan

The objectives of this study were threefold: 1) to develop an expression for the potential for algal growth in Illinois lakes and streams, 2) to gain some insight into the major chemical changes occurring in water during progressive algal growth, and 3) to compare the algal growth potentials of different water sources and of different locations on the same watercourse.

The first section of this report details the preparation of water samples and the methods developed for their quantitative determination. The second section presents the results and discussion in three parts. The first part deals with quantitative measurements for algal growth and discusses tests for reliability and some experimental techniques. The second part includes observations on quantitative changes in algal cultures as well as in water chemistry during algal growth. The third part includes algal growth potentials determined at the stream and lake sources previously noted.,

Acknowledgments

This study was conducted under the general supervision of Ralph L. Evans, Head of the Water Quality Section, and Dr. William C. Ackermann, Chief, Illinois State Water Survey. Many Water Survey personnel assisted in the study. Daniel J. Brabec and David L. Hullinger, Assistant Chemists, performed most of the chemical analyses. Davis B. Beuscher, Assistant Biologist, made the biological observations. Larry G. Epley performed most of the field work involving collection of samples. James C. Vaughn and his staff at the Water Purification Laboratory of the Central Water Filtration Plant in Chicago provided samples of Lake Michigan water; and Dr. Ching T. Hou, formerly of the Northern Regional Research Laboratory, USDA, at Peoria, assisted in the development of the algal fluorescence spectrum. Mrs. J. Loreena Ivens edited the final report; Miss Katherine Shemas typed the original manuscript; and John Brother, Jr., prepared the illustrations.

EXPERIMENTAL METHODS

Water Samples

Stream samples were collected in 1-gallon acid-washed plastic bottles near the surface and as close to the center of flow as possible. Lake samples were obtained from the intakes of water purification plants; sewage samples were collected at the mid-depth of channel flows.

The water samples were prefiltered through Whatman # 1 filter paper. This was followed by filtering through a 0.45 micrometer membrane filter with a large volume filter apparatus as shown in figure 2. The fabrication and perfor-

mance of the unit have been fully described by Wang and Schnepfer.⁸ The time required for filtration was in proportion to the particulate matter in the sample. After filtration, the samples were stored at 4C until inoculation, usually within 48 hours.

Inoculation and Incubation

The inoculum was prepared from a raw water sample collected from the Illinois River at Peoria. The sample was filtered immediately through crepe filter paper, which re-

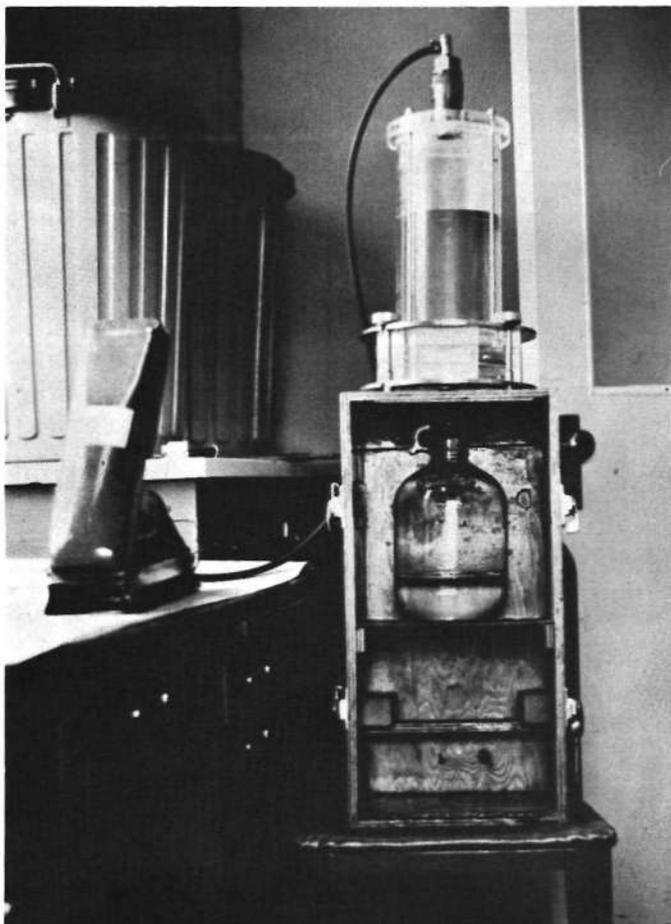


Figure 2. Membrane filter apparatus



Figure 3. Shaker platform and illumination for incubation

moved most of the gross turbidity. The filtrate, in a half-gallon Mason jar stoppered with polyurethane foam, was placed on a reciprocating shaker (figure 3) and agitated at 100 strokes per minute for 7 days.

A test was run to determine whether there was any significant water loss due to vaporization through the porous polyurethane foam. The test was made in the same manner as regular experimental runs but with daily volumetric measurement. The results showed that there was no measurable loss of water during a 2-week period.

Illumination, as shown in figure 3, was provided by cool-white fluorescent tubes suspended above and arranged around the shaker insuring an intensity of about 100 foot-candles on the incubation vessel. A thermostatically controlled air conditioner was used to regulate temperature and provide for adequate air circulation. A chart recorder provided a continuous record of air temperatures above the shaker platform; normal variations were less than 2C throughout the incubation period.

Abundant algal growth developed in the inoculum within 4 days, although incubation was continued for 7 days. The inoculum was prepared for use by first thoroughly scraping the interior of the Mason jar to detach adherent organisms. Cell clumps were dispersed, and a homogeneous suspension was produced by attaching a homogenizing blade assembly directly to the top of the Mason jar and mixing the contents

at a low speed for 20 seconds on a blender. The inoculum culture was then visually homogeneous.

Previously prepared water samples were brought to room temperature and 1500-ml volumes of each in half-gallon Mason jars were inoculated with 100-ml aliquots of the well-mixed culture. The samples were immediately placed on the reciprocating shaker and incubated in the same manner as the inoculum.

Algal Growth Measurements

Various methods were used to determine progressive algal growth in the inoculated water samples. Determinations began on the third day and were made daily thereafter through the seventh day of incubation. Following the homogenization of the incubated sample, subsamples were withdrawn and examined for changes in light absorption, fluorescence, and particulate matter. Changes in alkalinity and hardness were observed on the substrate. Other determinations involving principally water chemistry of the substrate, were made only on the day of inoculation and on the seventh day of incubation.

Absorbance. Light absorption on each unfiltered subsample was determined with a Bausch and Lomb Spectronic 20 spectrophotometer having a light path of 2 centimeters, at a wavelength of 680 nanometers. This wavelength was found to be the region of maximum absorbance.

Fluorescence. Two drops of a saturated magnesium carbonate suspension were added to a 10-ml unfiltered subsample in a centrifuge tube. This was centrifuged at 2000 rpm for 1 hour. The supernatant was discarded and 10 ml of a 9:1 (V/V) acetone-water solution was added to the residue. After vigorous shaking, the mixture was placed in a refrigerator for 24 hours to permit complete extraction of the fluorescent material. The acetone extract was then separated from the residue by centrifuging at 1000 rpm for 10

minutes. This extraction method is a modification of that described by Strickland and Parsons⁹ in which they suggested the use of a membrane filter for plankton separation instead of centrifuging.

The extract was examined with a Turner Fluorometer (Model 110) equipped with a Kodak Wratten blue 47B primary filter (maximum transmittance 430 nanometers) and a Kodak Wratten red 25 secondary filter (85 percent transmittance above 630 nanometers) as recommended by Bain.¹⁰ The chlorophyll content in the acetone extract was determined by light absorption as suggested by Strickland and Parsons.⁹

The same measurement procedures were applied to unextracted algal subsamples. Thus, four optical determinations were made, two light absorption measurements and two fluorescence measurements on extracted and unextracted subsamples.

Particulate Assessment. A gravimetric method was developed to determine inorganic and organic particulate matter. A 100-ml homogenized subsample was pipetted onto a 0.45-micrometer membrane filter that had been previously acid washed (0.5 N hydrochloric acid), repeatedly rinsed in deionized water, dried, and tare weighed. The total particulate matter on the membrane was dried at 90C for 1 hour. The weight of this material represented the combined weight of inorganic and organic particulate matter of 0.45 micrometer mean diameter or larger.

The membrane filter was next placed on a filter support and held in place by suction. This was then washed with four 5-ml volumes of 0.5 N hydrochloric acid drawn through the membrane (figure 4). The remaining residue and filter were further washed 3 times with doubly deionized water, then dried and weighed as before. The dry weight of this acid insoluble residue was taken to represent organic par-

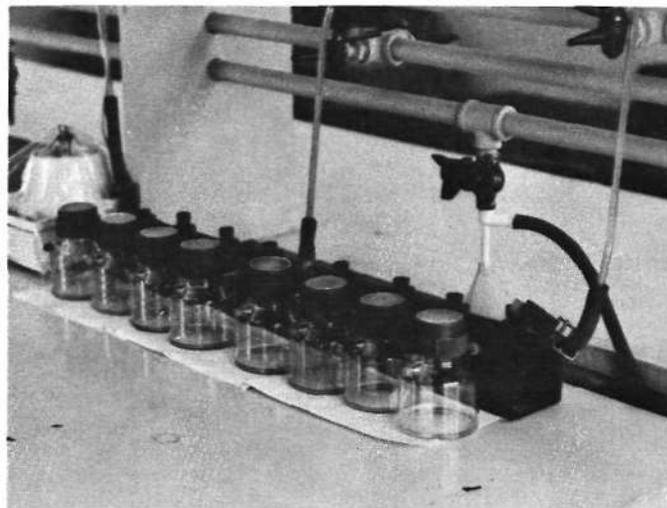


Figure 4. Acid-washing of membrane filters

ticulate matter. All weighings were made on a Cahn Gram Electrobalance[®] to a sensitivity of 0.01 mg.

Alkalinity and Hardness. The filtrate from the 100-ml subsample was titrated electrometrically to pH 4.5 for total alkalinity. Hardness was determined titrimetrically using EDTA and eriochrome black T indicator as described in Standard Methods.¹¹

Other Determinations. Chemical determinations performed on the substrate commencing on the day of inoculation and then again on the seventh day of incubation included: ammonia nitrogen by the phenate-hypochlorite method,¹² nitrate nitrogen by the chromotropic acid method,¹³ Kjeldahl nitrogen by the Kjeldahl method with titration of the distillate,¹¹ and orthophosphate, polyphosphate, and total phosphate by a number of modifications of the molybdenum blue method.^{1*}

RESULTS AND DISCUSSION

Algal Growth Measurements

Direct methods such as algal mass, packed cell volume, population counts, and pigment content, as well as indirect methods such as primary productivity and changes in the chemistry of the aqueous environment, have been used to express algal growth quantitatively. Various degrees of sophistication have been incorporated in each method, but none has satisfied all conditions. Platt and Rao,¹⁵ in their survey of the coast of Nova Scotia, concluded that no single parameter served as an ideal indication of the physiological vigor of a plant community. It seemed therefore advisable to try a variety of methods for ascertaining algal growth, if only to determine which method was best suited to the conditions imposed by the testing procedures.

Inoculum. As mentioned earlier, the inoculum used in the study was obtained from the Illinois River at Peoria. It contained mixed species of algae, and the distribution of the species was apparently seasonally dependent. Because of these characteristics there was a fundamental question to be

resolved regarding the effect of the mixed inoculum upon the reproducibility required for algal growth studies.

Traditionally single species algal cultures have been used for such investigations. The uniform composition is generally desirable for biochemical pathway studies, but is not necessarily desirable in many other cases. Johnson¹⁶ in his discussion on yeast fermentation works, stated that in many cases uniformity is "not particularly good for preparatory purposes." An important advantage of a mixed culture is its adaptability to differing aqueous environments where more than one organism species is available for potential growth dominance.

In an earlier study of the Illinois River¹⁷ the composition of plankton population was observed from March to October. A summary of these observations is depicted in figure 5. The majority of the population was made up of diatoms. Other types in order of abundance were flagellates, greens, and blue-greens.

During the AGP study, runs 8, 9, and 10 were planned

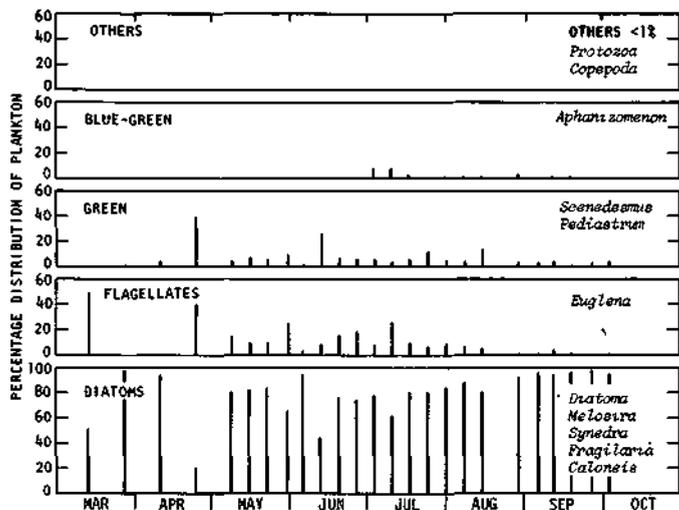


Figure 5. Seasonal distribution of plankton, Illinois River at Peoria

specifically to test the effect of natural inocula from four different sources in a water sample representative of a single source. Enumerations of organisms present in the inocula were made for runs 8 and 9 as shown in table 1. Diatoms were dominant in the lake and stream sources, whereas green algae and protozoa were the principal forms existing in the sewages. An analysis of variance performed on the assembled data (table 2) showed that there was no significant variation in the algal growth potential during the 3

Table 1. Composition of Various Inocula

| Inoculum | Classification | Genera | Composition (%) | |
|-------------------------|---------------------------|---------------------------|------------------|----|
| Run 8 | | | | |
| Lake Eureka | Diatom | <i>Fragilaria</i> | 95 | |
| Illinois River (Peoria) | Diatom | <i>Navicula</i> | 80 | |
| | Diatom | <i>Fragilaria</i> | 10 | |
| Tertiary sewage pond | Green | <i>Scenedesmus</i> | 10 | |
| | Green | <i>Chlorella</i> | 50 | |
| | Green | <i>Scenedesmus</i> | 30 | |
| | Green | <i>Coelastrum</i> | 10 | |
| Raw sewage | Protozoa | <i>Ciliates</i> | 10 | |
| | Green | <i>Oocystis</i> | 90 | |
| | Green | <i>Chlorella</i> | 5 | |
| Run 9 | | | | |
| Lake Eureka | Diatom | <i>Tabellaria</i> | 80 | |
| | Diatom | <i>Navicula</i> | 10 | |
| | Green | <i>Scenedesmus</i> | 5 | |
| | Protozoa | <i>Actinophrys</i> | 5 | |
| Illinois River (Peoria) | Diatom | <i>Navicula</i> | 30 | |
| | Protozoa | <i>Collodictyon</i> | 10 | |
| | Protozoa | <i>Loxophyllum</i> | 5 | |
| | Protozoa | <i>Stylonychia</i> | 25 | |
| | Green | <i>Chlorella</i> | 10 | |
| | Green | <i>Scenedesmus</i> | 5 | |
| | Green | <i>Ankistrodesmus</i> | 10 | |
| | Green | <i>Chlamydomonas</i> | 5 | |
| | Tertiary sewage pond | Flagellate | <i>Cyclidium</i> | 30 |
| | | Protozoa | <i>Colponema</i> | 5 |
| Green | | <i>Coelastrum</i> | 5 | |
| Green | | <i>Scenedesmus</i> | 10 | |
| Raw sewage | Green | (?) <i>Palmellococcus</i> | 30 | |
| | Blue-green | <i>Oscillatoria</i> | 20 | |
| Green | (?) <i>Palmellococcus</i> | 100 | | |

Table 2. Analysis of Variance of Inoculum Effect

| | Degrees of freedom | Mean squares | F-ratio |
|---------|--------------------|--------------|---------|
| Runs | 2 | 823 | 4.585* |
| Inocula | 3 | 89 | 0.496* |
| Error | 6 | 1795 | |

* Not significant at 95 percent level

runs for the 4 inocula used, F-ratios 4.585 and 0.496; otherwise the computed F-ratios would have exceeded the F-ratios required for significance (F-ratios of 19.33 and 8.94 at the 95 percent level, respectively), and the hypothesis that variations do not exist would not have held. It seems reasonable to conclude that if significant variations did not occur for AGP runs utilizing extremely diversified inocula, then there should be no question concerning the use of a more consistent but mixed inoculum as represented by figure 5.

Absorbance. Light absorption has long been used as an index of microorganism growth in a liquid medium. The method is rapid and easy. The precise wavelength at which absorbance of algal cultures is maximum has not been generally agreed upon: Staub¹⁸ used 610 nanometers, Eberly¹⁹ cited 650 nanometers, and Brown and Arthur²⁰ preferred 410 nanometers. Apparently differences in algal species, and particularly the pigment characteristics of each, caused the selection of differing wavelengths by these researchers. In this study the algal population of the inoculum, drawn from the Illinois River at Peoria, is naturally mixed and seasonally changing. Figure 6 shows that, except at terminal absorbance at the lower wavelengths, the maximum absorbance for 5- and 6-day-old cultures was 680 nanometers; the same wavelength was also found to give maximum absorbance for tertiary sewage pond inoculum. Thus a wavelength of 680 nanometers was chosen for determining the light absorption of algal cultures during this study.

Another question requiring resolution was whether or not light absorbance at the chosen wavelength showed a linear response to various algae concentrations. A calibration curve indicating the relationship of light absorption to varying

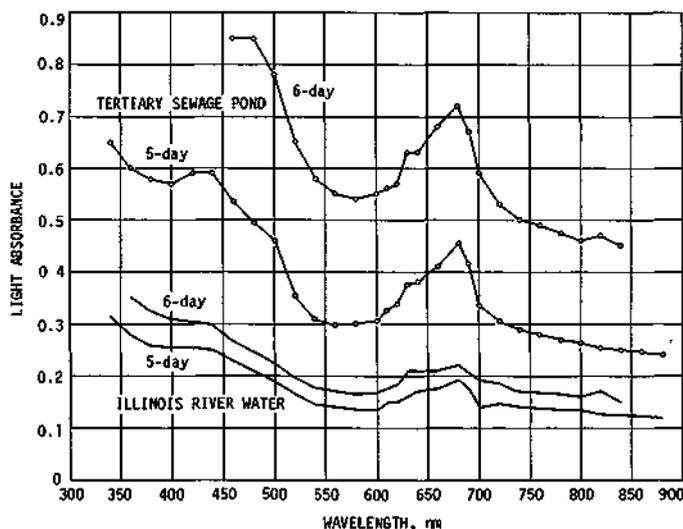


Figure 6. Light absorption spectrum of algal cultures

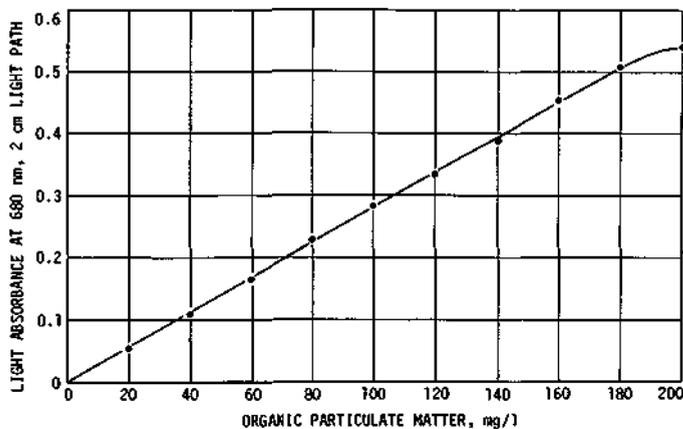


Figure 7. Calibration curve for light absorbance and organic particulate matter

algae concentration was prepared as shown in figure 7. The relationship was linear up to 180 mg/l of organic particulate matter with an absorbance of 0.500. Since the response leveled off at higher concentrations it seemed prudent to limit absorbance readings to 0.500.

Fluorescence. Fluorescence is a very sensitive method^{21,10} which should find its greatest application in oligotrophic waters. To test this method, a fluorescence spectrum was developed with an Aminco-Bowman spectrophoto-fluorometer. The results are depicted in figure 8. The optimum excitation and optimum emission were found to be 435 and 670 nanometers, respectively. This range generally coincided with the Kodak Wratten filters recommended by Bain.¹⁰ A calibration curve as shown in figure 9 indicates that the fluorescence response for an acetone extract is linear for all of the four apertures (1, X3, X10, and X30) used.

The fluorescence method, however, exhibited a distinct discrepancy in comparison with other methods, particularly the method related to particulate matter. Figures 10 and 11 show that algal growth expressed by organic particulate matter increased steadily with time, whereas that expressed by fluorescence in an acetone extract dropped off sharply; fluorescence for an unextracted sample leveled off with time.

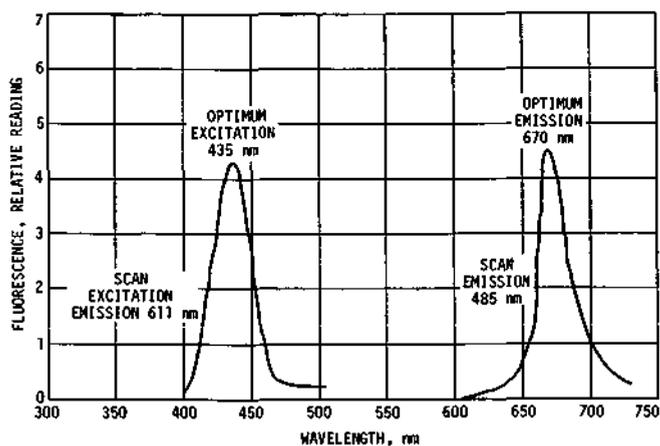


Figure 8. Fluorescence spectrum of algal extract, IP sample

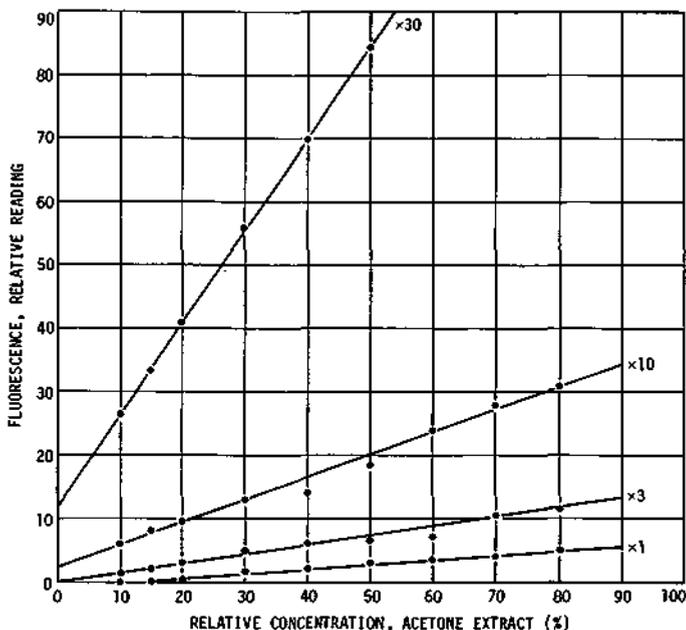


Figure 9. Calibration curve of fluorescence on acetone extract

This unlikely deviation is probably due to the incompleteness of the extraction process within a 24-hour period. This appeared to be the case from observations of residue in the centrifuge tubes. The extension of the extraction process beyond 24 hours, however, was not fruitful. According to

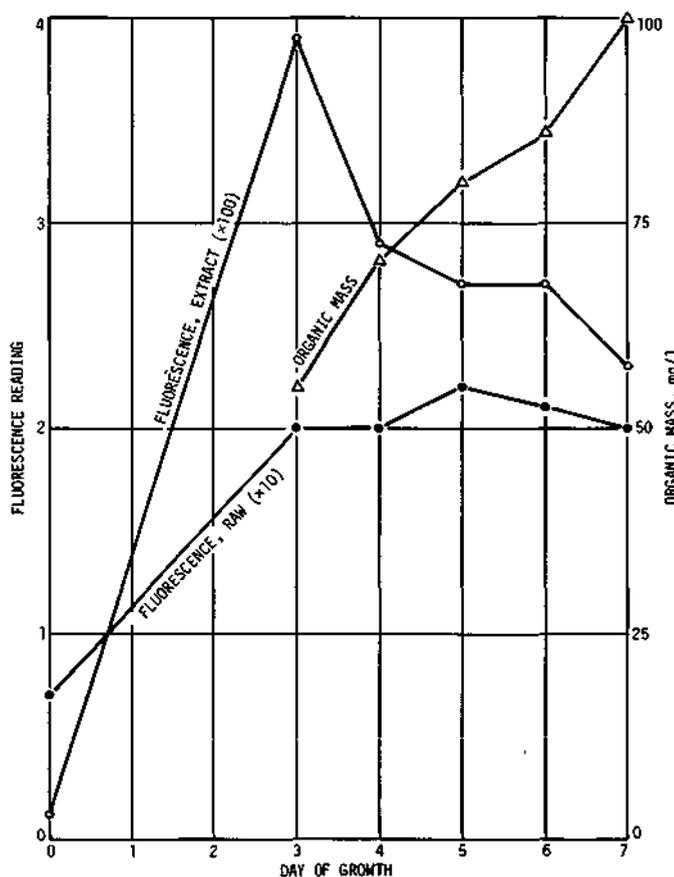


Figure 10. Algal growth expressed by various parameters, IP sample

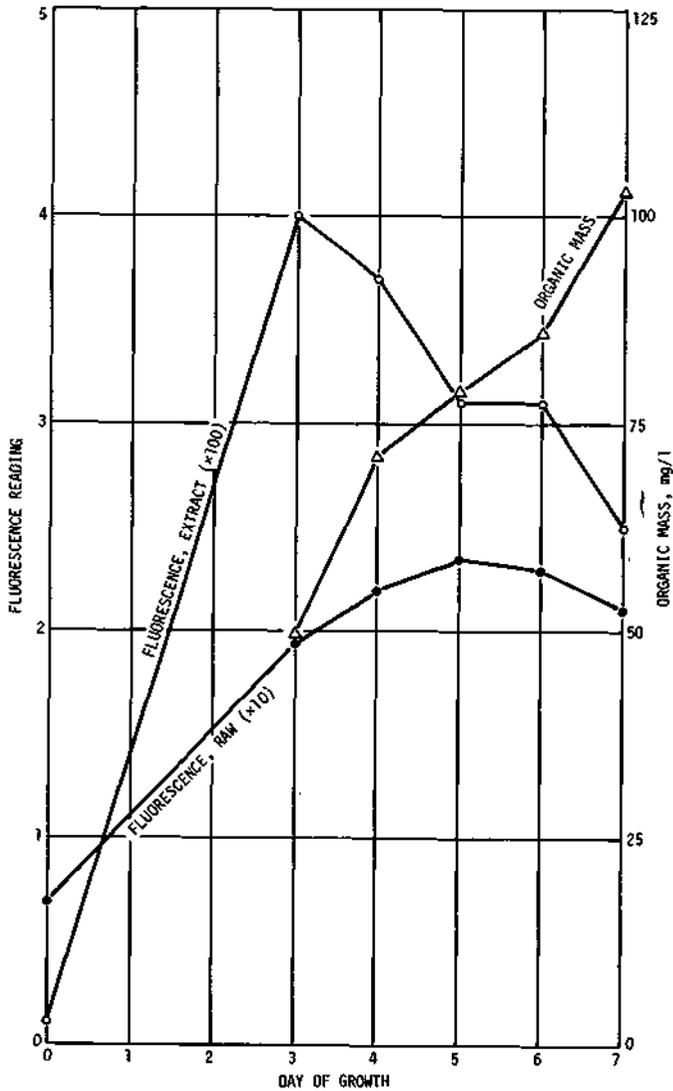


Figure 11. Algal growth expressed by various parameters, ID sample

Yentsch and Menzel²¹ and Bain,¹⁰ grinding of the tissue with an electric homogenizer is the only method that will insure nearly complete extraction of pigment from algal cells. This procedure was not attempted.

The deviation of the fluorescence method on unextracted samples may be due to variations in algal clump size, pigment content, and other natural conditions.

Particulate Matter. Since algal biomass has not been widely used as an indicator of algal growth, its merit was tested rather extensively. The first consideration involved the reliability of the weighing techniques. Table 3 shows that within the range of 29.1 to 186.3 mg/l of particulate matter, the experimental error was less than 5 percent, expressed by coefficients of variation. This range of experimental error included gravimetric determinations for total as well as organic particulate matter. Although the gravimetric determination for organic particulate matter consisted of three weighings (tare, total, and organic) in comparison with two weighings (tare and total) for the total particulate matter determination, there was no significant additional experimental error. Nevertheless it seems likely that experimental

Table 3. Reliability of Weighing Particulate Matter

| Particulate matter | Weight (mg/l) | Average (mg/l) | Coefficient of variation (%) |
|---|---------------|----------------|------------------------------|
| <i>Illinois River (Peoria)</i> Total (inorganic and organic) | 188.8 | 186.3 | 3.2 |
| | 180.8 | | |
| | 190.4 | | |
| Acid-washed (organic) | 82.9 | 85.7 | 2.1 |
| | 84.0 | | |
| | 88.2 | | |
| | 88.2 | | |
| <i>Illinois River (Peoria Dam)</i> Total (inorganic and organic) | 81.2 | 83.3 | 2.2 |
| | 82.0 | | |
| | 86.8 | | |
| Acid-washed (organic) | 26.4 | 29.1 | 4.8 |
| | 30.2 | | |
| | 30.8 | | |
| | 30.8 | | |
| <i>Illinois River (Kingston)</i> Total (inorganic and organic) | 178.2 | 181.4 | 1.2 |
| | 180.4 | | |
| | 185.6 | | |
| Acid-washed (organic) | 78.6 | 79.8 | 0.8 |
| | 81.6 | | |
| | 81.6 | | |
| | 79.2 | | |

error will increase at some low threshold concentration which would suggest a minimum limit of measurement. All the samples tested had an AGP well above the minimum limit.

The washing of algal residue on a membrane filter with 0.5 N hydrochloric acid is believed to be a new technique. This technique was employed when it became apparent that an inorganic carbonate precipitate increased with phytoplankton growth during photosynthesis in water samples representative of Illinois lakes and streams. The 0.5 N hydrochloric acid is of sufficient strength to totally dissolve the carbonate precipitate. There was some concern regarding whether or not the acid would rupture algae cells, with subsequent loss of filterable organic mass brought about by the dispersion of cellular contents. Such occurrences would subject the acid washing operation to serious error.

To test this possibility three concentrations of hydrochloric acid were used to dissolve the carbonate precipitate, i.e., 0.1 N, 0.5 N, and 1.0 N. In addition, various concentrations of sodium chloride were used to wash the residues. Presumably the sodium chloride solution would inflict plasmolytic damage to the algal cells, similar to that possibly done by the acid, but would not affect the carbonate precipitate. Results of this inquiry are shown in table 4. A comparison of the residual weights obtained after salt-washing (209.9, 208.3, and 218.1 mg/l) with the weight of the total particulate matter (219.8 mg/l) shows a variation of less than 5 percent, which was essentially within the range of experimental error. If we assume that the acid and salt solutions would have similar rupturing effects on the cells and that any material thus lost was of no significance, we may conclude that acid-washing would not significantly reduce the organic weight of the particulate matter. It was decided therefore to designate the acid-insoluble fraction of the particulate matter as organic cellular substance representative

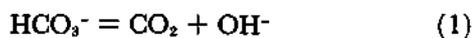
Table 4. Reliability Test of Acid-Washing Technique

| Particulate matter | Weight (mg/l) | Average (mg/l) | Coefficient of variation (%) |
|------------------------|---------------|----------------|------------------------------|
| Unwashed | 210 0 | 219 8 | 3 8 |
| | 217 6 | | |
| | 219 0 | | |
| | 216 0 | | |
| | 212.6 | | |
| | 219 2 | | |
| | 220 4 | | |
| | 218 8 | | |
| | 204 6 | | |
| | 224 4 | | |
| | 228 0 | | |
| | 224 2 | | |
| | 224.8 | | |
| | 225 4 | | |
| | 219.0 | | |
| | 212 4 | | |
| | 232 8 | | |
| | 227 0 | | |
| | 237 2 | | |
| | 211 8 | | |
| 209 2 | | | |
| Washed with 0 1 N HCl | 77 6 | 81 2 | 1 8 |
| | 83 2 | | |
| | 82 8 | | |
| | 79 2 | | |
| | 79 8 | | |
| | 80 8 | | |
| 0 5 N HCl | 79 2 | 79 9 | 0 5 |
| | 79 8 | | |
| | 80 8 | | |
| 1 0 N HCl | 81 2 | 81 8 | 0 9 |
| | 82 8 | | |
| | 81 4 | | |
| Washed with 0 1 N NaCl | 208 8 | 209 9 | 1 8 |
| | 215 0 | | |
| | 206 0 | | |
| | 208 8 | | |
| | 210 4 | | |
| | 205 8 | | |
| | 210 2 | | |
| | 224 0 | | |
| | 220 0 | | |
| | 220 0 | | |
| 0 5 N NaCl | 208 8 | 208 3 | 0 8 |
| | 210 4 | | |
| | 205 8 | | |
| | 210 2 | | |
| 1 0 N NaCl | 210 2 | 218 1 | 1 7 |
| | 224 0 | | |
| | 220 0 | | |
| | 220 0 | | |

of algae, and the acid-soluble fraction as inorganic carbonate.

Alkalinity and Hardness. During incubation minor exchanges of gases between the media and atmosphere were possible through the polyurethane stopper. No effort was made to aerate the cultures or to charge the media with carbon dioxide. All of the water sample sources were relatively high in both alkalinity and hardness, and at the initial pH levels the alkalinity was believed to be predominantly of the bicarbonate (HCO₃⁻) species. The role of carbon in algal growth has been reported recently by several researchers.^{22,23,24} During this study, algae were believed to have used dissolved bicarbonate as a major source of carbon for organic synthesis. The process is analogous to the formation of marl by submerged aquatic macrophytes, as the readily soluble bicarbonate is changed to the less soluble carbonate following the uptake of carbon dioxide.

Ruttner²⁵ suggested two pathways for the utilization of carbon dioxide by algae from soluble bicarbonates:



and



Table 5. Correlations between Methods of Measuring AGP

| | Absorbance | Fluorescence | Organic mass | Inorganic mass | Alkalinity |
|--|------------|--------------|--------------|----------------|------------|
| Lake Eureka (degrees of freedom—33) | | | | | |
| Organic mass | 0 934* | | | | |
| Inorganic mass | 0 767* | | 0 858* | | |
| Alkalinity | 0 359** | | 0 288 | 0 326 | |
| Hardness | 0 390** | | 0 924* | 0 320 | 0 961* |
| Lake Michigan (degrees of freedom—68) | | | | | |
| Fluorescence | 0.669* | | | | |
| Organic mass | 0 806* | 0 612* | | | |
| Inorganic mass | 0 566* | 0 420* | 0 785* | | |
| Alkalinity | 0 658* | 0 484* | 0 750* | 0 755* | |
| Hardness | 0 582* | 0 498* | 0 617* | 0 669* | 0 913* |
| Lake Bloomington (degrees of freedom — 73) | | | | | |
| Fluorescence | 0 878* | | | | |
| Organic mass | 0 862* | 0 866* | | | |
| Inorganic mass | 0 363* | 0 390* | 0 368* | | |
| Alkalinity | 0 636* | 0 532* | 0 614* | 0 694* | |
| Hardness | 0.692* | 0 557* | 0 628* | 0 725* | 0.944* |
| Money Creek (degrees of freedom — 73) | | | | | |
| Fluorescence | 0 907* | | | | |
| Organic mass | 0 829* | 0 772* | | | |
| Inorganic mass | 0 485* | 0 260** | 0 333* | | |
| Alkalinity | 0 479* | 0 309* | 0 351* | 0.850* | |
| Hardness | 0 515* | 0 350* | 0 405* | 0 836* | 0 935* |
| Mackinaw River (degrees of freedom — 78) | | | | | |
| Fluorescence | 0 863* | | | | |
| Organic mass | 0 895* | 0 863* | | | |
| Inorganic mass | 0 484* | 0 265** | 0 384* | | |
| Alkalinity | 0 379* | 0.173 | 0 259** | 0 770* | |
| Hardness | 0 177 | 0 097 | 0 128 | 0 271** | 0.344* |
| Six-Mile Creek (degrees of freedom — 73) | | | | | |
| Fluorescence | 0 737* | | | | |
| Organic mass | 0 624* | 0 418* | | | |
| Inorganic mass | 0 426* | -0 013 | 0 445* | | |
| Alkalinity | 0 462* | 0 229 | 0 452* | 0 672* | |
| Hardness | 0 511* | 0 334* | 0 372* | 0 481* | 0.975* |
| Illinois River at Peoria (degrees of freedom — 78) | | | | | |
| Fluorescence | 0 924* | | | | |
| Organic mass | 0 812* | 0 786* | | | |
| Inorganic mass | 0 648* | 0 643* | 0 693* | | |
| Alkalinity | 0 682* | 0 722* | 0 410* | 0.908* | |
| Hardness | 0 669* | 0 719* | 0 694* | 0 902* | 0 972* |
| Illinois River at Peoria Dam (degrees of freedom -78) | | | | | |
| Fluorescence | 0 881* | | | | |
| Organic mass | 0 820* | 0 791* | | | |
| Inorganic mass | 0 491* | 0 502* | 0 682* | | |
| Alkalinity | 0 725* | 0 562* | 0 660* | 0 882* | |
| Hardness | 0 551* | 0 576* | 0 613* | 0 852* | 0 924* |
| Illinois River at Kingston (degrees of freedom — 78) | | | | | |
| Fluorescence | 0 559* | | | | |
| Organic mass | 0 843* | 0 494* | | | |
| Inorganic mass | 0 612* | 0 235** | 0 633* | | |
| Alkalinity | 0 674* | 0 293* | 0 606* | 0 892* | |
| Hardness | 0 650* | 0 292* | 0 625* | 0 919* | 0 940* |
| Tertiary Sewage Pond (degrees of freedom — 18) | | | | | |
| Organic mass | 0 748* | | | | |
| Inorganic mass | 0 674* | | 0 821* | | |
| Alkalinity | 0 620* | | 0 889* | 0 860* | |
| Hardness | 0 687* | | 0 929* | 0 907* | 0 920* |
| Raw Sewage (degrees of freedom -18) | | | | | |
| Organic mass | 0 910* | | | | |
| Inorganic mass | 0 687* | | 0 564* | | |
| Alkalinity | 0.656* | | 0 831* | 0 928* | |
| Hardness | 0 307 | | 0 553** | 0 824* | 0 867* |

* Significant at 99 percent level
 ** Significant at 95 percent level

Under the conditions of this study, either route could have been functioning. The mechanism suggested by equation 2 is in good agreement with the observed data.

Correlation of Methods. With the exception of the fluorescence procedures, correlations were made between all methods for all water sources examined. Fluorescence measurements were limited to the waters of Lake Eureka, the tertiary sewage pond, and raw sewage samples. Only unextracted samples were used in the correlation analyses. A summary of the correlation coefficients is set forth in table 5. Six of the 11 sources investigated, i.e., Lake Michigan, Lake Bloomington, Money Creek, Illinois River at Peoria, Illinois River at the Peoria Lock and Dam, and the tertiary sewage pond, showed excellent correlation for all methods at the 99 percent significance level. In fact, of the correlation coefficients developed for the 11 sources and 6 parameters, 52 concerning absorbance had 2 below the 95 percent level of significance (Mackinaw River, absorbance:hardness 0.177; raw sewage, absorbance:hardness 0.307). Similarly, of 52 correlation coefficients for organic mass, 2 were less than the 95 percent level of significance (Lake Eureka, organic mass:alkalinity 0.288; Mackinaw River, organic mass:hardness 0.128). For all other comparisons, no more than 5 coefficients failed to achieve a 95 percent significance level. In these instances the lack of comparison appeared to be related to the source as much as to the methods.

In reaching a decision on the most meaningful method for determining algal growth, it seemed desirable to select a direct method over an indirect one, e.g., gravimetric rather than alkalinity determinations. Obviously a basic requirement was that the method be reproducible, and also that it be independent of the water chemistry of the sources as well as of the type of inoculum used. For these reasons, principally, the use of organic mass by gravimetric means appeared superior to all other methods explored.

Algal Growth Characteristics

Growth Rate. Algal growth rates were determined by gravimetric methods applied to organic mass. A graphic representation of typical growth rates observed for natural cultures of algae in samples of the water from one source is shown in figure 12. Daily growth maxima occurred in most cases from 3 to 5 days after inoculation; some maxima did occur, however, within 2 to 4 days. After peaking, the growth rate decreased substantially but did not completely cease, as illustrated in figure 13. The uptake of nitrogen was most pronounced during the initial 7 days, after which, according to Strickland et al.²⁶ and Foree,²⁷ carbohydrate and lipid assimilation are presumed to dominate.

The algal population was able to acclimate to the natural water substrates and attain its growth maxima within 7 days. Since this was the period encompassing the greatest nutrient uptake and the maximum rate of production of organic matter, the 7-day incubation period was selected for the measurement of AGP. An extension beyond 7 days could be

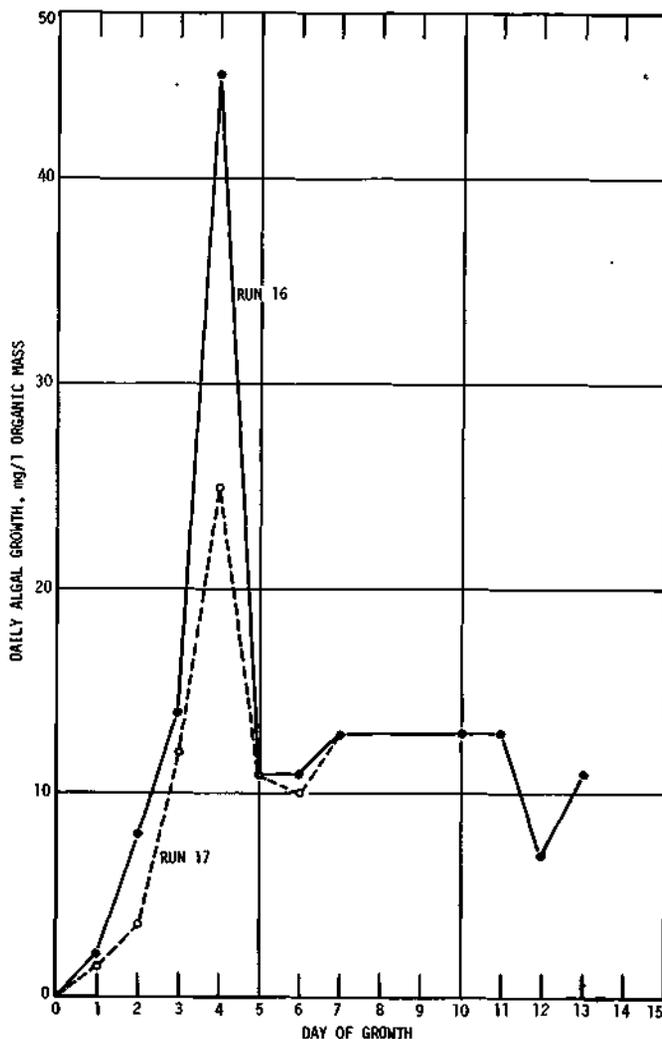


Figure 12. Algal growth rates, IP sample

expected to shift the primary mode of metabolism from protein synthesis to carbohydrate and lipid production, to increase the chance of zooplankton influence, to permit a shift to secondary climax species, and to allow some degree of decomposition and nutrient recycling.

Nitrogen and Phosphorus. Typical changes which occurred in nutrient content during algal growth in Illinois River water samples are depicted in figure 14. Here the ammonium-N level is shown to have dropped from 4.65 mg/1 to 0.6 mg/1 in 5 days and below a level of detection within 7 days. Nitrate-N, however, remained essentially unchanged through the first 5 days and notably diminished only after the sharp reduction of ammonium-N. These data, for the basal levels which are typical of Illinois River water, suggest that ammonium is the preferred source of nitrogen and that nitrate is not utilized until the ammonium is depleted. Similar findings have been reported for marine²⁶ and freshwater²⁸ plankton.

The major uptake of phosphate-P was also found to occur within 5 days with little subsequent utilization.

The mean atom ratio of total nitrogen to total phosphorus initially observed in water samples from the Illinois

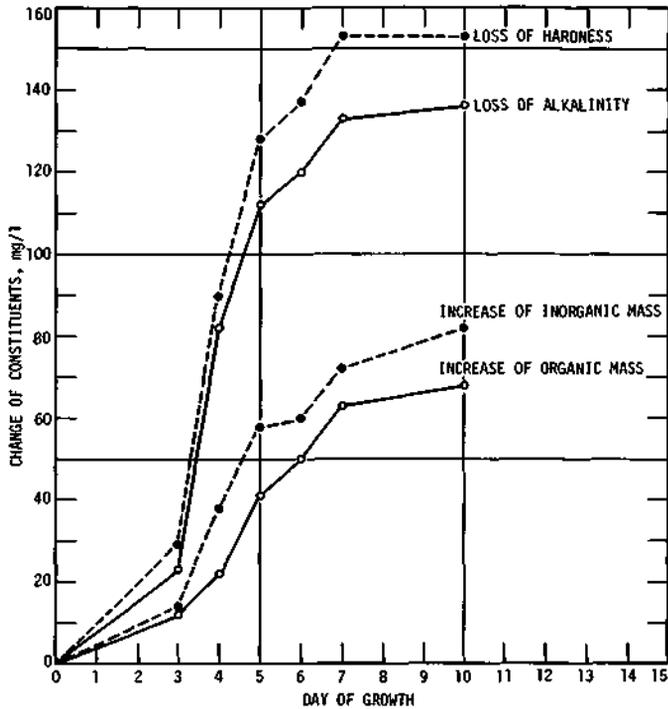


Figure 13. Parameters quantifying algal growth, IP sample

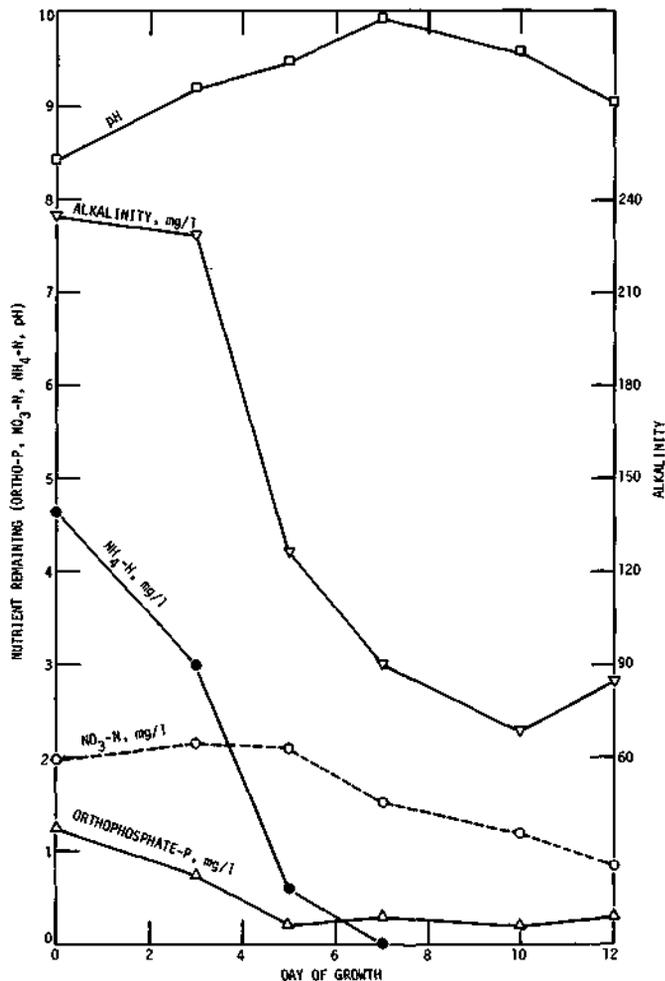


Figure 14. Nutrient concentration changes with algal growth, IP sample

River at Peoria was 8.4:1. This ratio is close to the working value of N:P atom ratio in the natural algal population reported by Ryther and Dunstan.²⁹ After the 7-day incubation period, the N:P atom ratio in the medium was found to be 220:1. The results support the contention of many researchers that algal cells possess the capability of accumulating phosphorus within their cell structure considerably in excess of their immediate nutritional requirements. It is thus unlikely that the low phosphorus concentration in the medium after 7 days was the primary cause of limiting algal growth. Phosphorus requirements could be satisfied by the regeneration of phosphorus from decomposing algal cells,²⁹ and such regeneration occurs more rapidly than the similar mechanism for ammonium regeneration. It is probable that a steady state condition was reached between the regeneration and algal uptake of phosphorus.

Alkalinity and Hardness. The general range of average alkalinity concentrations encountered at 8 of the natural water sources examined was 180-280 mg/l as CaCO₃; similarly the range of average hardness was 250-350 mg/l as CaCO₃. Lake Michigan waters exhibited a rather constant concentration of 115 mg/l and 130 mg/l of hardness and alkalinity as CaCO₃, respectively. The two sewage sources displayed considerable variation, reaching as high as 480 mg/l of hardness and 400 mg/l of alkalinity. Generally, these constituents were found in a lesser concentration in the raw sewage than in the tertiary sewage pond. The predominant form of alkalinity existing in midwestern waters is bicarbonate (HCO₃⁻), which as previously cited, may be a source of carbon for algal metabolism. In theory the reduction of alkalinity and hardness would proceed equally during progressive algal growth. Figures 15 and 16 show the loss of alkalinity compared with the loss of hardness for a 7-day incubation period for 10 water sources. The data in

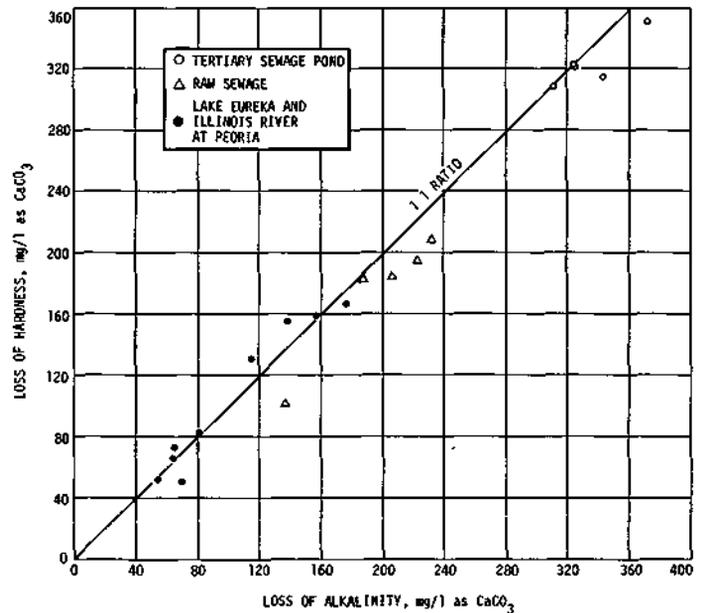


Figure 15. Loss of dissolved alkalinity vs dissolved hardness, LE, IP, RS, and TSP samples

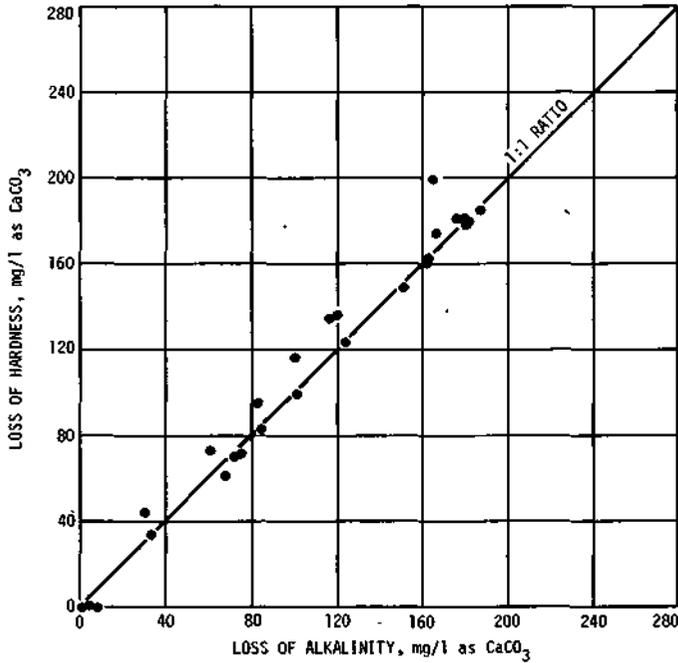


Figure 16. Loss of dissolved alkalinity vs dissolved hardness, LM, LB, MC, MR, SMC, and IP samples

figure 15 have been presented separately because these runs included samples of water from sewage sources.

The data are generally scattered along the line of equality. It is apparent in figure 15 that the distribution of the two sewage sources is invariably on or below the line. This indicates a loss of alkalinity in excess of the loss of hardness. The distribution of values from the eight natural water sources is generally on or above the line as shown in figures 15 and 16.

The array of the sewage samples may be attributable to an essential difference between sewage and natural waters. In sewage, there are organic acids such as formic, acetic, and propionic as well as more complex carbohydrates³⁰ readily available as carbon sources for algal growth. They may also contribute to the buffering capacity of the sample. In the determination for alkalinity by titration, it is assumed that the titration is of the carbonic species alone. The presence of organic acids and their salts introduces a degree of buffering capacity that must be overcome in the titration. The alkalinity titration may in this instance be taken as a measurement reflecting *apparent alkalinity* — a combination of the carbonic species and other titratable compounds. Any deviation from the true value would place the net alkalinity at a higher level than the net hardness titration would suggest. The distance below the line of equality may indicate the extent to which simple organic acids were used by the algae. The data distribution above the line for the natural waters is not so easily reasoned.

The relationship between the initial concentrations of alkalinity and hardness with the loss of these constituents during 7 days was generally linear and a positive function. That is, the higher the initial concentrations, the greater the loss.

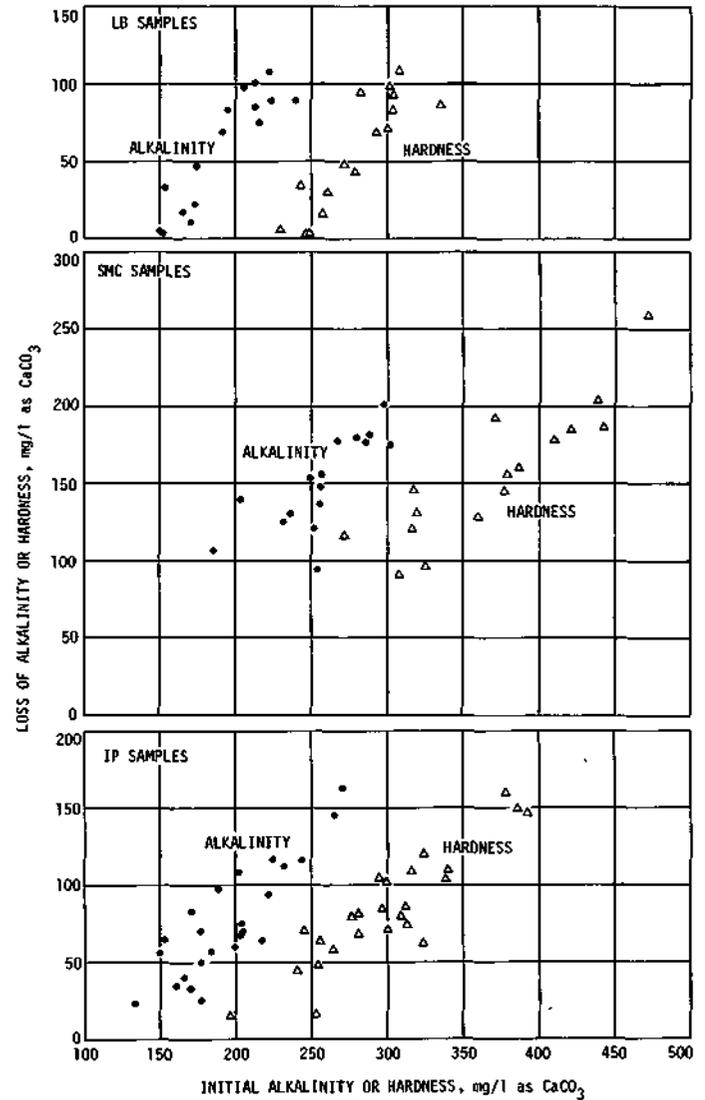


Figure 17. Relation between initial alkalinity or hardness and its decrease in 7 days

This relationship is depicted for 3 sources in figure 17, and the regression equations expressing this dependence mathematically are summarized in table 6 for all the natural sources investigated. These relationships for Lake Michigan and Lake Eureka waters are obscure. In the case of Lake Michigan the initial concentrations of alkalinity and hardness never exceeded 150 mg/l (CaCO_3). This could be the threshold limit below which a significant correlation between initial concentrations and loss could no longer be expected. The lack of a significant relationship between initial alkalinity and hardness and loss for Lake Eureka may be attributed to a generally poor growth response.

Why there should be a loss dependent upon the initial concentration is not clear. The implication is that the initial concentration of alkalinity has a significant effect upon algal metabolism because the major loss of this constituent is due to primary production. It is not reasonable to assume that the alkalinity, in the range observed during this study, is a

Table 6. Loss of Alkalinity and Hardness as a Function of Initial Alkalinity and Hardness

| | Regression equation* | r | n |
|------------------------------------|-----------------------|------|----|
| Lake Eureka | | | |
| Alkalinity | $Y = -1.40 + 0.36 X$ | 0.53 | 9 |
| Hardness | $Y = -2.32 + 0.31 X$ | 0.34 | 7 |
| Lake Michigan | | | |
| Alkalinity | $Y = -1.895 + 1.71 X$ | 0.65 | 14 |
| Hardness | Y = No correlation | | |
| Lake Bloomington | | | |
| Alkalinity | $Y = 1.385 + 1.05 X$ | 0.86 | 14 |
| Hardness | $Y = 1.761 + 0.85 X$ | 0.78 | 12 |
| Money Creek | | | |
| Alkalinity | $Y = -7.84 + 0.83 X$ | 0.79 | 15 |
| Hardness | $Y = -8.74 + 0.63 X$ | 0.81 | 15 |
| Six-Mile Creek | | | |
| Alkalinity | $Y = -3.39 + 0.72 X$ | 0.76 | 16 |
| Hardness | $Y = -9.39 + 0.68 X$ | 0.88 | 16 |
| Mackinaw River | | | |
| Alkalinity | $Y = -5.18 + 0.74 X$ | 0.84 | 17 |
| Hardness | $Y = -30.6 + 0.50 X$ | 0.66 | 17 |
| Illinois River (Peoria) | | | |
| Alkalinity | $Y = -1.140 + 1.22 X$ | 0.95 | 25 |
| Hardness | $Y = -7.26 + 0.69 X$ | 0.89 | 25 |
| Illinois River (Peoria Dam) | | | |
| Alkalinity | $Y = -7.27 + 0.98 X$ | 0.94 | 16 |
| Hardness | $Y = -7.05 + 0.68 X$ | 0.96 | 16 |
| Illinois River (Kingston) | | | |
| Alkalinity | $Y = -9.99 + 1.11 X$ | 0.97 | 16 |
| Hardness | $Y = -8.70 + 0.72 X$ | 0.96 | 16 |

* $Y = a + b X$
 $Y =$ loss in 7 days; $X =$ initial concentration; $a =$ constant; $b =$ slope
 Note: $r =$ correlation coefficient; $n =$ number of samples

limiting source of carbon. It is possible that other growth limiting factors are closely related to, and thus vary with, the concentration of alkalinity in the waters investigated.

Inorganic and Organic Mass. As mentioned earlier, the total mass of the algal culture during progressive growth was made up of two components, i.e., inorganic and organic material. The inorganic mass was of carbonate origin, as ascertained by the use of 0.5 N hydrochloric acid. The organic mass was that residue remaining after acid treatment.

A review of all the data indicated apparent variations in the ratios of the inorganic mass to the organic mass (mass ratio). The mass ratios ranged from 0.24 to 15.36 with an average of 2.24. A two-way analysis of variance (table 7) indicated that a variation of mass ratios did indeed exist when comparing the sources collectively, as indicated by the highly significant level of variation (99 percent, F-ratio 4.39) of the combined sources. Table 7 also indicates that a similarity exists between some sources. For example, the analysis indicated that the chances were 99 out of 100 that no variation existed between the mass ratios determined for Money Creek, Mackinaw River, and Six-Mile Creek, all in the Mackinaw River basin. Similarly, the analysis indicated that the three Illinois River sources could be considered nonvariable from the standpoint of the mass ratios. In the case of the Mackinaw River basin the mass ratio ranged from 0.80 to 5.29 with an average of 2.90, and for the Illinois River the range was 0.73 to 3.52 with an average of 1.72 (table 8).

The higher the numerical value of the mass ratio, the less

Table 7. Two-Way Analysis of Variance of Inorganic Mass to Organic Mass

| | Degrees of freedom | Mean squares | F-ratio |
|---|--------------------|--------------|---------|
| Combined sources (excluding LE, RS, TPS) | | | |
| Sources | 7 | 9.20 | 4.40* |
| Runs | 12 | 5.78 | 2.77* |
| Error | 84 | 2.09 | |
| MC, MR, and SMC | | | |
| Sources | 2 | 0.73 | 0.89 |
| Runs | 12 | 3.52 | 4.29* |
| Error | 24 | 0.82 | |
| IP, ID, and IK | | | |
| Sources | 2 | 0.13 | 1.08 |
| Runs | 12 | 0.69 | 5.75* |
| Error | 24 | 0.12 | |

*Significant at 99 percent level

Table 8. Atomic Ratio of Organic Carbon Synthesized to Inorganic Carbon Precipitated in 7 Days

| Source | Mass ratio I/O | Atom ratio IC/OC | C/C_p * |
|-----------------------------|---------------------|-----------------------|-----------|
| Lake Eureka | 1.910 | 0.458 | 2.2 |
| Lake Michigan | 0.896 | 0.215 | 4.7 |
| Lake Bloomington | 3.813 | 0.915 | 1.1 |
| Money Creek | 3.167 | 0.760 | 1.3 |
| Mackinaw River | 2.708 | 0.650 | 1.5 |
| Six-Mile Creek | 2.832 | 0.680 | 1.5 |
| Illinois River (Peoria) | 1.626 | 0.390 | 2.6 |
| Illinois River (Peoria Dam) | 1.823 | 0.437 | 2.3 |
| Illinois River (Kingston) | 1.711 | 0.411 | 2.4 |
| Raw sewage | 1.311 | 0.315 | 3.2 |
| Tertiary sewage pond | 1.712 | 0.411 | 2.4 |

* $C/C_p =$ Atom C synthesized/Atom C precipitated

effective the biosynthetic mechanisms of the culture became, i.e., more inorganic than organic material was produced. To put this conclusion in proper perspective and on a common basis, carbonate values (inorganic mass) were converted by computation to carbon by use of the ratio 0.12 of the atomic weight of carbon to calcium carbonate. The values obtained were considered illustrative of the carbon precipitated. Although the elemental composition of algae varies with species makeup, nutrition, age, etc., the carbon content, on a dry ash-free basis, is remarkably constant, varying between 45 and 55 percent.³¹ A value of 0.50 was used to mathematically convert the organic mass to a carbon which was considered representative of the organic carbon synthesized. The results of this computation are summarized in table 8.

For the lake waters, the atoms of carbon synthesized for every atom of carbon precipitated as carbonate varied from 1.1 in Lake Bloomington to 4.7 in Lake Michigan. For the stream sources, an average of 1.5 was derived for the Mackinaw River basin (MC, MR, SMC) and an average of 2.4 for the Illinois River (IP, ID, IK). The raw sewage and the tertiary sewage pond sources averaged 3.2 and 2.4, respectively. Megard³² has reported on several natural lake waters in north central Minnesota which suggest values ranging from 2.5 to 10.0 atoms of carbon synthesized per atom of carbon precipitated.

Since the streams in the Mackinaw River basin and the

Illinois River have shown distinct numerical differences in the quantity of carbon synthesized to that precipitated, i.e., 1.5 vs 2.4, it would seem worthwhile to mention the principal differences in their water quality. The alkalinity of the Mackinaw River basin is significantly higher than that of the Illinois River; the nutritional aspects of the Illinois River, as measured by ammonia-N and phosphate-P concentrations, are more favorable for algal growth than that for the streams in the Mackinaw River basin. It is probable that these factors contribute to the higher biosynthetic efficiency displayed by the Illinois River. An explanation of differences between Lake Bloomington and Lake Michigan samples is not possible on the basis of the existing data.

Algal Growth Potential

Algal growth potential (AGP) is here defined as the algal or organic mass resulting from a 7-day incubation of a culture grown on a natural water substrate under standardized laboratory conditions and expressed as milligrams of dry organic mass per liter of sample. Organic mass as a measure of AGP was chosen because it was, for all sample sources, the most reliable parameter.

Table 9 summarizes the AGPs of the sources examined, the number of runs performed, and the associated coefficients of variation. The lowest AGP was found in Lake Eureka at 12 mg/l followed closely by Lake Michigan and Lake Bloomington waters with average AGPs of 13 and 19 mg/l, respectively. The AGPs for Money Creek, Mackinaw River, and Six-Mile Creek were 34, 41, and 51 mg/l, respectively. The values for these streams, all in the same river basin, are relatively close. The potentials for the Illinois River samples were 77, 82, and 82 mg/l. The highest AGP values found were 120 and 135 mg/l for raw sewage and tertiary sewage pond sources, respectively.

An analysis of variance was applied to test the AGP relationships of the various sources. The results on 13 runs involving 8 sample sources are shown in table 10. In comparing all stations, it was found they could not be considered to be of the same origin but were quite diverse. However, a similar analysis defining mass ratio relationships showed that the variation between the 3 streams in the Mackinaw River

Table 9. Algal Growth Potential of Water and Sewage Samples

| Source | Number of runs | Mean AGP (mg/L) | Coefficient of variation (%) |
|-------------------------------|----------------|-----------------|------------------------------|
| Lake Eureka | 9 | 12 | 103 |
| Lake Michigan (Chicago) | 14 | 13 | 38 |
| Lake Bloomington | 16 | 19 | 54 |
| Money Creek | 16 | 34 | 51 |
| Mackinaw River | 17 | 41 | 48 |
| Six-Mile Creek | 16 | 51 | 31 |
| Illinois River (Peoria) | 25 | 77 | 30 |
| Illinois River (Peoria Dam) | 16 | 82 | 22 |
| Illinois River (Kingston) | 16 | 82 | 25 |
| Raw sewage (Washington) | 5 | 120 | 32 |
| Tertiary sewage pond (Peoria) | 5 | 135 | 7 |

Table 10. Analysis of Variance of Algal Growth Potential

| | Degrees of freedom | Mean squares | F-ratio |
|---|--------------------|--------------|---------|
| <i>Combined sources (excluding LE, RS, and TPS)</i> | | | |
| Sources | 7 | 10,018 | 51 11* |
| Runs | 12 | 10,402 | 53 07* |
| Error | 84 | 196 | |
| <i>MC, MR, and SMC</i> | | | |
| Sources | 2 | 688 27 | 3 18 |
| Runs | 12 | 1171 | 5 58* |
| Error | 24 | 210 | |
| <i>IP, ID, and IK</i> | | | |
| Sources | 2 | 50 0 | 0 79 |
| Runs | 12 | 1223 | 19 34* |
| Error | 24 | 63 23 | |
| <i>LM and LB</i> | | | |
| Sources | 1 | 40 1 | 9 44* |
| Runs | 12 | 68 58 | 16 14* |
| Error | 12 | 42 50 | |

*Significant at 99 percent level

basin was not significant and that they could be considered of the same origin. Similarly, the 3 sources on the Illinois River were found to be representative of the same origin. This would suggest that a meaningful relationship might exist between the AGP and the mass ratio of the same water source. Although the mean AGPs for the lake sources were numerically similar, the analysis of variance showed them to be distinctly different and variable.

Seasonal Changes. The similarity among certain source's is apparent also in the seasonal fluctuations of AGP. These changes are depicted in figures 18 and 19. For the streams located in the Mackinaw River basin (figure 18), a peak occurring on September 30, 1970, was followed by a relatively low AGP period until late February 1971. Two minor peaks occurred in late May and July of that year. Of particular interest was the pattern similarity for the 3 sources; also the maximum AGP seemed to occur preceding periods of runoff.

AGPs for Lake Michigan and Lake Bloomington (figure 18) depict a different pattern, with Lake Michigan producing a rather constant AGP throughout the study period. This would suggest that Lake Michigan is not as sensitive to seasonal fluctuations, whether they be influenced by temperature changes, nutrient input, or other factors. The 3 Illinois River sources, all within a 15-mile reach, behaved very much alike (figure 19). A plateau of relatively high AGPs occurred from October 1970 to April 1971. A review of the chemical history of the river shows that nutrient concentrations, particularly $\text{NH}_4 - \text{N}$, are considerably higher in this area of the river during low temperature periods. The nitrification capability of the river during summer months has been well documented.³³ With biological activity lessened during the cooler period (October to April) and continuing flows of treated waste effluents being discharged upstream, it may be expected that any nutrients added would remain essentially intact during their downstream passage.

The length of record for the sewage sources was not sufficient to permit speculation on seasonal effects.

Nutrient Levels. There is a tendency to assume that the

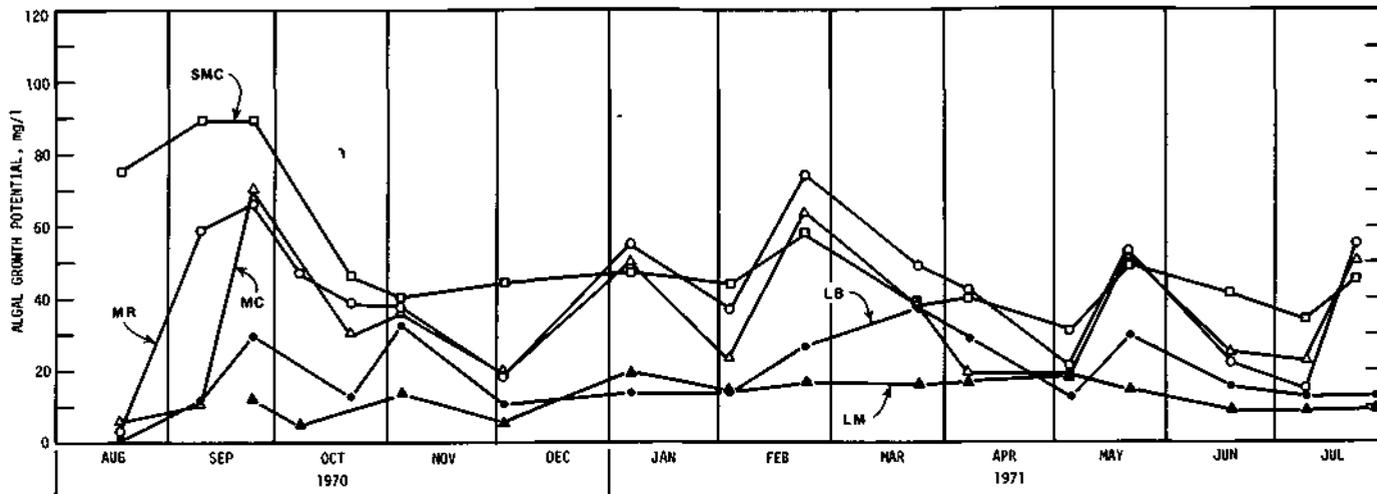


Figure 18. Seasonal change of AGP, Mackinaw River basin and lake sources

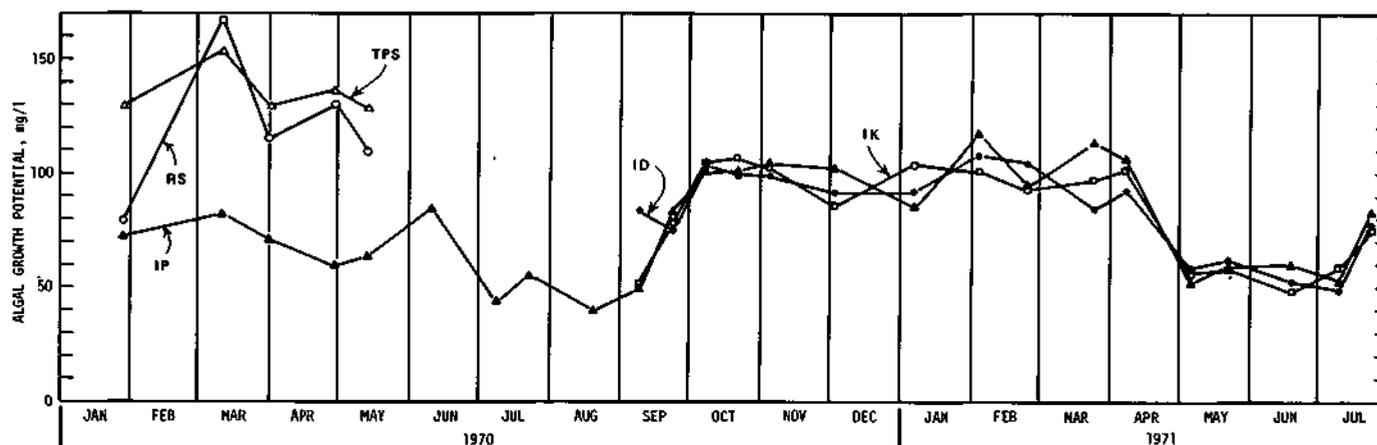


Figure 19. Seasonal change of AGP, Illinois River and sewage sources

variation of AGP, as determined under standardized laboratory conditions, reflects the nutrient level of the water source under examination. Among the nutrient elements essential for algal growth, nitrogen and phosphorus are generally regarded as limiting factors in most waters. Where a water source is particularly low in alkalinity, carbon is suspected of being a limiting nutritional factor.^{22,23,24} The mean concentrations of ammonium nitrogen, nitrate nitrogen, orthophosphate-phosphorus, and alkalinity as CaCO₃ for the water sources examined are shown in figure 20.

Average ammonium nitrogen levels varied from 0.07 mg/l for Lake Michigan to 15.7 mg/l for the raw sewage source. Average nitrate nitrogen levels ranged from 0.37 mg/l for Lake Michigan to 10 mg/l for Six-Mile Creek. That nitrate, like NH₄ — N, is a nutrient source is shown in figure 14; but on occasion nitrate in high concentrations has been shown to inhibit algal growth.³⁴ However, a maximum nitrate nitrogen level of 11.8 mg/l in Six-Mile Creek had no apparent detrimental effect upon the AGP at that source.

The alkalinity of the various water sources would appear to discount the possibility of carbon availability being a growth limiting factor.

Orthophosphate-P varied from less than 0.01 for Lake Michigan to 6.1 mg/l for the tertiary sewage pond. Some

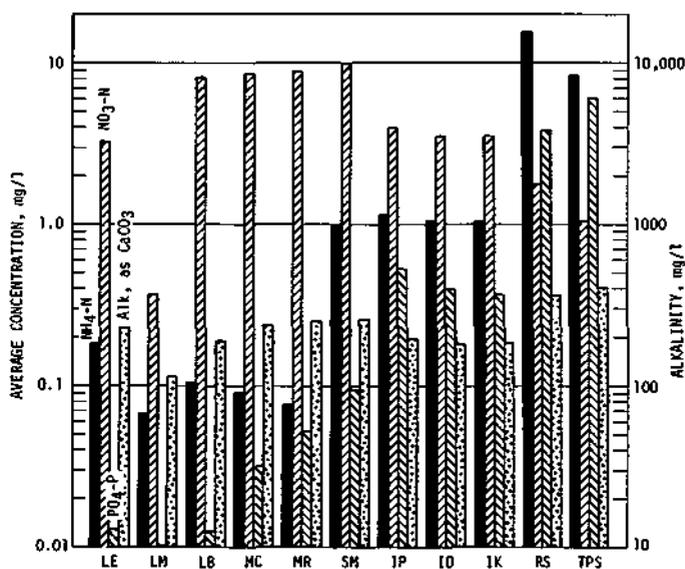


Figure 20. Nutrient levels of water sources

Lake Michigan samples were below the limit of detection for orthophosphate-P. Chu³⁵ has reported that 0.05 mg/l is a critical concentration for algal growth. In consideration of the experience of Clasen and Bernhardt³⁶ and the work of Hutchinson³⁷ in which the transitions of phosphorus from

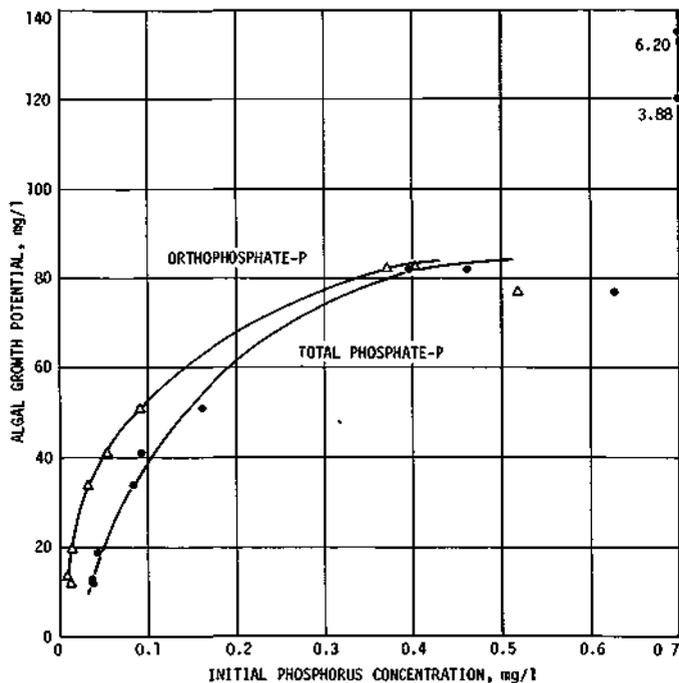


Figure 21. Effect of initial phosphorus concentration on AGP

one form to another are cited, a comparison of total phosphate-P, along with orthophosphate-P, to AGP was made, as depicted in figure 21. Although there appears to be an exponential relationship between initial phosphorus content and AGP up to 0.4 mg/l, any comparison thereafter appears meaningless. In general, a conclusion might be reached that phosphorus in orthophosphate form is more readily utilized for algal growth than other forms at concentrations lower than 0.4 mg/l.

Application. Although specific relationships between algal growth potential and observed nutrient levels are uncertain, several important points have been elucidated. Apparently, the concentrations of major nutrients are not the sole factors controlling algal growth. The form in which a nutrient occurs may be of as great importance as its concentration. At this time, the value of algal growth potential measurement appears to be in its use as a predictive technique. Assessment of the likelihood of excessive algal growth is in itself a potentially valuable tool for water resource management, especially in its application to impounded stream waters.

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