A LABORATORY STUDY OF THE EFFECTS OF NITROGEN AND PHOSPHOROUS ON FARM POND ALGAE

BY

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A LABORATORY STUDY OF THE EFFECTS OF NITROGEN AND PHOSPHOROUS ON FARM POND ALGAE

A Research Paper

Presented to

the Graduate Council of

Austin Peay State University

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

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by

Sherrie Clardy Richardson

March 1972

March, 1972

To the Graduate Council:

I am submitting herewith a Research Paper written by Sherrie Clardy Richardson entitled "A Laboratory Study of the Effects of Nitrogen and Phosphorous on Farm Pond Algae." I recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Biology.

Davis S. Findley
Major Professor

Accepted for the Council:

Wayne C. Stacy
Dean of the Graduate School

ACKNOWLEDGEMENTS

The author wishes to express appreciation to Dr. Davis Findley, Assistant Professor of Biology, Austin Peay State University, for helpful suggestions and constructive criticism during the study; to Dr. Haskell Phillips, Chairman of the Department of Biology, and Dr. Floyd Ford, Professor of Biology, for their criticism of the manuscript.

Appreciation is also expressed to Dr. Diane Findley and Mr. Billy Dean Cole for their suggestions.

The author wishes to thank her parents for their understanding and encouragement during the study.

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CHAPTER I

INTRODUCTION

Within the last few years, there has been much concern over the pollution of our environment. The air is filled with smoke and fumes from manufacturing plants and motor vehicles. The clean water supplies are threatened with untreated sewage and chemical wastes making them unsuitable for human consumption or recreation. With the sudden awareness that this excessive pollution must stop, companies have begun installing soot-trapping devices in smoke stacks. Cities are building more efficient sewage treatment plants. Some pollutants, such as chemical wastes, are more difficult to dispose of without harm to the environment. Mercury, herbicides, insecticides, and fertilizers contaminate the ponds and streams causing fish kills, foul smells, algal blooms and death of our aquatic organisms.

An immediate source of concern to farmers, manufacturers and other individuals has been the formation of very dense algal blooms on ponds and lakes during the warm months of the year. The toxicity of some blooms to farm animals, the clogging of water facilities and the presence of fishy tastes and odors have been incentives to investigate the cause and erradication of the blooms. Phosphates (Shapiro, 1970) and nitrogen compounds

(Mackenthun, Keup, and Stewart, 1968) have been indicated as key nutrients for these blooms. However, little work has been done to determine what concentrations and ratios will initiate an increased algal growth rate.

A farm pond situation was chosen for this study because of the great number of ponds with blooms readily available and because of the great interest among farmers in preventing these blooms. This research was undertaken to offer some information useful in determining the factors which might cause algal blooms. The main purpose is to assay the effects of various concentrations and ratios of nitrate and ammonium nitrogen and phosphate on algal growth rate. It is intended that the data collected will be beneficial to future investigators.

CHAPTER II

METHODS AND MATERIALS

Collection

The algae for this study were collected on October 14, 1971, from a pond on the Langford farm located on the northwest side of Port Royal road approximately eight miles northeast of Clarksville, Tennessee. Surface samples were collected by the use of a phytoplankton net (173 mesh to the inch) and carefully placed in glass containers with pond water for transport to the laboratory. Four pH measurements were made at various locations in the pond. For each collection the water surface temperature was measured and recorded.

The area around the pond had not been used in any manner for approximately one month. After consulting the owner, it was learned that no herbicides, insecticides, or fertilizers containing nitrates or phosphates had been applied in the drainage area of the pond within the last six months.

Culture Methods

For the culturing of the algae, Bristol's solution was chosen. The solution was prepared according to Starr (1964) and adjusted to the pond pH of 7.6. The algae were transferred from the collecting containers into

250 milliliter flasks containing 100 ml. each of sterile Bristol's solution and plugged with foam plugs. The flasks were incubated at 27°C which was the pond surface temperature. The algae were allowed to remain in the 250 ml. flasks for one month to allow growth of the organisms which would grow under laboratory conditions. After the one month growth period three drops of one of the cultures, chosen at random, were transferred using Pasteur pipettes into each of nine tubes of sterile complete medium. These first culture tube transfers were incubated for one month.

Stocks of Bristol's solution minus nitrogen and Bristol's solution minus phosphate were prepared. Stock solutions of nitrate, phosphate and ammonia in the concentration of one part per thousand nitrogen or phosphate were prepared by mixing 0.722 gm. potassium nitrate (KNO_3) and diluting to one liter, 0.143 gm. potassium monohydrogen phosphate (K_2HPO_4) and diluting to one liter, and 0.378 gm. ammonium chloride (NH_4Cl) and diluting to one liter. The stock solutions were each diluted with the appropriate medium to prepare 0.25 to 5.0 parts per million solutions. Eight ml. of the completed medium were put into five 6-inch test tubes. The test tubes were then autoclaved for 20 minutes under 18 pounds pressure. After cooling, three drops of algae from the first transfer tubes were transferred by sterile Pasteur pipettes into the tubes of test medium. These second transfer tubes

were also incubated for one month.

A nitrogen and phosphate ratio test was conducted simultaneously in Bristol's solution with a 10:1 nitrate nitrogen-to-phosphate ratio prepared in the same manner as above. Three drops of algae were transferred by sterile Pasteur pipettes from one of the first transfer tubes into the sterilized tubes of medium. These second culture tubes were incubated at 27°C for one month.

Counting Method

After the one month growth period, all of the second culture tubes were removed from the incubator and prior to counting were stirred on a Super-Mixer (Matheson Scientific) to break up any sheets and clumps of algae. A procedure similar to that used by Lange (1970) was chosen to determine algal growth. A Neubauer counting chamber (American Optical Company) was used to count a representative sample from each tube following the procedure identical to erythrocyte counting but without dilution. A percentage estimate as to genera present was made on each culture.

CHAPTER III

RESULTS

Genera Survey

A microscopic examination of the algae collected from the pond in October revealed three predominate genera: Aphanothece, Chlamydomonas, and Gleocystis (Prescott, 1951; Prescott, 1970; Smith, 1950). Pandorina was found less frequently. A second examination made simultaneously with the cell counts showed a majority of Chlamydomonas in all test cultures. Pandorina appeared less frequently in the nitrate tests from 1.25 to 4.0 ppm nitrate nitrogen.

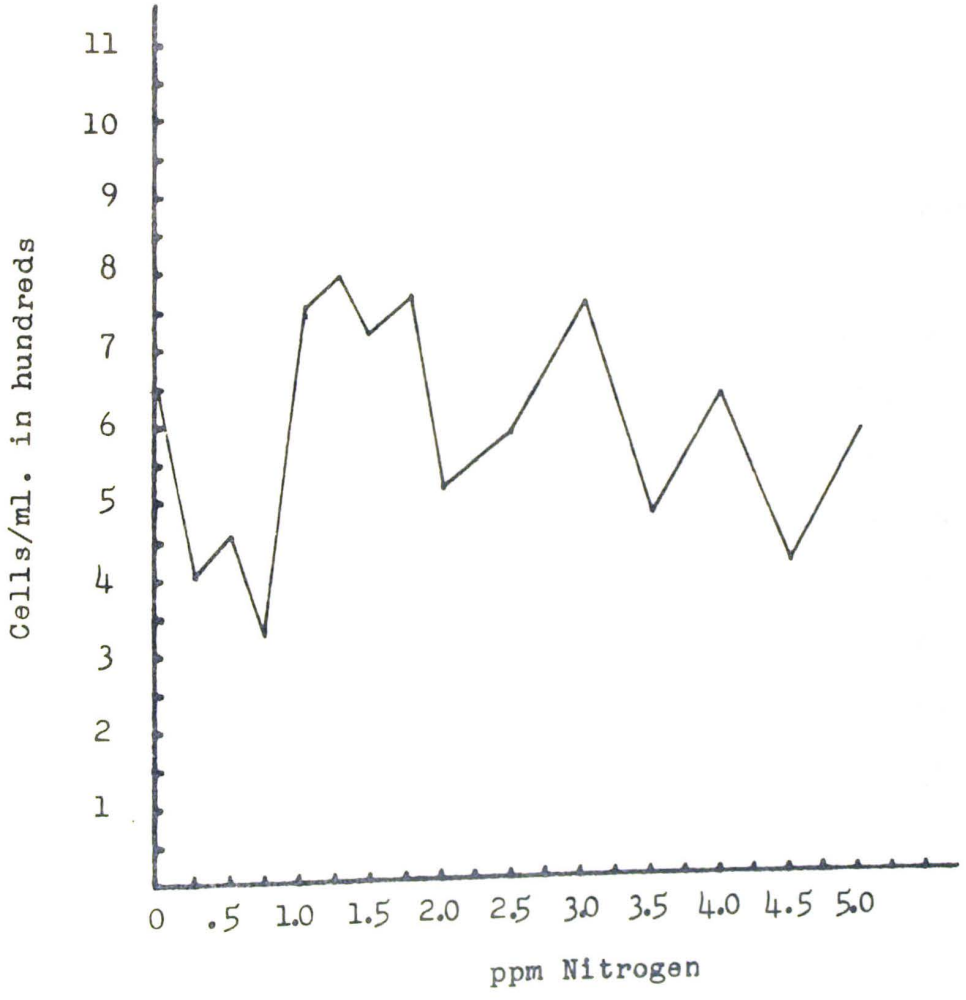
Count Results

The results of each test expressed as biomass of the algae or the number of cells/ml. for each concentration of nitrogen, or phosphate, or both, are shown in Figures 1-4.

As seen in Figure 1, a greater amount of biomass was supported by ammonium nitrogen in the lower concentration range of 1.0 to 2.0 and at 3.0 ppm nitrogen. The number of cells/ml. at these concentrations ranged between 720 and 800. Between 3.0 and 5.0 ppm nitrogen, the biomass dropped slightly to range from 425 to 650 cells/ml.

FIGURE 1

Ammonium Nitrogen Biomass Curve



Nitrate nitrogen (Figure 2) gave a biomass curve similar to ammonium nitrogen. The greatest biomass was between the concentrations of 0.75 to 1.25 ppm nitrogen where the number of cells varied from 850 to 1100 cells/ml. At the concentrations of nitrogen, 1.5 to 5.0 ppm, the biomass varied between 475 and 750 cells/ml.

The phosphate graph (Figure 3) shows one area of greater biomass. A concentration of 1.5 ppm phosphate supported a growth of 250 cells/ml. The low 0.0 to 1.25 ppm and the higher concentrations of 1.75 to 5.0 ppm phosphate show the cell number varying between 25 and 150 cells/ml. The phosphate curve shows considerably less biomass when compared to the nitrate and ammonium nitrogen curves.

The 10:1 nitrate nitrogen-to-phosphate ratio results (Figure 4) show that a much greater biomass is supported in the lower nitrate nitrogen concentration range. The cell number rises to 2025 cells/ml. in the 1.5 ppm nitrogen concentration. The higher concentrations of nitrate nitrogen with proportionately more phosphate show less biomass. The 10:1 ratio shown on Figure 3 at the 5.0 ppm phosphate concentration shows considerably less growth than the same ratio at 5.0 ppm nitrate nitrogen and 0.5 ppm phosphate (Figure 4). The 1:1 nitrogen-to-phosphate ratio shown at the 3.0 ppm nitrate nitrogen concentration on Figure 2 supports more biomass than either of the higher concentration 10:1 ratios.

FIGURE 2

Nitrate Nitrogen Biomass Curve

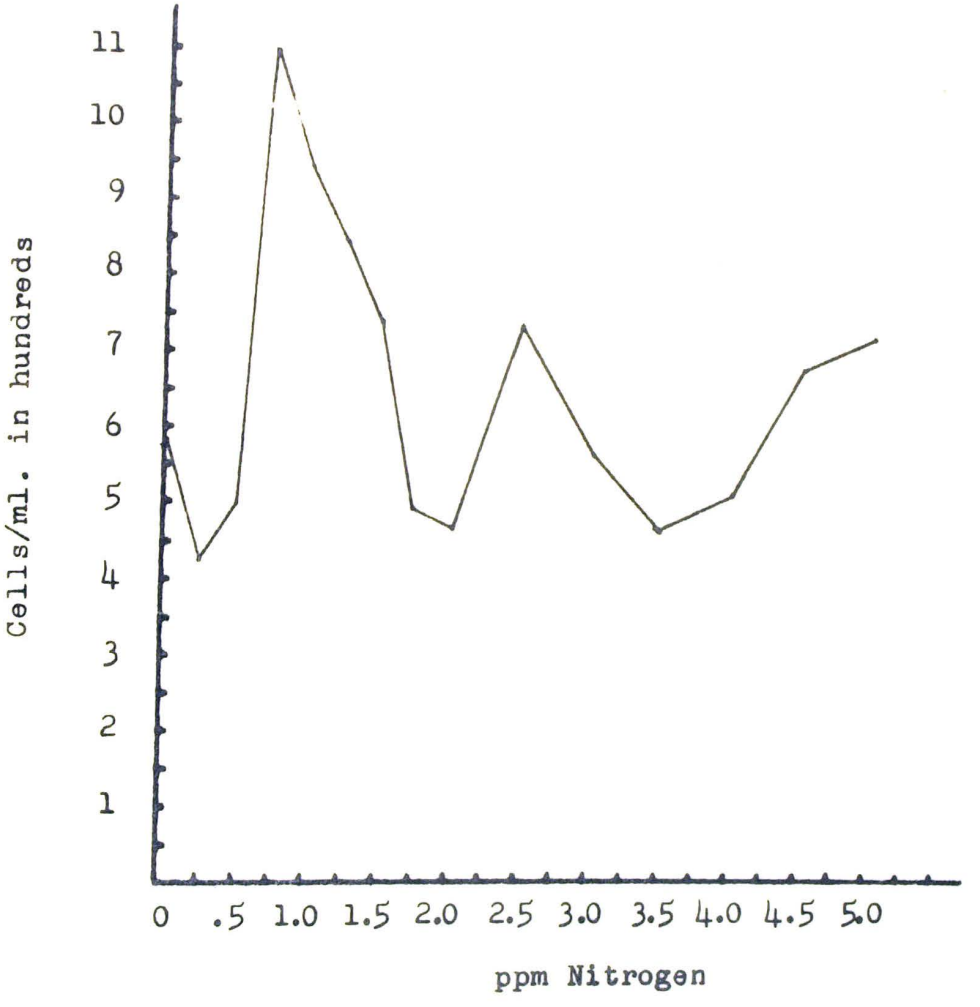


FIGURE 3

Phosphate Biomass Curve

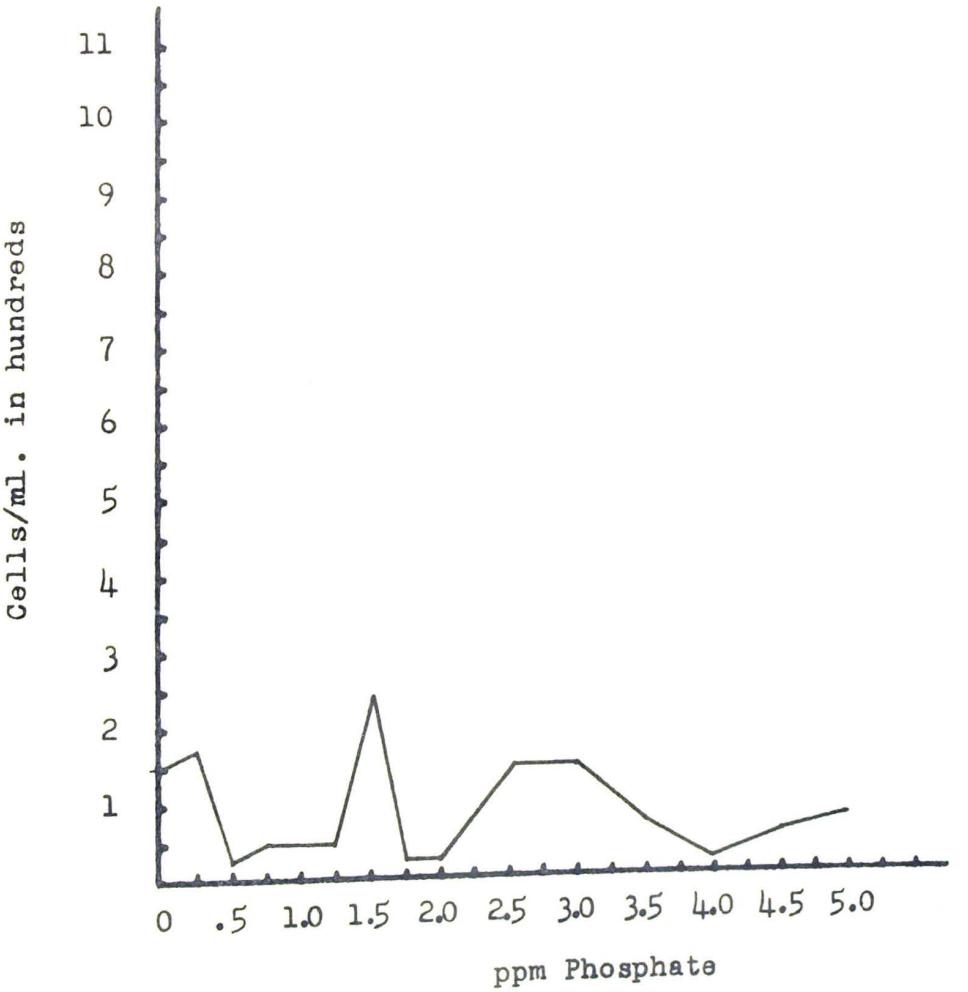
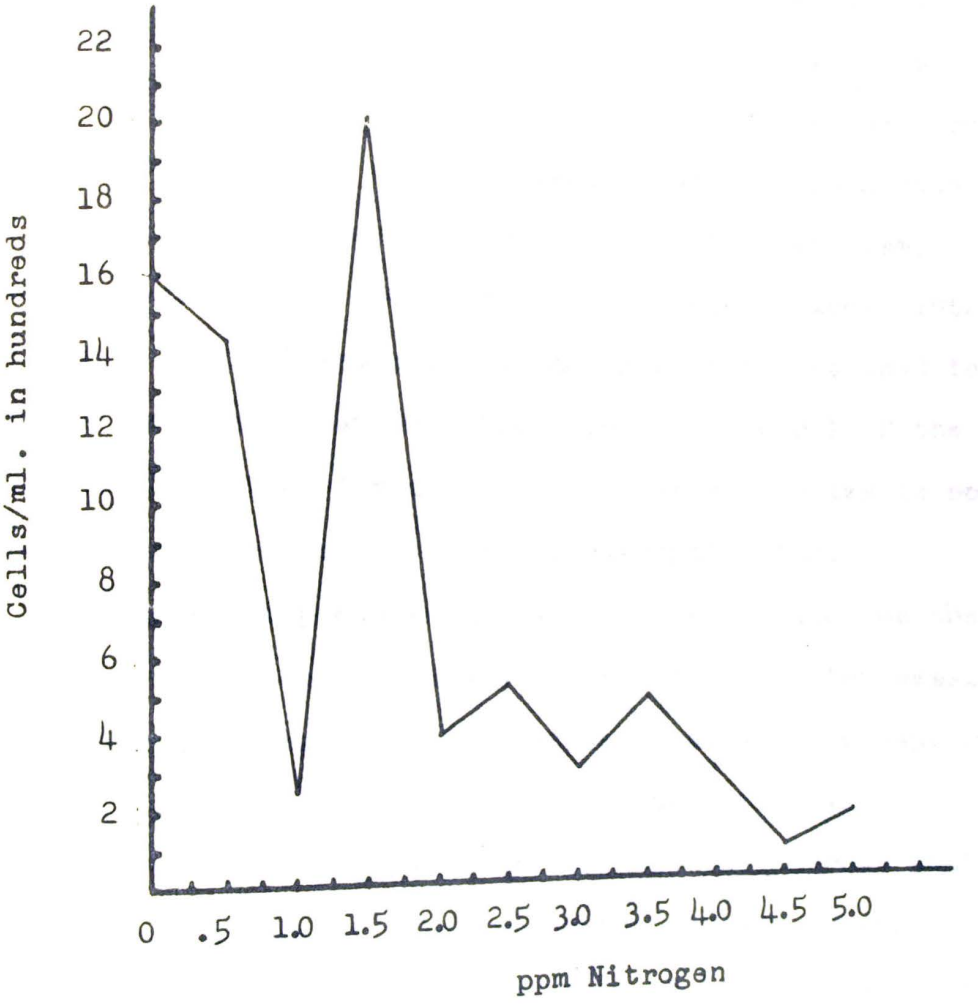


FIGURE 4

10:1 Ratio of Nitrogen to Phosphate

Biomass Curve



CHAPTER IV

DISCUSSION AND CONCLUSIONS

The eutrophication of lakes and ponds has renewed interest in algal nutrition from both a biochemical and an economic standpoint. Phosphorous and nitrogen, two major nutrients, are being closely examined for their roles in the production of massive algal blooms. Phosphorous plays an important part in most phases of metabolism, particularly energy transformation reactions (Kuhl, 1962). Nitrogen, assimilated only by the Cyanophyta, is used to produce amines and amides (Fogg, 1962). Control of the flow of these two elements into our water supplies is now thought to be one way to prevent eutrophication.

Economic pressures have initiated studies on the phosphorous content of waters presently supporting massive algal blooms. Shapiro (1970) states that since phosphorous is usually present in fresh water in the least amount relative to need, a rise in the soluble phosphorous content will allow the use of other nutrients already present in abundance. After conducting studies on Lake Sebasticook, Maine, Mackenthun (1968) recorded a direct correlation between the amount of soluble phosphorous in the lake and the phytoplankton present. However, other phosphate studies have contradicted these reports. Abbott (1969)

reported that increases in phosphate in his carboy microcosm studies did not upset the trophic balance. Kuentzel (1969) agrees that phosphorous is a necessary requirement for algal growth, but he also states that massive blooms have formed on waters with a soluble phosphate concentration of 10 parts per billion or less. He reports that Lake Minnetonka, Minnesota, has a higher soluble phosphate average than the more polluted Lake Sebasticook yet does not support an algal bloom.

Nitrogen is less frequently considered to be the nutritional factor initiating blooms. Algae can utilize either ammonium salts or nitrates when supplied at a suitable concentration (Syrett, 1962). However, very few studies have been made to determine what effect varying concentrations have on algal growth rates. Abbott (1969) reported that increasing the nitrates in his estuarine models did not cause an algal bloom. Shapiro (1970) says that the algal productivity of the north Pacific may be a result of the nitrate concentration since phosphorous is present in excess. Ryther and Dunstan (1971) report that nitrogen is the limiting factor in coastal marine waters since an excess of phosphate is present. They also state from unpublished results by Woods Hole Oceanographic Institution that algae cultures in water samples from Moriches Bay failed to respond to phosphate enrichment but increased greatly in number in ammonium nitrogen enriched cultures.

The ratio of nitrogen to phosphorous may be even more important than enrichment by either element by itself. It has been shown that deficiencies of either element in culture media may drastically alter the growth of the algae. Ryther and Dunstan (1971), checking the ratio of nitrogen to phosphorous, have reported finding ratios varying between 5:1 and 15:1 in coastal marine algae.

The results reported in this study seem to support the more recent idea that neither nitrogen nor phosphorous by itself will cause an algal bloom. The algal growth in the nitrogen enriched cultures here do not show the extensive growth reported in similar cultures by the Woods Hole Oceanographic Institute. However, more growth is shown than in Abbott's estuarine models. The lack of "bloom" formations in the phosphate enriched cultures agrees with Kuentzel's theory that phosphorous may not be the key nutrient. The excessive growth at 1.5 ppm nitrate nitrogen in the 10:1 ratio test substantiates Ryther and Dunstan's idea that the ratio of nitrogen to phosphorous may be the limiting factor in waters that do not produce blooms.

It is the opinion of this investigator, in view of the results obtained, that the ratio of nitrogen to phosphorous is more important than the increase of either element with all other nutrients present in excess. Lower concentrations of ammonium and nitrate nitrogen may support some excessive growth if the phosphate concentration is

sufficient. Phosphate alone in varying concentrations between 0.25 and 5.0 ppm does not appear to induce massive blooms if other nutrients are available in excess. The success of the laboratory bloom in this study seems to depend on a low but sufficient concentration of nitrogen and phosphate in an appropriate ratio. However, more work such as expanding the 10:1 ratio range between 0.0 and 2.0 ppm nitrogen and increasing the amount of carbon dioxide available to the algae in the culture tubes should provide more definite results.

CHAPTER V

SUMMARY

A study of various concentrations of nitrogen and phosphate between 0.25 and 5.0 ppm and a 10:1 nitrogen to phosphate ratio test were conducted on algae collected from a farm pond algal bloom. Chlamydomonas, growing best under laboratory conditions, was used for testing purposes. Growth in "bloom" proportions did not result from enrichment by either ammonium or nitrate nitrogen or phosphorous. Excessive growth did occur in the 10:1 ratio test at 1.5 ppm nitrate nitrogen.

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APPENDIX

APPENDIX A

CELL COUNT

Ppm	Ammonium Nitrogen	Nitrate Nitrogen	Phosphate	Ratio
5.0	10	12	0	3
	14	18	2	5
4.5	6	10	0	1
	11	18	1	5
4.0	15	10	1	13
	11	11	0	0
3.5	9	11	0	12
	11	8	3	8
3.0	13	10	1	3
	18	13	5	9
2.5	12	17	1	7
	12	13	5	14
2.0	11	14	1	2
	10	15	0	14
1.75	21	10	1	
	6	10	0	
1.50	17	13	8	30
	8	13	2	15
1.25	16	19	1	
	16	15	0	
1.0	19	20	1	6
	11	18	1	5
0.75	8	20	0	
	5	24	2	
0.50	10	9	0	26
	8	11	1	31
0.25	8	7	4	
	8	10	3	
Control	20	14	3	32