

AUG 11 1953

83

ABSTRACT

INVESTIGATION OF EXTRAMETABOLITES PRODUCED BY ALGAE IN CULTURE

by Elaine H. Hurst

Four species of algae, Microcystis incerta Lemmermannii, Nostoc sp., Oscillatoria rubescens de Candolle, and Oscillatoria Agardhii Gomont, were isolated and cultured. The first three of these plus Microcystis aeruginosa Kuetz. emend Elenkin (Strain NRC-1), Aphanizomenon flos-aquae (L.) Ralfs (Strain NRC-23), and Anabaena flos-aquae (Lyng.) De Brébisson (Strain NRC-44) were tested for the production of inhibitory extrametabolites against several non-algal organisms. The blue-green algae were not bacteria free. The non-algal organisms included: the fungi, Glomerella cingulata (Stonem.) Spauld. and Schrenk, Fusarium oxysporum Schlecht. ex Fries, Rhizopus nigricans Ehrenberg, + strain, and Trichophyton rubrum (Castellani) Sab.; the yeast, Candida albicans (Robin) Berkh.; and the bacteria, Staphylococcus aureus Rosenbach, Escherichia coli (Migula) Castellani and Chambers, and Pseudomonas aeruginosa (Schroeter) Migula.

Aphanizomenon flos-aquae demonstrated the greatest activity, inhibiting Rhizopus nigricans and Pseudomonas aeruginosa slightly. This species showed stronger inhibition

toward Fusarium oxysporum and Staphylococcus aureus. The inhibitory activity was shown not to be due to the bacterial symbionts alone but could have been formed by the combination of the alga and the bacteria.

Two bacteria were isolated from Aphanizomenon flos-aquae. These were identified as Bacillus cereus var. mycoides Flügge comb. nov. Bacillus mycoides Flügge and Flavobacterium diffusum (Frankland and Frankland) Bergey et al.

The effect of changing the concentration of nitrogen, phosphorus and manganese in cultures of Aphanizomenon flos-aquae was studied. Growth in the control medium, designated as ASM, was satisfactory. Increasing the concentration of nitrogen inhibited growth. In the medium containing three times the amount of available nitrogen growth was inhibited almost completely. Increasing the amount of phosphorus also brought about a decrease in growth but the effects were not as great as in the culture medium containing increased amounts of nitrogen. Decreasing the amount of phosphorus made little change in growth.

The manganese concentration appeared to be the limiting factor in the medium used. Growth increased when this mineral was omitted from the medium. Growth was greatest in medium containing 0.0005 ppm of manganese indicating that a small amount of this element was stimulatory.

INVESTIGATION OF EXTRAMETABOLITES

PRODUCED BY ALGAE IN CULTURE

By

Elaine H. Hurst

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Botany and Plant Pathology

1965

6-35-963
1-26-66

ACKNOWLEDGEMENTS

The investigator wishes to express thanks for the inestimable assistance of Dr. G. W. Prescott for his continued directive suggestions and his constructive evaluation during the entire course of this study.

The valuable guidance of Dr. E. S. Beneke as well as his kindness in providing cultures and special equipment is sincerely appreciated as is the advice received from Dr. W. E. Wade and other members of my committee, Dr. I. W. Knobloch and Dr. B. T. Sandefur.

The bacterial identification and nomenclature by Mr. M. R. Wiseman and Mr. A. R. Barbiers, Biology Department, Western Michigan University, is gratefully acknowledged.

The investigator is indebted to Dr. P. R. Gorham, National Research Council, Ottawa, Canada; to Miss L. Neu, Department of Microbiology and Public Health, Michigan State University; and to Dr. J. L. Lockwood, Department of Botany and Plant Pathology, Michigan State University, for contributing various cultures.

Additional expressions of thanks are due Mr. D. F. Jackson, Dr. F. R. Uyenko, and Dr. J. Sieminska for accompanying the investigator on field trips and to Mrs. P. E. Rutherford, Biology Department, Western Michigan University, and Mrs. M. C. Hagerman, Nazareth College, for proof reading this manuscript.

Special gratitude is due my husband, Orville, and my children, Marie and Phillip, for their patient endurance and cooperation over the years devoted to this endeavor.

TABLE OF CONTENTS

Chapter	Page
I. INTRODUCTION	1
The Problem	1
Historical Discussion	3
Definition of terms	3
Evidence of extrametabolites	9
Terrestrial organisms	9
Higher plants	9
Soil inhabitants	14
Interrelationships between soil organisms and higher plants	18
Aquatic organisms	21
Protozoa	21
Higher animals	24
Algae	27
Evidence of extrametabolite production in general	27
"Red tides"	34
Blue-green algae	37
Evidence of toxicity produced by blue-green algae	37
Conditions conducive to blue-green blooms	50
Relationship of the bacteria living in association with the blue-green algae	54
Antibiotic activity produced by blue-green algae	55
II. METHODS AND MATERIALS	58
Organisms Used	58
Collection - Habitats and Related Features	59
Isolation of Algae	69
Medium	70
Growth Conditions	73
Testing for Antibiotic Activity	76
Isolation of the Bacterial Symbionts	78
Effects of Variations in Mineral Concentra- tions in the Medium	79

Chapter	Page
III. RESULTS	83
Growth of Algae	83
Inhibitory Activity	100
Identification of the Bacteria	112
Effects of Various Concentration of Minerals on the Growth of <u>Aphanizomenon flos-aquae</u>	115
IV. DISCUSSION	158
Ecological Considerations in the Production of Water-Blooms	158
Isolation of Algae	164
Succession of Algae on Agar	164
Growth in Liquid Cultures	165
Temperature and Light	166
Inhibitory Activity	168
Bacterial Symbionts	170
Effects of Varying the Mineral Concentrations	175
Changes in pH	179
V. CONCLUSIONS	182
BIBLIOGRAPHY	184

LIST OF TABLES

Table	Page
1. ASM medium	71
2. The effects of the spent medium of <u>Anabaena flos-aquae</u> on the non-algal organisms . . .	102
3. Inhibitory effects of the spent medium of <u>Oscillatoria rubescens</u> on the non-algal organisms	103
4. Inhibitory effects of the spent medium of <u>Microcystis aeruginosa</u> on the non-algal organisms	104
5. Inhibitory effects of the spent medium of <u>Microcystis incerta</u> on the non-algal organisms	105
6. Inhibitory effects of the spent medium of <u>Nostoc</u> sp. on the non-algal organisms . . .	106
7. Inhibitory effects of the spent medium of <u>Aphanizomenon flos-aquae</u> on the non-algal organisms	108
8. Experiment I - Growth of <u>Aphanizomenon flos-aquae</u> in control medium on shaker . .	116
9. Experiment I - Growth of <u>Aphanizomenon flos-aquae</u> in mg. (control medium in growth chamber)	118
10. Experiment I - Growth of <u>Aphanizomenon flos-aquae</u> in medium containing twice the available nitrogen	120
11. Experiment I - Growth of <u>Aphanizomenon flos-aquae</u> in medium containing three times the available nitrogen	122

Table		Page
12.	Experiment II - Growth of <u>Aphanizomenon flos-aquae</u> in control medium	128
13.	Experiment II - Growth of <u>Aphanizomenon flos-aquae</u> in medium containing twice the amount of available phosphorus	130
14.	Experiment II - Growth of <u>Aphanizomenon flos-aquae</u> in medium containing three times the amount of available phosphorus	132
15.	Experiment III - Growth of <u>Aphanizomenon flos-aquae</u> in control medium	138
16.	Experiment III - Growth of <u>Aphanizomenon flos-aquae</u> in medium containing 0.01 the amount of available manganese	140
17.	Experiment III - Growth of <u>Aphanizomenon flos-aquae</u> in medium containing 0.001 the amount of available manganese	142
18.	Experiment III - Growth of <u>Aphanizomenon flos-aquae</u> in medium containing no added manganese	144
19.	Experiment IV - Growth of <u>Aphanizomenon flos-aquae</u> in control medium	150
20.	Experiment IV - Growth of <u>Aphanizomenon flos-aquae</u> in medium containing 0.5 the amount of available nitrogen	152
21.	Experiment IV - Growth of <u>Aphanizomenon flos-aquae</u> in medium containing 0.1 the amount of available nitrogen	154

LIST OF FIGURES

Figure	Page
1. Waterhole from which <u>Microcystis incerta</u> was isolated	60
2. Same waterhole showing the fence erected to prevent cattle from drinking the water (lower right corner)	60
3. Sixth Lake showing vegetation along the southeast shore taken when a bloom of <u>Anabaena limnetica</u> was present	63
4. Sixth Lake taken from western shore looking toward the inlet of the lake	64
5. Sixth Lake taken from southern shore where cottages are located	64
6. Alward Lake taken from eastern shore just after disappearance of the bloom of <u>Oscillatoria Agardhii</u> in 1963	67
7. Alward Lake - northern shore	68
8. Alward Lake - southern shore and outlet	68
9. Growth chamber	74
10. Agar plates inoculated with mud from the bottom of Sixth Lake	84
11. Photomicrograph of culture on the right in Figure 10	84
12. Maintenance of cultures on agar slants	87
13. Photomicrograph of <u>Microcystis aeruginosa</u> growing on agar	88
14. <u>Microcystis aeruginosa</u> on agar	88

Figure	Page
15. <u>Microcystis incerta</u> in liquid culture	90
16. <u>Microcystis aeruginosa</u> in liquid culture . . .	91
17. <u>Microcystis aeruginosa</u> in liquid culture . . .	91
18. <u>Nostoc</u> sp. on agar	92
19. Photomicrograph of <u>Nostoc</u> sp. from agar plate on left in Figure 18	92
20. <u>Nostoc</u> sp. in liquid culture	94
21. <u>Oscillatoria rubescens</u> in liquid culture . . .	96
22. <u>Oscillatoria Agardhii</u> on agar	98
23. Photomicrograph of <u>Oscillatoria Agardhii</u> on agar	98
24. <u>Aphanizomenon flos-aquae</u> in liquid culture	99
25. Photomicrograph of <u>Aphanizomenon flos-</u> <u>aquae</u> growing on agar	99
26. <u>Anabaena flos-aquae</u> in liquid culture	101
27. Spent medium of <u>Aphanizomenon flos-aquae</u> against <u>Staphylococcus aureus</u> 2 days after inoculation	109
28. Spent medium of <u>Aphanizomenon flos-aquae</u> against <u>Candida albicans</u> 2 days after inoculation	110
29. Spent medium of <u>Aphanizomenon flos-aquae</u> against <u>Glomerella cingulata</u> 2 days after inoculation	111
30. Growth increments of <u>Aphanizomenon flos-aquae</u> in mg./35 ml. of medium. Exp. I - Control medium on shaker	117
31. pH of spent medium at time of harvesting Exp. I - Control medium on shaker	117

Figure		Page
32.	Growth increments of <u>Aphanizomenon flos-aquae</u> in mg./35 ml. of medium. Exp. I - Control medium in growth chamber	119
33.	pH of spent medium at time of harvesting. Exp. I - Control medium in growth chamber	119
34.	Growth increments of <u>Aphanizomenon flos-aquae</u> in mg./35 ml. of medium. Exp. I - Medium containing twice the amount of nitrogen as in the control	121
35.	pH of spent medium at time of harvesting. Exp. I - Medium containing twice the amount of nitrogen as in the control	121
36.	Growth increments of <u>Aphanizomenon flos-aquae</u> in mg./35 ml. of medium. Exp. I - Medium containing three times the amount of nitrogen as in the control	123
37.	pH of spent medium at time of harvesting. Exp. I - Medium containing three times the amount of nitrogen as in the control	123
38.	Comparison of growth increments of <u>Aphanizomenon flos-aquae</u> . Exp. I - Variations in nitrogen concentration	124
39.	Comparison of pH of spent medium at the time of harvesting. Exp. I - Variations in nitrogen concentration	125
40.	Millipore filters from Experiment I	126
41.	Growth increments of <u>Aphanizomenon flos-aquae</u> in mg./35 ml. of medium. Exp. II - Control medium	129
42.	pH of spent medium at time of harvesting. Exp. II - Control medium	129
43.	Growth increments of <u>Aphanizomenon flos-aquae</u> in mg./35 ml. of medium. Exp. II - Medium containing twice the amount of phosphorus as in the control	131

Figure	Page
44. pH of spent medium at time of harvesting. Exp. II - Medium containing twice the amount of phosphorus as in the control . . .	131
45. Growth increments of <u>Aphanizomenon flos-</u> <u>aquae</u> in mg./35 ml. of medium. Exp. II - Medium containing three times the amount of phosphorus as in the control	133
46. pH of spent medium at time of harvesting. Exp. II - Medium containing three times the amount of phosphorus as in the control .	133
47. Comparison of growth increments of <u>Aphanizomenon flos-aquae</u> . Exp. II - Variations in phosphorus concentration . . .	135
48. Comparison of pH of spent medium at time of harvesting. Exp. II - Variation in phosphorus concentration	136
49. Millipore filters from Experiment II	137
50. Growth increments of <u>Aphanizomenon flos-</u> <u>aquae</u> in mg./35 ml. of medium. Exp. III - Control medium	139
51. pH of spent medium at time of harvesting. Exp. III - Control medium	139
52. Growth increments of <u>Aphanizomenon flos-</u> <u>aquae</u> in mg./35 ml. of medium. Exp. III - Medium containing 0.01 the amount of manganese as in the control	141
53. pH of spent medium at time of harvesting. Exp. III - Medium containing 0.01 the amount of manganese as in the control . . .	141
54. Growth increments of <u>Aphanizomenon flos-</u> <u>aquae</u> in mg./35 ml. of medium. Exp. III - Medium containing 0.001 the amount of manganese as in the control	143
55. pH of spent medium at time of harvesting. Exp. III - Medium containing 0.001 the amount of manganese as the control	143

Figure	Page
56. Growth increments of <u>Aphanizomenon flos-aquae</u> in mg./35 ml. of medium. Exp. III - Medium with no manganese added	145
57. pH of spent medium at time of harvesting. Exp. III - Medium with no manganese added	145
58. Comparison of growth increments of <u>Aphanizomenon flos-aquae</u> . Exp. III - Decrease in manganese concentration	146
59. Comparison of pH of spent medium at time of harvesting. Exp. III - Decrease in manganese concentration	147
60. Millipore filters from Experiment III	149
61. Growth increments of <u>Aphanizomenon flos-aquae</u> in mg./35 ml. of medium. Exp. IV - Control medium	151
62. pH of spent medium at time of harvesting. Exp. IV - Control medium	151
63. Growth increments of <u>Aphanizomenon flos-aquae</u> in mg./35 ml. of medium. Exp. IV - Medium containing one-half the amount of nitrogen as in the control	153
64. pH of spent medium at time of harvesting Exp. IV - Medium containing one-half the amount of nitrogen as in the control	153
65. Growth increments of <u>Aphanizomenon flos-aquae</u> in mg./35 ml. of medium. Exp. IV - Medium containing 0.1 the amount of nitrogen as in the control	155
66. pH of spent medium at time of harvesting. Exp. IV - Medium containing 0.1 the amount of nitrogen as in the control	155

Figure	Page
67. Comparison of growth increments of <u>Aphanizomenon flos-aquae</u> . Exp. IV - Decrease in nitrogen concentration	156
68. Comparison of pH of spent medium at time of harvesting. Exp. IV - Decrease in nitrogen concentration	157

CHAPTER I

INTRODUCTION

The Problem

The natural aging of bodies of water brings about environmental conditions that are responsible for changes in numbers and kinds of organisms present in respective ecosystems. Man, because of his changing social structure and his increase in numbers, has hastened this aging process by adding ever increasing amounts of sewage, industrial, and agricultural wastes to natural bodies of water. Occasionally these environmental changes are conducive to an accelerated growth of aquatic organisms, especially algae. Such an accelerated algal growth leads to various problems which effect the economy of man directly or indirectly. One such problem involves the effects on other organisms of substances produced by these algae during periods of accelerated growth. In many parts of the country the only water available for human use is surface water such as that stored in reservoirs. The presence of these substances, under certain conditions, if concentrated, might render surface water unfit for human consumption.

In reference to these problems the objectives of this study were:

1. To isolate and culture several species of blue-green algae suspected of being producers of such substances.
2. To test the spent medium from cultures of these blue-green algae for the possible presence of substances inhibitory to selected non-algal organisms.
3. To select for further study one of the blue-green algae which showed inhibitory activity; these studies to include (a) isolation and identification of the bacterial organisms associated with the blue-green alga, (b) investigation of the possibility that these bacteria are responsible for the inhibitory activity, and (c) determination of the effect on growth of the organism produced by changing the concentration of the minerals in the medium.

Historical Discussion

The complex interrelationships of organisms in an ecosystem pose a number of problems which have invited both speculation and investigation. Not only are the organisms affected by various factors in the environment but each organism itself is capable of producing changes in the environment. It has been demonstrated repeatedly that organisms may liberate substances into their immediate surroundings. These substances are referred to as by-products of metabolism. Webster's New International Dictionary defines a by-product as "something produced, as in the course of a manufacture, in addition to the principal product." The results, or "products," of metabolism are growth, maintenance, and functioning of a living organism. Any unused element or compound resulting from these metabolic processes, whether it be beneficial to the organism or a waste product, will be considered as a by-product.

These by-product are called extrametabolites, extracellular products, external metabolites, or free metabolites. These terms are considered to be synonymous. Lucas (1961) states that the term "extrametabolite," interpreted liberally, could "include all of the by-products of metabolism." He further states that all cells, living, or after death, are capable of releasing metabolic by-products. On the other hand, Fogg (1962) defines extrametabolites as "soluble

substances liberated from healthy cells as distinct from substances set free from injured cells or by autolysis or decomposition of dead ones." In the following work an extremetabolite will be considered as any by-product of metabolism released during the life of the organism, after the death of the organism, or both during life and after death.

It is relatively easy to detect the role of carbon dioxide and oxygen in the environment and their effects on the organisms in an ecosystem. The effects of simple phosphates and nitrates, although more difficult to follow, can also be detected. The effects produced by the more complex substances on the biota, however, are much more difficult, if not impossible, to determine and understand. Many such complex extrametabolites have been isolated and characterized chemically including organic acids, amino acids, polypeptides, fatty acids, various carbohydrates, enzymes, vitamins, and auxin-like substances.

Extrametabolites may be either beneficial or harmful to members of the biota. If an extrametabolite is beneficial it may be referred to as a growth factor or a growth substance. The usage of these terms is not precise, having slightly different meanings in various branches of biological science. These substances may promote the growth of the organism from which they originate (auto-stimulant).

It is more likely, however, that their presence in the environment promotes the growth of other organisms. As used here any substance that is an absolute requirement without which no growth can occur is considered to be a growth factor. These substances include enzymes and vitamins which regulate definite metabolic activity in a living organism. A growth substance is a hormone or auxin-like substance that changes the form or the rate of growth. Growth, although slow, can occur in the absence of a growth substance. The presence of a growth factor, however, increases the total yield. The term "growth factor" is synonymous with growth stimulator, growth promoter and growth regulator (Conrad and Saltman, 1963).

A harmful extrametabolite, in the broadest sense, may be regarded as an inhibitor. An inhibitor is "an agent which restrains, checks, or stops an activity in an organism" (Steen, to be published). Inhibitors are sometimes referred to as "toxic substances" or toxins. A precise definition that is universally accepted is as yet unknown. Microbiologists define a "toxin" as a poisonous product produced by a microorganism which can induce an antitoxin. Muir states that the term "toxin" is "usually applied to the poisonous products produced by microorganisms though it includes closely similar poisons of animal and vegetable origin" (Muir, 1941). More broadly defined, toxins are "poisonous

substances formed as secretion products of cells" which are "normal waste products or actual components of cells that have the power to damage plant and animal cells" (Wedburg, 1963). Akehurst (1930) defines a toxin as an excretion product or products which "may serve as an accessory food or may inhibit or stimulate growth," a definition that does not adhere to the basic interpretation of other researchers. The suggestion here is that the effect of a toxin may be gradational, inhibiting or stimulating depending on the concentration.

Toxins are divided into two major groups depending upon the mechanism of their release. "When a living cell excretes its poison during active metabolism and the toxin is liberated free into the medium" the toxin is an exotoxin. If the toxin "is not released except through physical and biological forces (autolysis) after the death of the cell" it is an endotoxin (Wedbury, 1963).

In this dissertation the term "toxin" refers to any poisonous substance released during the life of an organism or/and after the death of the organism. These substances are capable of inhibiting a specific activity and of producing a specific pathological change in a specific living organism or possibly in several species of organisms. In the most extreme cases death may be induced.

A more specific inhibitor is the antibiotic. Bacteriologists define an antibiotic as an antimicrobial substance produced during the metabolism of a living organism (Burrows, 1963). The term "antibiotic" is, therefore, used to denote substances that exhibit inhibitory activity toward organisms placed by some taxonomists under the Protista. The organisms included in this group are Protozoa, bacteria, Rickettsia, viruses, Actinomycetes, molds, yeasts, and algae (Whaley, et al., 1964). Inhibitors, as discussed in the history are produced by a variety of organisms, including higher plants. These inhibitors in higher plants are not referred to as antibiotics unless they react on microorganisms.

Antibiotics are referred to more specifically in terms of the microorganisms inhibited. If the inhibition is directed against bacteria it is an antibacterial. It may be bacteriostatic, reducing or checking growth, or bacteriocidal, producing death. Antifungal substances are those which exhibit inhibitory activity toward fungi; anti-algal exhibit inhibitory activity against algae. Autoinhibitors are substances which adversely effect the growth of the organism producing them.

Agriculture is an old and practical science. Much of the knowledge in this field has been acquired from observations made by many generations of farmers. It is not

surprising then that some of the earlier references pertaining to extrametabolites have to do with agricultural plants. In 1832, de Candolle, interested in improving agriculture, theorized that poor yields of a crop planted in successive years on the same soil were the result of inhibitors produced by the plants themselves. He cited the effects of Euphorbia sp. on various grasses and of thistle on Avena sp. He advocated crop rotation using species which were not inhibited by toxic substances left in the soil by previous crops. Although based primarily on observation this early work by de Candolle did cite experimental evidence to support his theory of crop rotation.

De Candolle cited the work of M. Macaire on kidney beans. Macaire watered growing kidney bean plants and collected the water after it had passed through the soil in which these plants were growing. He found that this water when applied to younger seedlings inhibited their growth. The same water had no effect on wheat seedlings. This experimental evidence suggested that extrametabolites were produced by the growing plants and that these products were selectively inhibitory to other plants (cited by Bonner, 1950; by Rose, 1960).

At first Leibig accepted de Candolle's theory but later abandoned it because exhaustive chemical analyses of soils suggested that mineral balance in the soil was the

important factor in producing good crop yields (cited by Bonner, 1950). From the middle of the Nineteenth Century until the early part of the Twentieth Century plant growth was interpreted in terms of available minerals. Renewed interest in the production of toxic substances by plants resulted after application of fertilizers to low-productive soils did not increase yields. The literature now contains numerous references concerning extrametabolites produced both by plants and animals.

Evidence of Extrametabolites Produced by Terrestrial Organisms

Higher Plants

Early reports of harmful interactions between plants include: (1) the inhibition of shrubby cinquefoil (Potentilla fruticosa L.) by the butternut (Juglans cinerea L.) (Jones and Myers, 1902); (2) the inhibition of grapes by rye (Secale cereale L.) (Cubban, 1925); and (3) the adverse effects on rice produced by common weeds growing in rice paddies (Peralta and Estioko, 1924). Pickering (1903, 1907) attributed the injury to apple trees by grass to competition for oxygen and nutrients in the soil. Later, experimentation showed that the growth of apple trees was inhibited by something produced by the roots of the grass (Pickering, 1919).

In 1907 and 1908, Schreiner and his co-workers isolated picolonic acid, salicylaldehyde, and dihydroxystearic acid, the first extrametabolites proved to be inhibitory. These workers also isolated two substances that were inhibitory to wheat seedlings. These were vanillin from the vanilla bean and arbutin from the leaves of Bergenia crassifolia Fritsch (Schreiner and Reed, 1907, 1909; Schreiner and Sullivan, 1909; Schreiner and Shorey, 1909, 1910; Schreiner and Lathrop, 1911).

From an economic standpoint knowledge of the production of extrametabolites would be beneficial. Crop plants could be selected so that inhibition would not occur or would be minimal. The knowledge of inhibitory activity would also be helpful in such fields as horticulture and landscape architecture where species selected for plantings should be compatible.

The production of an inhibitory extrametabolite may have been responsible for the difficulty encountered in attempts to replant peach orchards. Although not demonstrated under field conditions, alcohol extracts of the roots of old trees remaining in the soil were found to be toxic to young peach trees (Proebsting and Gilmore, 1941; Patrick, 1955).

Growth of many cultivated plants is severely inhibited by couch grass (Agropyron repens (L.) Beauv.) whereas others are not affected. Work by Burmester in 1914 showed

that this species had no effect on oats if both were germinated at the same time. If couch grass is planted 14 days before the oats the latter is severely inhibited (cited by Grümmer, 1961). Hamilton and Bucholtz (1955), working in an alfalfa field (Medicago sativa L.) infested with couch grass, found that the removal of the rhizomes of couch grass retarded the growth of some weeds and simultaneously increased the seedlings of others. Wedland found that washings from the roots and rhizomes of couch grass stimulated the growth of tomato and kale seedlings but inhibited the seedlings of cereals. Several phenolic substances have been isolated from the roots and rhizomes of couch grass. An essential oil, agropyrene, has been found to be antifungal and antibacterial but its effects on higher plants is not known (Grümmer, 1961). The work of Wedland suggests that the study of extrametabolite production might be economically important in the control of weeds.

Some plants are known to produce auto-inhibitors. The roots of guayule (Parthenium argentatum Gray) produce an organic acid, trans-cinnamic acid. In nurseries where plants grow close together the roots intermingle and development may be effected. Young plants growing under older plants are inhibited and there is a high mortality of seedlings. Trans-cinnamic acid is unstable in unsterilized soil suggesting that it is destroyed by micro-organisms in the soil (Bonner

and Galston, 1944). Experimentally this substance was found to inhibit peas but not tomatoes. In nature the production of an auto-inhibitor may prevent overpopulation.

A stand of brome grass (Bromus inermis Leyss) may thin out and eventually die back. Washings from old brome grass cultures when used to irrigate seedlings proved to be inhibitory to the seedlings of brome grass (Benedict, 1941). Lower yields of brome grass were produced in soils in which this species had been grown (Myers and Anderson, 1942).

The species of plants growing in the vicinity of the black walnut (Juglans nigra L.) are very different from the species growing fifty feet away. Davis (1928) identified the inhibitory substance as juglone, an alkaloid. The inhibitor is not produced until the black walnut is about two years old (McDaniels and Münscher, cited by Brooks, 1951).

From a practical and from a purely scientific standpoint an increased knowledge of extrametabolite production could help ecologists to understand the make-up of plant communities. The presence or absence of certain species in certain areas, not explainable by edaphic or climatic factors, might be related to the production of extrametabolites.

The ecology of desert regions suggests that the survival of certain species may be the result of inhibitors that prevent the growth of other species. In North American deserts some annual plants grow only beneath shrubs while

others are found between widely spaced shrubs. The shrub, Franseria dumosa A. Gray, harbors a growth of annuals but the composite Encelia formosa A. Gray does not (West, 1942). Tomatoes, corn and peppers were inhibited when mulched with the leaves of Encelia. Annuals which normally grow in the desert shrub associations were also inhibited. No inhibition of Encelia itself or of barley, oats and sunflower occurred. In nature the toxin remained active in the soil two or more years (Went, 1955).

The production of inhibitors also may explain why Ailanthus grandulosa Desf., tree of heaven, is often found growing in single stands. The leaves of this tree were found to contain a substance that was inhibitory to 45 species of trees but not to Fraxinus americana L., the white ash. Extracts of the leaves were also toxic to animals (Mergen, 1959).

An interesting extrametabolite ecologically is parasorbic acid produced by the fruits of the mountain ash (Sorbus Aucuparia (L.) Ehrh.). This acid is released from the fruits on the ground and provides a small area in which no other plant can grow thus giving the seedling a chance to grow without competition from other plants (cited by Garb, 1961).

The type of soil modifies the effect of metabolites. The glandular hairs of wormwood (Artemisia Absinthium L.)

secrete a glucoside, absinthin. This substance, washed off by the rain and distributed, is capable of inhibiting plants a mile away (Bode, 1939; Funke, 1943). The inhibitory activity is much greater in sand than in soils containing more humus. The extrametabolite is probably absorbed by the organic material rendering it inactive. Four other inhibitors have been isolated from this species (Grümmer, 1961).

Soil Inhabitators

The relationships between the soil-inhabiting bacteria, actinomycetes, fungi, nematodes, earthworms, insect larvae, algae and protozoa with one another and with roots of higher plants and humus in the soil are extremely complex. The soil population reflects the amounts and kinds of food available as well as the pH, temperature, and the moisture content. Food includes the extrametabolites secreted by the organisms into the environment some of which may be inhibitory or stimulatory to specific organisms. The interrelationships within the soil are difficult to study because any disturbance in the soil itself brings about many changes in environmental conditions.

Much of the knowledge of the production of extrametabolites by soil-inhabiting organisms has been the result of man's search for antibiotics. As early as 1878 Pasteur believed that there must be germ killers among substances

produced by "friendly" microbes. He came close to the truth about antibiotics but his techniques were inadequate (Epstein and Williams, 1946). Interest in antibiotics continued at various intensities until the discovery of Penicillin by Fleming. After 1939 intensive research projects were initiated and soil samples from all over the world were examined for organisms that were able to produce useful antibiotics. Only a few antibiotics have proved to be of clinical importance. Useful antibacterials may be produced by bacteria, by higher fungi (molds), by actinomycetes or synthesized. Those of bacterial origin are bacitracin, produced by Bacillus subtilis Cohn emend. Prazmowski, tyrothricin, gramicidin, and tyrocidine, produced by Bacillus brevis Migula emend. Ford, and polymyxin, produced by Bacillus polymyxa (Prazmowski) Migula. The penicillins, produced by Penicillium notatum Westling and P. chrysogenum Thom, are the most useful of the antibacterials from the higher fungi. Most of the antibacterials are produced by the actinomycetes and include streptomycin from Streptomyces griseus Krainsky, chloramphenicol, now synthesized, but originally produced from S. venezuela Ehrlich, Gottlieb, Burkholder, Anderson, and Pridham, aureomycin (Chlorotetracycline) from S. aureofaciens Duggar, terramycin (Oxytetracycline) from S. rimosus Sabin, Finlay and Kane, neomycin from S. fradiae Waksman and Curtiss, erythromycin from S. erythreus Waksman, and streptothricin from S. lavendulae Waksman and Curtiss. The

antibiotic, tetracycline, was discovered during catalytic reduction studies on aureomycin and is referred to as the parent compound of aureomycin and terramycin (Sokoloff, 1949; Robinson, 1953; Cooley, 1954; Burrows, 1963).

It is interesting to note that, with the exception of classic actinomycosis, the pathogenic fungi are not effected by the antibacterial antibiotics. The converse of this is also true: bacteria are not appreciably effected by antifungal substances. Several antifungal substances used clinically are produced by actinomycetes including actidione from Streptomyces griseus Krainsky, nystatin, from S. noursei Hazen and Amphoteric B from S. nodosus Goldstout, Pagano and Donovich. The mold, Penicillium griseofulvin Dierckx., produces the antifungal agent, griseofulvin.

The ability to produce an inhibitor would give an organism a competitive advantage in a community (Brian, 1957). A substantial number of organisms isolated from the soil are able to produce inhibitors: perhaps many more do. It must be remembered, however, that even if an inhibitor is produced it may be released in such small quantities that it would not be effective. If it is produced in large enough quantities to be effective it is likely that it would not spread very far from the site of its release before it would be absorbed by soil particles or utilized as a source of food by other organisms to which it was not inhibitory. The

amounts of extrametabolites produced by organisms in the soil would certainly not be as great as the amounts produced in culture media which have been carefully developed to produce maximum growth.

Many pathogenic organisms find their way into the soil but do not remain long because they are unable to compete with the free-living, non-pathogenic organisms normally present in the soil. Undoubtedly they are also destroyed by extrametabolites produced by soil-inhabitants. Escherichia coli (Migula) Castellani and Chambers is able to multiply rapidly in sterile soil but dies out quickly in unsterilized soil. Hutchinson, Weaver, and Scherara (1943) found that three strains of Pseudomonas aeruginosa (Schroeter) Migula, one strain each of Sarcenia sp., Micrococcus sp. and Flavobacterium sp., two actinomycetes and three unidentified non-spore-forming, gram-negative rods showed antagonism toward E. coli.

It must be remembered that some substances that are inhibitory in larger amounts are stimulatory in smaller amounts. Pringsheim (1949) found that on agar plates Corynebacterium diphtheriae (Flügge) Lehmann and Neuman, the causative organism of diphtheria, was inhibited by a strain of Bacillus mesentericus-vulgatus Flügge. Beyond the zone of inhibition the diphtheria colonies were larger than normal showing that small amounts of the inhibitory substance activated growth.

Protozoa make up only a small part of the living organisms in the soil. They exist either in the trophic or the encysted stage depending on the conditions in the environment. Crump (1950) found that the excystment of a soil amoeba occurred in the presence of two strains of bacteria belonging to the genus Aerobacter. Evidently the bacteria released an extrametabolite that caused the return to the trophic state.

Interrelationships Between Soil Organisms and Higher Plants

This brief discussion points out the complex situation encountered by a soil microbiologist when he attempts to find some of the answers to the relationships between the soil-inhabiting organisms. Add to the organisms already present in a particular environment the growing roots of the higher plants and a whole new set of problems is created. The roots determine the population in their immediate vicinity. Those organisms closer to the roots have lower synthetic abilities and are more dependent on the extracellular products given off by the roots. Rovira (1956) identified numerous amino acids and sugars from media containing sterile root systems. The release of these materials make it possible for root-invading organisms to be more competitive with the less selective saprophytes, and, therefore, they are able to grow. The population near the roots of higher plants, the

rhizosphere, is always greater than it is in root-free soil (Rangaswami and Vasantharajan, 1962).

Recent studies of the rhizosphere of crop plants have resulted in valuable information on the ecology of soil organisms. The nematode population is governed by the amino acid concentration in the soil. Some nematode species grow better in soil in which grains are grown whereas others occur in soil where legumes are grown (Henderson and Katznelson, 1961). Stevenson (1960) reported that root-rot of wheat was controlled to some extent by the antibiotic activity of actinomycetes. Buxton (1960) reported that root zone conditions are favorable for the production of minute but effective amounts of inhibitory substances. He found that pea root extract enhanced the ability of rhizosphere fungi to inhibit Fusarium oxysporum.

Not only does the invading root effect the organisms present in the soil but these organisms do, in turn, effect the higher plants. Polypeptide enzymes, which are extracellular products of many soil organisms, can injure the root tissues at low concentrations. The polypeptides are quickly absorbed at particular sites on the root surfaces bringing about a change in permeability which then allows leakage of cell metabolites (Norman, 1955, 1960). The damage to the root tissue is irreparable and these areas serve as portals of entry for the weakly pathogenic pathogens which have the ectotrophic habit unless they are antagonized

by some antibiotic (Norman, 1960).

One of the most interesting groups of organisms that inhabit the soil are the nitrogen-fixers. These include the free-living bacteria belonging to the genera Nitrosomonas, Nitrobacter, Azotobacter, and a few Closteridium, several blue-green algae, and the nodule producer, Rhizobium (Thimann, 1955). Recent research has been aimed at a better understanding of the biochemistry of these organisms. Species of Rhizobium invade the roots of legumes after the host roots secrete a substance that stimulates the infective process. Nodulation of younger plants is stimulated by the additions of solutions which have been in contact with the roots of older plants (Thornton, 1929a, 1929b; Clark, 1957). Elkan (1961) found that root excretions of mutant non-nodulating soybeans decrease the nodulation of normal plants. Other inhibitory actions with reference to the nitrogen-fixers include the reports by Nickell and Burkholder (1947) that Azotobacter was inhibited somewhat by the actinomycetes in the soil, and one by Chan et al. (1963) which described the inhibitions of Azotobacter by soil and root extracts of oats, soybeans and wheat.

Aquatic Organisms

In observing aquatic environments it is easy to see the changes that occur from season to season and even from day to day. Many changes in the population are responses to the seasonal changes but there are many that are not understood. In aquatic habitats extrametabolites may be carried farther from their origin and thus produce effects at greater distances than those produced by terrestrial organisms. On the other hand extrametabolites may be dispersed and diluted to such an extent that they have no apparent effect on the environment.

Protozoa

The protozoa are important inhabitants of aquatic environments. Very little is known of the interrelationships between the various protozoa and between the protozoa and other members in the community. Most of the facts pertaining to the physiology of these organisms have been obtained from work carried out in culture and may or may not be pertinent to their natural ecology.

An important factor in the survival and distribution of protozoa is the ability to encyst. Many factors in the environment, including lack of oxygen and food, extremes in temperature, and evaporation of water are responsible for encystment. Several investigators have reported encystment

as a response to the accumulation of metabolic products (Bela, 1921; Mast and Ibara, 1923; Beers, 1926: cited by Kudo, 1951). Others have reported that encystment occurred when certain bacteria were present (Mouton, 1902; Belar, 1926: cited by Kudo, 1951).

Likewise excystment has been reported to be caused by substances in the culture medium. Thimann and Barker (1934) and Haagen-Smith and Thimann (1938) reported that Colpoda cucullus Müller excysted in response to specific substances from plant infusions. Beers (1945) reported that the primary cause of excystment in Tillinia magna Grüber was a change in osmotic pressure and that the secondary cause was inducing substances in the medium.

Among species of Ciliata which undergo various types of sexual reproduction, such as autogamy and conjugation, there seems to be several mating types. Sonneborn (1943) identified seven varieties of Paramecium aurelia Ehrenberg. His experimental work showed that some types of this species, "sensitive" types, were killed by a substance liberated into the medium by other races or types. The types that produced this substance were called "killers" and were immune to this substance. The extrametabolite was called paramecin and was identified as a desoxyribonucleo-protein. Evidence of another extrametabolite was found by Kimball in cultures of Euplotes patella Müller. He found that water taken from the medium in

which Euplotes were conjugating would induce conjugation in other mating types (Kimball, 1942, 1943). Culture medium from some varieties of Paramecium bursaria Ehrenberg brought about autogamy in single animals (Chen, 1945).

Metz (1949) reported that mating activities involve substances present on the surface of the cilia. The interactions of that substance results in mating. Subsequent investigations suggested that the substance is a protein because the mating activity was destroyed by the addition of proteolytic enzymes (Metz and Butterfield, 1951). In one protozoan, Nycotatherus cordiformis Ehrenberg, conjugation takes place only among those individuals that live in metamorphosing tadpoles. This suggests that some extrametabolite produced by the tadpole is responsible for conjugation in this species (Wichterman, 1926).

Asexual reproduction in protozoa occurs by binary fission. Robinson, in work done between 1921 and 1926, found that the division rate of Enchelys and Colpoda was increased as much as ten times when more than one animal was present. He assumed that an agent was secreted into the medium and that the concentration was increased when more than one animal was present. Mast and Price confirmed these results for Chilomonas paramecium Ehrenberg (1939) but Phelps (1935) found that this was not true for Tetrahymena.

Higher Animals

Fish in overpopulated lakes and ponds are stunted. The lack of growth has been attributed to various factors such as insufficient food and lack of living space. From observation and experimentation it would seem that one factor in the retardation is the production of inhibitory extrametabolites. Vernon (1899) found that culture water from a species of sea urchin inhibited the larvae of the same species and the growth of closely related species while that of more distantly related species was stimulated. Evidently the extrametabolite promoted the growth of some species while inhibiting the growth of others.

The lack of growth and delay in metamorphosis of frogs grown in culture has been attributed to several factors. Young (1878, 1885), attributed the effect on metamorphosis to lack of aeration. Adolph (1931) attributed the lack of growth to the fact that tadpoles do not feed as readily in crowded cultures as they do in uncrowded situations although food is available. Lynn and Edelman (1936), rather curiously, attributed the lack of growth to crowding itself. Rugh (1934) concluded that the rapid movement of tadpoles after collisions expended energy that might otherwise have been used for growth. Altogether Rugh listed ten factors as possible reasons for the effect of growth, one of which was the production of growth inhibiting substances (autotoxins).

Rose (1960) found that tadpoles of Rana pipiens Schreber grew at different rates and that, although all grew after hatching, only the most rapidly growing and largest survived to metamorphose. The stunted ones stopped growing, failed to eat and died if left with the larger. He found that by withholding food from the larger the water did not become inhibitory to the smaller. Evidently the higher the metabolism the greater is the amount of inhibitor produced. Large tadpoles which naturally have a higher metabolic rate can make the culture water inhibitory in a few minutes. Experimental evidence indicated that in all cultures varying in size from 1 to 75 liters the small grew more slowly than the larger and some failed to metamorphose. The inhibitor appears to be long lasting and proteinaceous in nature. The presence of other organisms and ultraviolet light reduce the inhibitory effects (Rose, 1961).

Richards, working with this same species, found that the inhibition was associated with large particles, larger than bacteria. She found that treatments that destroyed cells removed the inhibitory products (Richards, 1958). Later she found a cell, perhaps an alga, to be present in the intestines of the growing tadpoles. The source of the inhibitory substance was also the intestine. This cell was not present in the intestine of another tadpole belonging to the genus Scaphiopus. The cell was introduced into cultures of Scaphiopus with the result that species specific

inhibition was produced. Cultures of Scaphiopus were inhibited by water from cultures in which Scaphiopus tadpoles had been living but not by water from cultures of Rana sp. (cited by Rose, 1961). In culture three urodels, three fish and the snail, Physa, failed to inhibit the growth of the tadpoles. In most cases growth increased perhaps because the inhibitors were removed or at least neutralized by these organisms.

Brown (1946) has reported the same phenomenon for trout, Salmo trutta Linn. Fry grown under favorable conditions show a great variation in size within a few months. The smaller ones when separated from the larger showed this same variation within a few months. Something was produced by the larger trout that inhibited the growth of the smaller as was evidenced by the fact that it was the smallest that exhibited the most marked retardation. In both groups the smaller eventually died.

Breder and Coates (1932) found that in a 5.5 liter aquarium at first containing 50 guppies (Lebistes reticulatus Peters) no more than nine ever survived. If the water was not changed no amount of feeding prevented infanticide and cannibalism. The slow swimmers were chased relentlessly while the fast swimmers were not molested. Slowness may be the result of extrametabolites released into the water. The addition of unrelated species to the aquarium increased the survival of the guppies.

Similar results have been obtained with White Cloud Mountain fish (Tanichthys albonabes Lin.). Never more than 20 hatched eggs reached the one-centimeter size. After feeding started the smaller stopped eating and eventually died even when food was plentiful. If only twenty fish were allowed to remain in an aquarium all survived. Similarly not more than fifteen Barbus tetrazona (Puntius partipentazona Fowler) of a spawning of over 200 survived to the one-centimeter size in a 15 liter aquarium. More survived if half of the water was replaced two or three times daily indicating that dilution of the inhibitor reduces its effectiveness (cited by Rose, 1959). The literature contains many more examples of inhibitory substances which are produced by animals.

Algae

Evidence of Extrametabolite Production in General

Algal populations vary from one body of water to another and from season to season. This variation seems to be primarily the result of chemical factors in the water but physical and biological factors are also important. Akehurst (1931) was one of the early observers of the seasonal distribution of algae in small ponds. He found that the oil-producing species belonging to the Bacillariales, Chrysophyceae,

Heterokontae, and Chloromonadales occurred in the largest numbers in the spring and that their numbers decreased during the summer. This decrease was accompanied by an increase in the starch-producing groups, the Myxophyceae, Eugleninae, Isokontae, and Cryptophyceae. He believed that the oil-producers secreted a substance that limited its own growth while at the same time stimulated the growth of the starch producers. This product would, therefore, be an inhibitor and a growth substance or growth promotor.

The fluctuation of planktonic populations in both fresh water and marine environments has been investigated many times and the investigators have attempted to correlate their findings with observed factors in the environment. Harvey (1935) suggested that the decrease in phytoplankton was caused by overgrazing by zooplankton. Observations by Hardy and Gunther (1935) and Harvey (1936) led these investigators to believe that during periods of phytoplankton domination animals move away from the area because the environment becomes unfavorable to their growth. They believed that chemicals produced by the phytoplankton were detrimental to the zooplankton and were responsible, therefore, for the scarcity of animals. Lucas (1938, 1947, 1949) concluded that the relative amounts of phytoplankton and zooplankton were the results of external metabolites which were able to stimulate the growth of some species while inhibiting the growth of others. Ryther (1954) found that

Daphnia stopped feeding and eventually died in a senescent culture of Chlorella but continued to grow and reproduce in an actively growing Chlorella culture. His work thus substantiated that of previous investigators.

Many investigators have endeavored to correlate the seasonal distribution with the amounts of major inorganic nutrients available (Chandler, 1940; Chandler and Weeks, 1945; Hutchinson, 1944; Ketchum and Keen, 1948; Ketchum, 1953; Rao, 1953; Brooks, 1953). Still others have demonstrated that minor elements play an important part in the physiology of the algae and have investigated these elements as possible factors in the fluctuations (Holm-Hansen et al., 1954; Kratz and Myers, 1955; Lund, 1957; Provasoli and Pintner, 1953).

More recently dissolved organic materials have been investigated as possible reasons for seasonal changes in algal populations (Saunders, 1957; Hartman and Graffius, 1960; Anderson, 1961; Krauss, 1961).

Vitamin B₁₂, thiamine and biotin are the only vitamins known at the present time to be of importance for the growth of algae. Vitamin B₁₂ is given off by several algae particularly diatoms and may, in part, account for the seasonal distribution in fresh water lakes. Vitamin B₁₂ content has been measured in fresh water by Hutchinson in 1943 and by Benoit in 1957, and in sea water by several

investigators (Burkholder and Burkholder, 1953; Cowey, 1956; Daisley and Fisher, 1948; Droop, 1957, 1961). The only evidence of the production of extracellular thiamine by algae was reported in the culture medium of Coccomyxa (Lewin, 1958). The algal dynamic substance reported by Lefevre and Jacob (1949) may be vitamin-like in nature. It may be that the greatest amount of these growth substances is produced by bacterial activity because these cultures were not bacterial-free.

Another factor accounting for seasonal succession of algae may be inhibitors that are auto-inhibitors or alga-inhibitors (algastatic or alga-cidal). Many workers have investigated the phenomenon of alga-inhibitor production, including Flint and Moreland (1946), Lefevre and Jacob (1949), Lefevre et al. (1952), Rice (1954), and Jorgensen (1956). Some of these substances have been shown to be algastatic while others have been algacidal, and, again, perhaps the effectiveness is proportional to the amount present in an ecosystem. Of all the alga-inhibitory substances reported, few have been characterized chemically. Proctor (1957) demonstrated that a fatty acid produced by Chlamydomonas reinhardtii Dang. inhibited the growth of Haematococcus pluvialis Reichenow. Auto-inhibitors have been reported as being produced by Nostoc punctiforme (Kuetz.) Hariot (Harder, 1917), by a strain of Chlorella vulgaris Beyerinck (Pratt and Fong, 1949), and by Nitzschia palea Kütz (von

Denffer, 1948; Jørgensen, 1956). None of these have been characterized chemically. Recently Scutt (1964), using the same strain of Chlorella vulgaris (Columbian strain) as Pratt and Fong, found that there was no auto-inhibitor production. This was attributed to the fact that auto-inhibitor production occurs only under certain conditions.

The growth of algae may also be effected by auxin-like substances. Auxins have been detected in the culture medium of Chlorella sp. and of Anabaena cylindrica Lemm., and in lake water which supported a nearly unialgal bloom of Oscillatoria (Bentley, 1958, 1960). Bentley separated one substance by paper chromatography and identified it as ammoniacal isopropanol. Another substance, a water-soluble, unstable complex, was found to break down into a product similar to indoleacetic acid.

There is some evidence that hormone-like substances released into the medium are responsible for sexual activity (Roper, 1952, 1957). Hoffman (1960) reported that substances produced by oogonia of Oedogonium which attract the sperm were species-specific. Evidence of the same type of substance was reported by Cook and Elvidge (1951) for Fucus serratus L. but was not species-specific. Sperm of two other species of Fucus were attracted. Although not completely analyzed the substance was thought to be a volatile hydrocarbon.

Diwald (1939) showed that the gametes of the dino-flagellate Glenodinium lubiniensiforme (Diwald) H. P. were released only after being treated with the culture filtrate from a compatible strain indicating that a hormone-like substance must be produced. The gametes of Pandorina morum (Muell) Bory are not released from the colonial matrix unless there are colonies of a complimentary mating type present. Culture medium from a complimentary type brought about the release of the gametes (Coleman, 1959).

Extensive work by Förster and his co-workers (1954a, 1954b, 1956: cited by Coleman, 1962) has characterized the male and female substances liberated by Chlamydomonas eugametos Moewus that result in clumping as glycoproteins of high molecular weight. Similar clumping substances have been demonstrated in the culture medium of C. moewusii Gerl. and C. reinhardtii. The substances from C. moewusii and C. eugametos are interchangeable but have no effect on C. reinhardtii. Also the substances produced by C. reinhardtii have no effect on the other two species. The substances seem to be released from the flagella (Förster, 1959; Förster and Weise, 1955: cited by Coleman, 1962).

Amino acids seem to be a common extrametabolite produced by algae. Culture filtrates of Oscillatoria splendida Greville were found to contain oxalic, tartaric, succinic, and other acids (Goryunova, 1950: cited by Fogg, 1962).

Species of Chlamydomonas were found to liberate glycolic, oxalic and possibly pyruvic acid (Allen, 1957). Glycolic acid liberation was reported by Lewin (1957) for two species of Chlamydomonas. Actively growing cultures of Chlorella pyrenoidosa were found to liberate three to eight milligrams per liter depending upon the environmental factors influencing the uptake of the bicarbonate (Tolbert and Zill, 1956, 1957).

Polysaccharides are also secreted by some species of algae. Oscillatoria splendida Greville excretes a polysaccharide which Goryunova (1950) believes has something to do with trichome production (cited by Fogg, 1962). Eighteen species, mostly of Chlamydomonas, were found to secrete a polysaccharide consisting mostly of galactose and arabinose. The polysaccharide secreted by C. ulvaensis, however, consisted mostly of glucose and xylose (Lewin, 1956). During short-termed photosynthesis the polysaccharide secreted by Chlorella pyrenoidosa was mostly sucrose (Tolbert and Zill, 1956). Polysaccharides have also been detected in the culture medium of several marine and brackish-water flagellates (Guillard and Wangersky, 1958).

Under certain ecological conditions about 65 species of algae have been known to reproduce so rapidly that the surface of the water becomes covered and the water is discolored. This phenomenon is referred to as a "water bloom."

Of interest to man and his welfare are the "red tides," a term used in reference to the discoloration of water caused by blooms of marine dinoflagellates. These blooms may or may not be accompanied by death of organisms. It is possible that the "red plague" referred to in the Bible in Exodus 7:17-18 was such a bloom. Darwin, in 1832, reported the occurrence of discolored water and the death of small animals off the coast of Chile (cited by Galtsoff, 1949). Between 1899 and 1934, twenty-four reports of red tides were made from Japan. Sixteen of these were accompanied by the death of fish and shellfish. Of these, three were due to Nocticula, nine to other flagellates, five to diatoms and one to a blue-green (Galtsoff, 1949).

The dinoflagellate, Gymnodinium brevis Davis, is the causative organism of the red tides that appear periodically along the coast of Florida. Besides causing high mortality of commercial and non-commercial fish, these blooms have caused the death of turtles, porpoises, shrimp, barnacles, oysters, coquinas, and the common blue, the fiddler, and the mud crabs (Davis, 1948). This same species has produced lethal blooms along the Gulf of Mexico on both the Mexican and Texan shores (Wilson and Ray, 1954, 1956; Ray and Wilson, 1957). The action of the fish are similar to those reported by Prescott in blue-green blooms (Prescott, 1948). Ray and Wilson (1957) showed that the filtrate was toxic and that

the toxicity increased when the organisms were subjected to treatment which caused the cells to rupture. This work indicated that the toxin was an endotoxin.

Blooms of Gonyaulax catenella Whedon and Kofoed have been responsible for red tides in the water off Galveston, Texas, and along the west coast of the United States and as far north as British Columbia. This organism is responsible for the clinical entity known as "mussel poisoning." The danger involved in eating mussels was known before the Sixteenth Century. In 1528, Cabeza de Vaca found that the aborigines referred to one season as the "time when fish die" (Connell and Cross, 1951). Many Indian tribes were forbidden to eat mussels during the time of the red tides. On the west coast between 1934 and 1941 there were 346 cases of mussel poisoning, 24 of which resulted in death. Anderson (1950) recorded 60 cases of poisoning after an outbreak of this organism in the vicinity of the Straits of Georgia (British Columbia). People along the west coast of the United States are now forbidden to gather shellfish during the season when these organisms may be poisonous (Davis, 1948).

The toxin produced by this alga is ten times more powerful than strychnine when given to mice. In man, the first symptoms produced are a numbness of the lips, tongue and fingertips. This is followed by muscular incoordination, paralysis, and, in severe cases, death in from two to twelve

hours. The mussels are not effected by the toxin but accumulate it in their digestive glands. Chemically, it seems to belong to the class of alkaloids to which strychnine, muscarine, and acontine belong (Galtsoff, 1948). Somner et al. (1948a, 1948b), extracted the poison from the livers and digestive glands of the California clam, Mytilus californianus Conrad, and called it a neuropoison.

Although these two species have been reported as the causative organism involved in the majority of lethal blooms other organisms have been reported to produce toxicity. A toxic bloom of Pyridinium phoneus occurred in Belgium in 1938-1939. The poison extracted from this organism was highly toxic, killing white mice in 60 to 90 seconds. Normal mussels placed in water containing this organism acquired toxicity in direct proportion to the concentration of this organism (Galtsoff, 1948). For 15 years the blooms occurring seasonally along the eastern coast of Canada have been studied. The toxicity of these blooms seems to be produced by Gonyaulax tamarensis Lebour. Six persons became ill from eating poisonous shellfish in 1961 (Needler, 1949; Medcak, 1960, 1961; Prakash, 1963).

Blue-Green Algae

The first report of a toxic water bloom may well have been made by Dwight when he was president of Yale College. Observing the organisms that floated on the lakes in New England he tried to correlate their occurrence with the prevalence of certain diseases. In 1796 he wrote:

I suppose vegetable putrefaction to be especially considered the cause of autumnal diseases. That (it) may be an auxiliary cause of these evils may, I think, be rationally admitted, but that it is the sole cause, or even the principal cause, may be fairly questioned. This putrefaction exists regularly every year; the diseases, in any given place, rarely. The putrefaction exists throughout the whole country; the diseases, whenever they exist, are confined to a few particular spots. (They cannot be due to stagnant waters, because they) are found on plains, in vallies, on hills and even on the highest inhabited mountains (cited by Baker, 1948).

The first accurate report of a "toxic" water bloom was made by George Francis of Adelaide, Australia. He wrote a letter to the editor of Nature describing the low water level and the high temperature and the bloom of algae which occurred at the same time as a loss of domestic animals along the banks of a lake. The letter was published in the May 2, 1878 edition of this magazine and stated:

A conferva that is indigenous and confined to the lakes has been produced in excessive quantities, so much as to render the water unwholesome.

It is, I believe, Nodularia spumigena, allied to Protococcus. Being very light, it floats on the water except during breezes, when it becomes diffused. Thus floating, it is wafted to the lee

shores, and forming a thick scum like green oil paint some two or six inches thick, and as thick and pasty as porridge, it is swallowed by cattle when drinking, especially such as suck their drink at the surface like horses. This acts poisonously, and rapidly causes death: symptoms --stupor and unconsciousness, falling and remaining quiet, as if asleep unless touched, when convulsions come on, with head and neck drawn back by rigid spasm, which subsides before death. Time--sheep, from one to six hours; horses, eight to twenty-four hours; dogs, four to five hours; pigs, three to four hours (cited by Olson, 1951).

Lethal water blooms were reported at Waterville, Minnesota, in 1882, 1883, and 1884 on Lakes Tetonka, Sakatah and Cordova. The lakes were well-drained by the Cannon River and had been used for watering stock for many years. Porter, Professor of Agriculture at the University of Minnesota, investigated the bloom which occurred in July of 1882. He found the lakes filled with floating particles of vegetable origin. Another bloom in 1883 led him to believe that the plant material poisoned the cattle. In 1884 Porter took J. C. Arthur, botanist of the New York Experimental Station, and Stalker, Professor of Veterinarian Science at Iowa State, with him to examine the lakes. Arthur described the condition as follows:

The facts illicited were that quite a number of animals, largely cattle, had died at the time when the lakes were filled with minute algae (then called Rivularia fluitans but now referred to as Gloeotrichia pium), disseminated through the water and forming a thick, dark-green scum when collected by the wind. That some of the animals had drunk of the water and scum a few hours before they died was positively known, and that all had done so seemed from the circumstances

quite probable. After a most careful examination the only plausible hypothesis that could be advanced to account for the death of animals was that the algae present possessed some toxic or other baneful properties sufficiently powerful to kill a cow in a half an hour or more after freely drinking of it. The well-established reputation of all algae for innocuousness made this hypothesis appear from the very first extremely improbable but for want of the slightest hint in any other direction it was thought worthwhile to bear in mind, and to investigate the matter further.

Professor Stalker described the outbreak as follows:

Coincident with the loss of livestock, a peculiar vegetable growth makes its appearance in the water of these lakes and the testimony goes to show that only those animals which obtain water from the lake at this season are effected with the disease. The testimony of all farmers is to the effect that:

First, there have been no losses except among animals obtaining water from the lakes;

Second, in every instance where deaths have occurred the wind had for some days previous blown shoreward where the animals drank and carried the plants to the margins of the lakes in large quantities;

Third, no losses have occurred after the odor from the lakes became offensive (cited by Fitch et al., 1934).

From 1900 to the present time toxic water blooms have been reported from several parts of the world. An especially large number involved bodies of water in mid-continental North America including parts of Canada and the United States. Fitch et al. (1934) described several blooms which occurred in various Minnesota lakes from 1900 to 1930. Deaths of cattle, sheep, hogs, and chickens occurred at the

time that these blooms were present. The blooms were produced by Aphanizomenon flos-aquae, Coelosphaerium Kuetzingianum Naegeli, Anabaena flos-aquae, Microcystis flos-aquae (Wittr.) Kirchner, and Microcystis aeruginosa.

In 1933 a series of three blooms involving Microcystis flos-aquae occurred in Hall Lake north of Fairmont, Minnesota. The first resulted in the death of ten sheep and the second, eight lambs and some chickens. Tests for copper sulfate and cyanide were negative. A half-gallon of water from the lake killed five sheep in the laboratory. Material from the lake given orally and interperitoneally produced death in guinea pigs, rabbits and chickens. Autopsy of the sheep disclosed no gross pathology. This was the first time that algal toxicity was demonstrated in the laboratory.

Not all deaths attributed to algal blooms have been those of domesticated animals. Toxicity in a small lake in Colorado resulted in the death of ducks, wild birds, snakes, fish, and salamanders. A black-crested heron, partially paralyzed when found, recovered in a few days (Deem and Thorp, 1939). A heavy fish kill involving carp, northern pike, yellow perchpike (walleye), black crappies, bluegills, suckers, hog suckers, buffalo, and an eel occurred in the Yahara River, Wisconsin. A bloom of Aphanizomenon flos-aquae, three or four acres in area and several inches thick, had developed above Lake Kegonsa Lock. This bloom had been allowed to pass over the lock during a six-hour period.

Experimental work showed that water taken from the lake 14 miles downstream from the lock was lethal to fish. All experimental fish placed in this water died within 9 days while none of those placed in spring water died (Mackenthun et al., 1948). There was no direct evidence that Aphanizomenon flos-aquae was responsible for the fish mortality. There was, however, the possibility that it was more critical than the depletion of oxygen caused by the decay of the algal mass.

Reports indicate that blooms produced by the same species vary in toxicity. Two blooms consisting of Microcystis flos-aquae, Aphanizomenon flos-aquae, and Anabaena flos-aquae occurred in the fall of 1933 in Lake Lac que Parle at Milan, Minnesota. Turkeys, ducks and geese died after drinking from the lake at the time of the first bloom. Other animals had pawed at the water before drinking and had not been effected. At the time of the second bloom not only poultry but also horses, cattle and pigs died (Fitch et al., 1934). It is possible that the animals by pawing at the water had dispersed the algae thus reducing the amount ingested to a sublethal quantity. It seems likely, however, that the two blooms varied in toxicity. The variation in toxicity may have been the results of differences in ecological factors at the time of the blooms.

The blue-green algae had thus been implicated in the death of many kinds of animals. Researchers next turned their efforts to obtaining more information concerning the formation of these blooms and the toxin and its effects. Deem and Thorp (1939) found that the toxic material was heat stable and that it remained effective after being stored in the refrigerator for three weeks. Prescott (1948) observed that fish which had died in an Iowa lake that supported a bloom of Aphanizomenon flos-aquae exhibited no symptoms of suffocation or disease. He demonstrated that fish placed in lake water containing this species died even when the dissolved oxygen content was as high as 12.6 ppm. Death occurred in small aquaria as well as in excavated ponds constructed in such a way that water from the lake was able to enter and leave. Death occurred in two to six hours in these various experiments. Chemical analysis of the water showed that hydroxylamine, a decomposition product of the algae, and hydrogen sulfide were present; the latter in large enough amounts to kill (Prescott, 1948).

Using the material collected during a water bloom which occurred in Lake Dauphin in Manitoba in 1951. McLeod and Bodnar (1951) concluded that the toxin was present in the plants and might be released into the water upon the disintegration of the algae. The toxin was not destroyed by air drying at 37°C, by freezing or by ultraviolet light.

The algae present in the bloom were: Aphanizomenon flos-aquae, 99 per cent; Anabaena flos-aquae, 0.9 per cent; and Microcystis aeruginosa, 0.1 per cent. The species producing the toxin, the fate of the toxin and the length of time it was effective were not determined. On the basis of quantity it might be assumed that Aphanizomenon was responsible.

The symptomology of the toxin is based largely on observations made by the owners of the animals involved. In only a few cases have veterinarians arrived in time to see the effects. Convulsions of varying severity occurred with extreme salivation in some cases. Symptoms and effects reported from autopsies have not been constant. One veterinarian reported a reddening of the gastrovascular tract. A piece of liver from one victim made a dog violently ill five minutes after it was ingested. The animal, after being made to regurgitate, recovered after a long convalescence. The liver appeared normal (Olson, 1951).

Ashworth and Mason (1949) used extracts from a naturally occurring bloom of Microcystis aeruginosa to study the effects of the toxin on white rats. The effects produced were similar to those produced by the poison of Amanita phalloides Fr. The liver was the first organ damaged undergoing marked cellular necrosis. The damage to the heart and kidneys which followed was not caused by necrosis of the liver tissue but by the poison itself. No damage to the

spleen, the suprarenal glands, the intestine or the brain was apparent.

Steyn, reporting on lethal blooms from South Africa, believed that the toxin contains two principles. One of these he thought effected the central nervous system. The second principle, found in phycocyanin, he believed caused skin lesions. This substance, reaching the blood vessels of the skin, absorbed ultraviolet rays resulting in burns and lesions. Chronic cases lived as long as a month before dying (Steyn, 1945: cited by Olson, 1951; and Prescott, 1960). This photosensitivity was also described in cattle which survived a lethal bloom in 1946. The bloom occurred in the Des Lac Lake, North Dakota. The skin in the white-haired portions blistered and skin sloughed off (Brandenburg and Shigley, 1947).

Effects of the toxic algae collected from naturally-occurring blooms have varied in toxicity. It was not clear whether the material was an endotoxin or an exotoxin or whether it was produced by the algae, by organisms living with the algae or by a combination of these. Louw, working with Microcystis toxica Stephens, found that the toxin was an endotoxin released upon the death and decay of the algae (Louw, 1950). Work by Mason and Wheeler (1942), by Olson (1951), and by Shelbusky (1951) supposed that the material from Microcystis aeruginosa was an exotoxin. Mason and Wheeler found, however, that freezing the algae increased

the toxicity. Freezing and thawing altered the permeability of the cells.

Research to this point had indicated that the toxin (1) existed in the water surrounding the algae; (2) dialyzed through cellophane and animal membranes; (3) was non-volatile; (4) was relatively heat stable (not losing potency by autoclaving at 15 pounds pressure for an hour and a half or by dry heat at 100°C for 100 hours); (5) was soluble in water, 95 per cent alcohol, and methanol; (6) was insoluble in benzene, ether, and acetone; (7) was resistant to pH changes; (8) was readily absorbed on activated charcoal from which it was removed with difficulty; (9) reacted negatively to tests for cyanide, nitrate, nitrite, philocarpine, saponins and strychnine; (10) was not inactivated by human blood; and (11) was neither an antigen nor a hapten (Olson, 1951; Shelbusky, 1951).

The development of culture techniques made blue-green algae available in large enough quantities for the laboratory study of water bloom production and the variation in toxicity. Olson isolated a strain of Microcystis aeruginosa that exhibited low toxicity to white mice. The toxicity varied considerably in the cultures grown. Thompson et al. (1957) isolated a strain (Western Strain) that was not toxic to mice. They did, however, isolate a bacterial contaminant that produced slow death in 3 to 48 hours.

This clearly indicated that more than one substance can be involved in algal toxicity.

Hughes, Gorham and Zehnder (1958) isolated Microcystis aeruginosa Strain NRC-1 from a lake in Ontario and found that it was more toxic than the strain isolated by Olson. Cells from a 40-day old culture killed mice in 24 to 52 hours. Cultures of this strain produced death in 30 to 60 minutes after the cells had been subjected to rapid freezing followed by rapid thawing, to sonic disintegration, or to darkness and reduced oxygen supply. Two toxins were produced by this strain: one that produced slow death and referred to as the slow death factor (SDF) and one that produced fast death and referred to as the fast death factor (FDF). The latter appears to be an endotoxin presumably released when the cells become more permeable. Decomposition may be the mechanism by which FDF is released in naturally-occurring water blooms.

Several strains of Microcystis aeruginosa have been isolated by Gorham and his associates from the same and different water blooms and were found to produce SDF, FDF, or SDF and FDF. Upon centrifuging cultures of strain NRC-1, the FDF was found to be in the algal fraction and the SDF in the bacterial fraction. When bacteria from this strain were added to cultures of non-toxic strains no FDF was produced. The variability in toxicity seems to be controlled by genetic as well as physiological factors (Gorham, 1962).

The toxin was present in such small amounts that it was necessary to concentrate it before chemical analyses could be made. Separation of the toxin was made by electrophoresis at a pH of 9.1. Five peptides were separated, one of which proved to be toxic. The toxic peptide was made up of asparatic acid, glutamic acid, serine, valine, ornithine, alanine and leucine in the ration of 1:2:1:1:1:2:2. This peptide seems to have a cyclic configuration as indicated by the fact that it was difficult to hydrolyze with enzymes. No hydrolysis occurred with pepsin, papain, trypsin, chymotrypsin, carboxypeptidase, polidase, takadiastase, or takadiastase/papain. This resistance to hydrolysis could explain the effectiveness of the toxin when taken orally in water blooms (Bishop et al., 1959). Structurally this toxin may be similar to bacitracin and gramicidin which are both peptides. It did not show any antibacterial activity.

Gorham (1962) reported the deaths of mice in one to ten minutes after materials from five strains of Anabaena flos-aquae were administered intraperitoneally. The origin of this very fast death factor (VFDF) was probably the algae and its production appeared to be genetically controlled.

The toxicity produced by the blue-green algae has been reported to effect 69 animals representing 21 families. There are reasons to suspect that man may also be susceptible. Man would probably not ingest water supporting an algal

bloom because of the odor and taste. Water supplies might be effected without his knowledge. Underground water could contain toxic material if a shallow well tapped a water-bearing stratum in a low-lying area where the underground flow comes from a body of water at a higher level in which an algal bloom occurred.

Reports indicate that algal blooms may be the cause of intestinal disturbances. Many people were effected in the Ohio Valley and Charleston, West Virginia, during the drought years of 1930 and 1931. The waters in the rivers were low and warm, conditions conducive to algal blooms. The disturbances were not of bacterial origin because the water met the bacterial standards. The outbreaks occurred just before or at the same time as the blooms (Tisdale, 1931a, 1931b). A dysentery outbreak just outside of Washington, D. C., was suspected to be caused by algae. This outbreak occurred at the same time as an algal bloom in the Anacostia Reservoir which supplies water to this area.

The syndrome known as Haff's Disease has been reported several times along the shores of the Koenigsberg Haff in East Prussia. The disease is associated with the consumption of fish and fish livers from lakes which supported blooms of the same species of algae responsible for toxicity in the United States and Canada. It is possible that the fish ingested the algae and concentrated the toxin

in their livers. Injection of the scum from one of these blooms produced toxic symptoms in animals (cited by Olson, 1951).

Danger may also exist in consuming animals which have died during algal blooms. A mother cat brought a duck to feed her kittens. A few hours later all of the kittens were dead. The mother, having eaten some of the duck herself, became violently ill. Her fate was not known. Dead birds or other animals should never be picked up with the thought of eating them.

Blue-green algae have also been responsible for allergies and skin rashes. Heise (1949) reported that an antigen had been successfully prepared from algae and had been used to treat a woman who had developed symptoms of hay fever after swimming in a lake. The algae retained a high titer of antigenicity even after storage in formaldehyde for two and a half years. Heise (1951) also showed that extracts of Microcystis produced the same symptoms of hay fever in susceptible people which had been produced by Lyngbya. A child, after swimming in a lake in which a blue-green bloom was thriving, developed a rash. The child was evidently sensitive to the blue pigment phycocyanin (Cohen and Rief, 1953). More recently Banner (1959) reported a type of dermatitis that was produced by Lyngbya majuscula Harv., a marine alga.

Conditions Conducive to Blue-Green Blooms

Among fresh water algae the Cyanophyta often produce blooms. Ecological conditions favoring the growth of these algae are nutrients such as nitrogen and phosphorus in high content, high carbon dioxide reserves in the form of bicarbonates, and high temperatures of 26 to 30°C (Prescott, 1948, 1960). Lakes which receive drainage from tilled soil, sewer effluents, barnyards, city streets, factories, or summer resorts are able to produce these blooms. As lakes become older, shallower, and warmer for longer periods of time during the year they are more likely to support blooms. Blooms of the blue-greens often impart obnoxious odors and tastes to water.

Phosphorus and nitrogen are the elements most likely to limit the growth of algae (Hutchinson, 1944; Rodhe, 1948). Nitrogen is the mineral required in the greatest concentration (Gerloff et al., 1952, 1959a, 1959b). Guseva, in 1937, when first investigating a water bloom in the Ucha Reservoir of the Moscow-Volga Canal, found that Anabaena Lemmermannii Richter was present in the largest numbers. The decrease in this species was followed by an increase in Aphanizomenon flos-aquae. This species reached a maximum and then declined in numbers. The decline in Aphanizomenon flos-aquae was followed by a fall bloom of Asterionella formosa Hass. He attributed the decline of Anabaena Lemmermannii to a

decrease in availability of nitrogen, phosphorus and iron. The increased growth of Aphanizomenon flos-aquae indicated to him that the requirements of this species for these three elements is lower than the requirements of Anabaena Lemmermannii. The decline of Aphanizomenon flos-aquae was attributed to the decrease in concentration of phosphorus and iron and the disappearance of this species was attributed to an increase in ammonia and manganese. The fall bloom of Asterionella formosa he attributed to a replenishing of essential nutrients by the fall turnover.

Water from the reservoir was collected periodically and used as a basis for a medium to study the mineral nutrition of the species involved in the bloom. The water was not filtered. The effects of varying the concentrations of nitrogen, phosphorus, iron and manganese were studied by adding these elements to cultures made with medium based on the water from the reservoir. In some of the experiments only one element was added and in others, combinations of these elements were added.

Guseva found that the maximum growth of Anabaena Lemmermannii occurred with the addition of 0.6 to 0.8 ppm of nitrogen supplied as $\text{Ca}(\text{NO}_3)_2$ while only 0.4 ppm produced maximum growth in Aphanizomenon flos-aquae. Chu (1943) found that the effect of nitrogen concentration on seven plankters varied considerably. He found that the lower

limits that produced maximum growth varied from 1.3 ppm for Pediastrum boryanum (Turp.) Menegh. to 3.5 ppm for Botryococcus Braunii Kütz. and that the upper limit varied from 5.3 ppm for Staurastrum paradoxum Meyen to 17.0 ppm for Asterionella gracillima Hantzsch. No growth occurred at concentrations of 0.1 ppm or less or at concentrations of 42 ppm or more. Gerloff et al. (1950b) reported that the maximum growth of Coccochloris Peniocystris (Kuetz.) Drou. and Daily occurred at a concentration of 13.6 ppm of nitrogen supplied as nitrate. It seems that the nitrogen concentration requirement varies considerably from species to species and that the requirement is perhaps species-specific.

McLachlan, using a medium based on one developed by Gerloff et al. (1950b), found that the maximum growth of Aphanizomenon flos-aquae occurred at a nitrogen concentration of 15 ppm although the alga became chlorotic. A concentration of 25 ppm neither improved nor inhibited growth. The final medium contained 20 ppm of nitrogen (McLachlan, 1957).

Guseva (1937) found that a concentration of 2 ppm of P_2O_5 (0.9 ppm of phosphorus) was essential for the growth of Anabaena Lemmermannii while only 0.6 ppm of P_2O_5 (0.27 ppm of phosphorus) was required by Aphanizomenon flos-aquae. Rodhe (1948) stated that the phosphorus concentration is able to limit the growth of algae in nature. According to Chu (1943) the amount of this element that normally occurs

in nature, 0.003 to 0.02 ppm, is not enough to inhibit growth. He found that the lower limits for maximum growth varied from 0.018 ppm for Tabellaria flocculosa (Roth.) Kütz. and Nitzschia palea (Kütz.) W. Sm. to 0.9 ppm for Botryococcus Braunii, Pediastrum boryanum and Staurastrum paradoxum. The upper limits varied from 8.9 ppm for Nitzschia palea and Tabellaria flocculosa to 17.8 ppm for Pediastrum boryanum, Staurastrum paradoxum, and Botryococcus Braunii. Below concentrations of 0.009 ppm no growth occurred.

Gerloff et al. (1950a, 1952) found that the growth of Coccochloris decreased at phosphorus concentrations below 0.45 ppm and that the growth of Anacystis marina (Hansg.) Drouet and Daily decreased at concentrations below 0.18 ppm.

McLachlan, working with Aphanizomenon flos-aquae, varied the concentration of phosphorus as K_2HPO_4 in steps of 0.5 ppm from 0 to 2.5 ppm. He found that the addition of more than 0.5 ppm had little effect on the growth. This amount was sufficient to make sure that phosphorus was not a limiting factor in the growth of this species (McLachlan, 1957). Zehnder and Gorham (1961) reported that the maximum growth of Microcystis aeruginosa occurred at a concentration of 3.5 ppm of phosphorus.

Blue-green algae grow in neutral waters but are more profuse in alkaline waters (Provasoli, 1958). Gerloff et al. (1950, 1952) reported that the maximum growth of Coccochloris

occurred at a pH of 10.5 to 11.0 and *Anacystis* grew best at a pH of 10.00. Allen (1952) reported that a pH of 10.00 to 11.00 was conducive to maximum growth of Oscillatoria sp. McLachlan (1957) reported that the best growth of Aphanizomenon flos-aquae in his final medium occurred at a pH of 10.00 to 11.00 and that poor growth occurred at a pH of 9.00. He found that adjusting the pH daily did not promote growth and attributed this to the toxic effect produced by the acid and base used in making these adjustments.

Relationship of the Bacteria Living in Association With the Blue-Green Algae

Very few investigations of the bacterial contaminants of fresh water algae have been conducted. The emphasis has been on the culturing of bacterial-free organisms. It is possible that the physiology of the bacterial-free alga is very different from the physiology of the alga plus the bacterial symbionts.

Guseva (1937) reported that bacteria were present in old cultures of Anabaena Lemmermannii and that these were "in the form of small pencils attached to the side of the algae." Correll (1961) reported the development of one type of bacterial colony from a culture of Anabaena variabilis Kuetz. He found that the organism was a rod-shaped, gram-negative bacterium which was strongly inhibited by tetracycline, aureomycin, terramycin, and tetramycin, but was not

inhibited by chloromycetin, penicillin, polymyxin, erythromycin, and distreptomycin.

Thompson and his co-workers isolated 26 strains of bacteria from 25 algal samples consisting of Microcystis aeruginosa (Western Strain) and Anacystis montana f. minor Drou. and Daily. The majority of these were gram-negative rods. Their work showed that the toxicity produced by these two algae was of bacterial rather than algal origin (Thompson et al., 1957; Thompson, 1959).

Antibiotic Activity Produced by Blue-Green Algae

An extensive search of the literature disclosed that little is known of the effects produced by algal extrametabolites on bacteria and fungi in natural bodies of water. Studies that have been conducted on aquatic bacteria and fungi have been quantitative (Fred et al., 1924; Graham and Young, 1934; Potter and Baker, 1946, 1961). Studies conducted by Gaukjman and Ryabov in the Dnepr Reservoir (Russia) indicate that blue-green algae are able to suppress the growth of saprophytic fungi. Their results were obtained using data on the biomass of the river and the reservoir (1962).

Antibiotic activity produced by blue-green algae has been reported by several investigators. Wurtz (1949) and Lefevre et al. (1952) reported that Microcystis aeruginosa inhibited some gram-positive and gram-negative

bacteria. Bishop et al. (1959) found that this species did not inhibit one strain of Staphylococcus aureus, Escherichia coli and Pseudomonas hydrophilia and two strains of Bacillus subtilis. Gorynova (1955) found that Oscillatoria splendida Greville secreted a volatile substance that killed bacterial symbionts and other micro-organisms in culture. Davidson (1959a) reported that pigment formation in the bacterium Serratia marcescens Bizio was inhibited by extracts and/or filtrates of bacterial-contaminated cultures of several Cyanophyta. Microcystis aeruginosa and Nostoc rivulare completely inhibited pigment formation. Partial inhibition was produced by Nostoc ellipsosporum (Desmaz.) Rabenhorst, N. punctiforme (Kuetz.) Hariot, N. calcicola Bréb., Oscillatoria formosa, Calothrix membranaceae Schmidle, and Anabaena variabilis Kuetzing. The same results were obtained when bacterial-free cultures of Oscillatoria formosa Bory and Microcystis aeruginosa were used. This factor, referred to as APF (antipigment factor) by the author, was produced in those algae which were toxic to mice. Although this substance did not kill the bacteria, it did effect the physiology of the bacteria in some way.

An amino acid, α - ϵ -diamino-pimelic acid (DAP), has been isolated from Anabaena cylindrica Lemm., Oscillatoria sp. and Mastigocladus luminosa Cohn (Work and Dewy, 1953). This substance demonstrated antibacterial activity against

Staphylococcus aureus, Escherichia coli and Streptococcus pyrogenes Rosenbach (Simonds, 1954).

An extract of Oscillatoria formosa was found to be active against Salmonella enteritidis (Gaertner) Castellani and Chalmers, S. typhosa (Zopf.) White and Staphylococcus aureus, in the logarithmic growth phase of the bacteria. The growth inhibiting substance was found to be unstable in crude extract if stored in water (Davidson, 1961). Further studies by Davidson (1962) showed that Anabaena variabilis, Phormidium lucidum var. olivace (C. A. Ag.) Kuetzing, Nostoc sp., and Lyngbya sp. inhibited the growth of Staphylococcus aureus. Extracts from the same alga either had no effect on the growth of Salmonella enteritidis or stimulated it.

Very little has been learned of the antifungal activity of the blue-green algae. Welch (1962) reported that of 35 species of marine algae screened for antifungal activity practically all exhibited a trace of activity against one or more of the test organisms. Most of the algae used were Rhodophyta and Phaeophyta. She did find, however, that the blue-green, Lyngbya majuscula, showed consistent activity against molds (fungi) and yeasts. This is the same species reported by Banner (1959) as the cause of a certain type of dermatitis.

CHAPTER II

METHODS AND MATERIALS

Organisms Used

The blue-green algae used in this study were obtained from several sources. Microcystis aeruginosa Kuetz. emend Elenkin (Strain NRC-1), Aphanizomenon flos-aquae (L.) Ralfs (Strain NRC-23) and Anabaena flos-aquae (Lyng.) De Brébisson (Strain NRC-44) were obtained from The National Research Council, Ottawa, Ontario, Canada. Four species were isolated from various sources; Microcystis incerta Lemmermannii from a culture collected from a water hole in a pasture near Mt. Pleasant, Michigan, (T.14N.;R.4W); Oscillatoria Agardhii Gomont from a bloom on Alward Lake, a lake just off U. S. 27 near DeWitt, Michigan, (T.5N.;R.2W.); Nostoc sp. and Oscillatoria rubescens de Candolle from a culture collected at Sixth Lake, northwest of Edmore, Michigan, (T.12N.;R.7W.). Aphanizomenon flos-aquae was also collected from Sixth Lake and attempts were made to culture it. Identification of the algae was confirmed by Dr. G. W. Prescott. None of the cultures was bacterial-free.

The non-algal organisms were obtained from the Botany and Plant Pathology Department and from the Microbiology Department, Michigan State University. These included: the fungi, Glomerella cingulata (Stonem.) Spauld. and Schrenk, Fusarium oxysporum Schlecht. ex Fries, Rhizopus nigricans Ehrenberg, + strain, and Trichophyton rubrum (Castellani) Sab.; the yeast, Candida albicans (Robin) Berkh.; and the bacteria, Staphylococcus aureus Rosenbach, Escherichia coli (Migula) Castellani and Chambers, and Pseudomonas aeruginosa (Schroeter) Migula. The fungi and the yeast were maintained on Difco Potato Agar and the bacteria on Difco Bacto Nutrient Agar.

Collection - Habitats and Related Features

In September, 1962, the Michigan Water Resources Commission sent a sample of water to Dr. G. W. Prescott at Michigan State University. This sample had been collected from a water hole located in a pasture near Mt. Pleasant, Michigan (Figures 1 and 2). The water hole had been covered with an algal bloom in which the dominant species was identified as Microcystis incerta. Late that summer, four cattle, part of a herd pastured near the water hole had died. The dead cattle included a three month old calf that was still nursing, an eleven month old heifer and a three year old. The fourth, about a year and a half in age, found limping



Figure 1. Waterhole from which Microcystis
incerta was isolated.



Figure 2. Same waterhole showing the fence
erected to prevent cattle from
drinking the water. (Lower right
corner.)

and staggering, died the next day. In 1961, three cattle had been found dead in the same pasture.

None of the dead animals had been seen by a veterinarian. The farmer had his cattle immunized against black leg and the water hole fenced off so that the animals could not drink the water. Black leg, suspected as the cause of death, is caused by an anaerobic bacterium, Closteridium chauvoei Arloing, Cornevin and Thomas (Burrows, 1963). This organism survives several years as a spore in almost all countries of the world and in the United States except possibly the southeast portion. It effects especially those cattle that are 6 to 18 months old. High-grade cattle are more susceptible than scrubs and well-fed more than those on poorer diets. Sheep and goats are susceptible and a few cases have been reported in swine. Horses, dogs, cats, and man are immune (Birkeland, 1949).

Before the work of Pasteur, this disease was considered to be anthrax. The organism produces a weak exotoxin. The disease is gangrene-like in that gas is produced causing subcutaneous swellings, mostly in the thigh and shoulders. The area of swelling becomes discolored, giving the disease its common name. Although a few recover most cattle die within 12 to 36 hours. There is no treatment. The exact method of infection is not known. Spores may enter through small wounds or abrasions on the skin or possibly through

the mucous membranes of the mouth, tongue and intestinal tract. Spores do not germinate in large wounds because the organism is an anaerobe. Soils that are heavily manured, such as those found in pastures, are more likely to contain these spores. To reduce infection carcasses of animals dying from this disease should be burned and pastures should be burned several years in succession (Birkeland, 1949). The State's Veterinarian's Office receives from six to ten reports of this disease a year. This is not a true picture because undoubtedly some cases are not reported and other cases are not seen by a veterinarian (G. L. Walker, personal communication).

Aphanizomenon flos-aquae was collected from Sixth Lake three times between September 2, 1962, and October 31, 1962, and four times between August 18, 1963, and October 15, 1963 (Figures 3, 4, 5). This species was not present in late March or early April of 1963 when the lake was still covered by ice. Neither was it present in May of 1963 or in late August of 1964. The alga never survived more than three weeks in laboratory culture.

Sixth Lake is the first in a series of six lakes. It is fed by a stream from Edmore, Michigan, that enters the lake on the east and by another stream that enters the lake from the northwest. The drainage from Edmore contains wastes from a pickle factory as well as septic tank overflow



Figure 3. Sixth Lake showing vegetation along the southwest shore taken when a bloom of Anabaena limnetica was present. August, 1964.



Figure 4. Sixth Lake taken from western shore looking toward the inlet of the lake.



Figure 5. Sixth Lake taken from southern shore where cottages are located.

and other pollution. The six lakes are connected by channels and the outlet of the last lake, First Lake, is Flat River. Investigation of this lake by the Michigan Water Resources Commission in August, 1959, disclosed a bloom of Oscillatoria sp. In July of 1962, a bloom involving Aphanizomenon flos-aquae was investigated (Fetterolf and Carr, 1962). This alga was apparently present in the lake in healthy condition from July until into November of 1962. In August, 1963, this species was present in large numbers but examination of plankton samples disclosed a Volvocalean bloom. Volvox aureus Ehrenberg was the dominant species in this bloom. Male and female colonies, colonies containing zygotes, colonies undergoing asexual reproduction and young daughter colonies recently liberated were all present. Eudorina elegans Ehrenberg, Gonium pectorale Mueller and Pandorina morum (Mue11.) Bory were also present in large numbers. Other algae identified included Ceratium hirundinella (O. F. Mue11.) Dujarden, Mougeotia sp., Coelosphaerium Naegelianum Unger, Microcystis aeruginosa, Nostoc sp., and Oscillatoria rubescens. Very little zooplankton was present. Large numbers of Volvocales were present in the channel between Sixth Lake and Fifth Lake. The number, however, decreased considerably in Fifth Lake. None of these species were present in plankton from Fourth Lake. Samples from Fourth Lake contained large numbers of Ceratium hirundinella.

Plankton collected in August of 1964 showed that the bloom present at this time consisted of Anabaena limnetica G. M. Smith with small amounts of Spirogyra sp., Microcystis aeruginosa, and Melosira sp. present.

The bloom from which Oscillatoria Agardhii was isolated was first observed in Alward Lake in December, 1962. In January, 1963, there were 3,000 to 4,000 filaments per cubic centimeter. These were dark blue-green and occurred singly or in irregular fascicles. This species had never before been reported as occurring under ice (Alexander and Sieminska, unpubl.). In May, when the sample was collected, the bloom had become more concentrated along the south and west shores of the lake. After rowing out into the lake the alga was found to be present in such large numbers that the water had the appearance of green "soup." The bloom, presumably present since December, 1962, disappeared in early July.

Alward Lake is a relatively shallow, eutrophic lake with a maximum depth of approximately 20 feet (Figures 6 - 8). It is fed by a creek which enters from the northeast and is drained by a creek toward the west. The lake is used extensively for fishing and there is a resort along the southeast shore. Owners of the resort stated that blooms had occurred in the lake for the last nine or ten years; one occurring in the spring and a second one usually in August.



Figure 6. Alward Lake taken from eastern shore
just after disappearance of the bloom
of Oscillatoria Agardhii in 1963.



Figure 7. Alward Lake - northern shore.



Figure 8. Alward Lake - southern shore and outlet. Bloom of Oscillatoria Agardhii became concentrated in this area in May, 1963.

These blooms in the past had lasted one to three days and then disappeared. The bloom of Oscillatoria Agardhii had been present since the ice broke. The entire surface of the lake had been covered with a thick mass of algae. No bloom had occurred in the spring of 1964.

Isolation of Algae

Two methods of isolation were used. In one, the material from samples collected was streaked on agar by means of an inoculating loop. Desired species which developed were removed and streaked on fresh agar. This transfer process was repeated until microscopic examination indicated that only one species was present (Lewin, 1959; Gerloff, Fitzgerald and Skoog, 1950).

The second method involved removal of the desired species by means of a micropipette. Micropipettes were made by drawing out glass tubing to a very fine diameter. A drop of the culture containing algae was placed on a slide. The slide was placed under a binocular microscope and the desired alga was drawn up into the micropipette. The alga was washed five times in each drop by picking it up with and expelling it from the micropipette. The washing process was continued in seven more drops and finally transferred by means of a fresh pipette to five milliliters of sterilized medium in a screw-cap test tube. Several test tubes were inoculated with

the same algal species. These were incubated at room temperature under constant light of approximately 500 foot candles. Those containing undesirable species were discarded. Uni-algal cultures were transferred to 125 milliliter Erlenmeyer flasks containing 35 milliliters of medium and kept under the same conditions.

Medium

The algal cultures were maintained in a medium devised by McLachlan and Gorham which they designate as ASM (Table 1) (McLachlan and Gorham, 1961). This medium was used after several others had been tried because it proved to be the most satisfactory for growth. Stock solutions of each of the chemicals in the medium were prepared by weighing a convenient amount of each compound, placing the weighed amount into a liter volumetric flask, and adding enough distilled water to make a liter. Each solution was then diluted until one liter contained one thousand times the concentration required in the medium. The stock solutions were stored in one-liter polyethylene screw-cap bottles. In preparing the medium one milliliter of each stock solution was transferred by means of a sterile one-milliliter pipette to a liter volumetric flask. The volume was then increased to one liter by the addition of Pyrex distilled water.

Table 1. ASM medium

Constituent	Molecular Weight	Weight Used	Number of Dilution	Final Concentration of Medium in Micromoles
$\text{MgSO}_4 \cdot 7 \text{H}_2\text{O}$	246.498	49.29	1	200
$\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$	203.33	40.66	1	200
CaCl_2	110.994	11.099	1	100
NaNO_3	85.01	85.01	1	1000
K_2HPO_4	174.183	17.418	1	100
Na_2EDTA^*	372.252	7.445	1	20
FeCl_3	162.22	3.244	2	2
H_3BO_3	61.844	.6184	1	10
$\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$	197.918	1.385	1	7
ZnCl_2	136.29	1.09	2	0.8
$\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$	237.95	.4759	2	0.02
$\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$	170.486	.341	2	0.0002

*Disodium ethylenedinitrilotetraacetate.

Pipettes were sterilized by wrapping in brown paper and placing them in an oven where they remained for three hours at a temperature of 120°C . All water used was triply distilled. De-ionized water was redistilled twice in Pyrex glassware. The pH of the medium was adjusted to 7.5 ± 0.1 with 0.1 normal potassium hydroxide.

Cultures were maintained in liquid and on agar. Pyrex glassware was used exclusively. All glassware was cleaned with a mixture of concentrated sulfuric acid and potassium dichromate, washed twice in hot water to which a detergent had been added, rinsed twice in tap water and three times in de-ionized water. Liquid cultures were grown in thirty-five milliliters of medium in 125 milliliter Erlenmeyer flasks. The flasks were stoppered with non-absorbent cotton plugs and autoclaved for 15 minutes at 120°C and 15 pounds pressure. The autoclaved flasks were allowed to stand at least 24 hours but no longer than 48 hours before inoculation. They were inoculated by adding one milliliter from a week-old culture by means of a sterile one milliliter pipette. The culture from which the inoculum was taken was agitated thoroughly to insure equal distribution of the alga.

Agar cultures were maintained on plates and on slants. These were prepared by adding 1.5 per cent of Difco Bacto-agar to the liquid medium. The agar plates were prepared by autoclaving the medium plus the agar for 15 minutes

at 120°C and 15 pounds pressure. The medium was then poured into sterile 15 x 100 millimeter Petri dishes. These Petri dishes had been wrapped in brown paper and sterilized by dry oven heat at 120°C for three hours. The agar was allowed to solidify and the plates were stored in quart plastic food bags to prevent contamination.

Agar slants were prepared by filling 25 x 200 millimeter Pyrex test tubes about one-fourth full of the medium-agar mixture, stoppering them with non-absorbent cotton plugs and sterilizing as above. When removed from the autoclave the test tubes were slanted so that the agar did not quite reach the cotton plug and then allowed to cool. Inoculation of all agar cultures was made by streaking the surface with a sterilized platinum inoculating loop.

Growth Conditions

The cultures, both liquid and agar, were grown under three sets of environmental conditions. Part of the culturing was done in a refrigerator that had been converted to a growth chamber by the installation of three shelves, lights, and a thermostatic device (Figure 9). These shelves were approximately fourteen and a half inches by nineteen and one-half inches. A fluorescent light had been installed above each shelf. Illumination was by means of a General Electric cool, white 15-Watt fluorescent light.



Figure 9. Growth chamber.

Only the central section was used because the amount of illumination at the extreme ends and the extreme front and back of the shelves was considerably lower than in the center. The range of illumination used was 175 ± 25 foot candles. The light intensity was determined by a Weston Illuminometer. The liquid cultures were agitated daily and the position of the flasks were rotated in an effort to give each culture the same amount of light. The cultures were illuminated continuously. Temperature of the growth chamber was recorded by means of a maximum-minimum thermometer over a period of several weeks. The thermostatic control was adjusted until the temperature remained at $21 \pm 1^{\circ}\text{C}$. The temperature was checked periodically to make sure that it remained within this range.

Other cultures were maintained at room temperature, $26 \pm 2^{\circ}\text{C}$, under a bank of four cool white 40-Watt fluorescent bulbs. The illumination from this bank of lights was 480 ± 15 foot candles at a distance of approximately twenty inches. All were subjected to periods of 16 hours light alternating with eight hours of darkness. Part of these were grown on a rotary shaker (New Brunswick Scientific Co. Model S35315) with the period of illumination coinciding with the period during which the shaker was in operation. The tops of the flasks were about 14 inches from the lights. The remainder of the cultures were stationary and were approximately 30 inches from the lights.

Testing for Antibiotic Activity

Testing the algae for the production of extrametabolites inhibitory to the non-algal organisms was carried out using the following procedures. The entire contents of each culture was passed through a 1,000 milliliter Seitz filter using Size D filter pads (0.3 to 0.5 micron porosity). The filter disc was placed in the filter and the top of the filter was plugged with non-absorbent cotton. The filter was placed on top of a 500 milliliter Pyrex filtering flask and a piece of heavy rubber hose was attached to the arm of the flask. The hose was clamped shut by means of a screw clamp. The whole apparatus was sterilized by autoclaving for 15 minutes at 120°C and 15 pounds pressure. Thus, the entire inside of the apparatus was sterile. When used, the hose was attached to the house vacuum line and the stopcock of the vacuum line was turned on. The clamp on the hose was released and the contents of the culture was passed through the filter.

After passing through the filter the spent medium was transferred to a Petri dish containing filter paper assay discs (Schleicher and Schull, No. 74-e, 12.7 millimeters in diameter). The Petri dish containing the discs had been sterilized in the same manner as the filtering apparatus. The non-algal organisms were streaked by means of a sterilized platinum inoculating loop across two diameters at right angles to each other. By means of a flamed

forceps a sterilized disc saturated with the spent medium was placed at the point of intersection of these two steaks (Figures 27, 28, 29). Controls for each culture were prepared in the same way but sterilized medium was used to saturate the discs. Observations of growth were made at 16, 24, 36 and 48 hours. The results are recorded in Tables 2 through 7.

After screening all of the algae for inhibitory activity an attempt was made to determine whether the algae had produced the activity or whether it had been produced by the bacterial contaminants. To do this the non-algal organism was streaked across the diameter of the agar and the alga was streaked on either side of it. Given an organic source of food the bacterial symbionts grew profusely, soon covering the algal cells. Any inhibition by bacterial contaminants should thus be evident.

Aphanizomenon flos-aquae, as pointed out in the introduction, is often the dominant species in a "water bloom." For this reason and also because of the results of screening the algae for inhibitory activity against the non-algal organisms, this species was selected for further study.

Isolation of the Bacterial Symbionts

There has been much speculation about the relationship between the bacterial symbionts and certain blue-green algae. In an attempt to learn more about the bacteria growing in association with Aphanizomenon flos-aquae, the alga was streaked by means of a sterilized platinum loop on Difco Nutrient Agar. The organisms resulting were then streaked on fresh agar plates and isolations were made. Smears of the bacteria were made on slides and these were stained with Gram's differential stain. One species was identified after staining. The standard tests used by bacteriologists in the identification of bacteria were performed on the bacterium isolated. These tests included growth in litmus milk, nitrate broth, tryptophane broth (indol test) and on gelatin. The bacterium was grown at 25 to 30°C and also at 37°C and motility was observed. The sensitivity of the bacterium to antibiotics was determined by using Difco Sensitivity Discs. Agar plates for the determination of sensitivity were inoculated by means of a sterile swab which had been dipped into a liquid culture of the bacterium. The plates were incubated at 25°C.

Effects of Variations in Mineral
Concentrations in the Medium

The amounts of nitrogen, phosphorus and manganese were varied in four series of cultures to determine the effects of different concentrations on the rate of growth. The limited amount of space available for growing cultures under the previously described conditions made it necessary to conduct the experiments in duplicate rather than in triplicate.

Determination of growth was made by weighing the entire algal content of each culture. The algal growth was removed from each culture by passing the entire contents of the culture through a tared millipore RA filter and washing the filter with distilled water. Weight of each filter had been determined after the filters had been in a Scheiber desiccator for several days to insure their dryness. The weight of each was obtained by determining the difference in weight between the filter and the filter plus a disk of coated paper of the same size as the filter. A chain-o-matic balance was used for weighing. The filters were handled by means of a forceps and were never placed on the balance or any other surface without the accompanying coated disk. These precautions were taken to prevent the additions of materials which might add to the inaccuracies inherent in this method of weight determination. The tared filters and the coated paper disks were placed in individual 15 x 60

millimeter Petri dishes which were numbered for easy identification. After the filters were used they were returned to the Petri dishes and were dried in a dessicator. Until the weight remained constant filters were weighed at one day intervals. The weights of the filters are low which allows for reasonable accuracy in using them to determine growth increments. The weight of the alga was determined by using the same method of differences in weight between the coated paper disk, the filter and the alga. The medium as previously described was used as the control (Table 1).

In the first series of cultures the amount of nitrogen was varied. Four series of ten cultures each were prepared. Two series contained the control medium. One of these series was placed in the growth chamber and the other on the shaker in order to compare growth under different environmental conditions. A third series was prepared in which the amount of nitrogen was doubled and a fourth in which the amount of nitrogen was increased to three times that in the control medium. These last two series were placed on the shaker. Conditions of light and temperature have been described previously. The cultures were harvested in duplicate on the 5,7,12,16 and 19 days. The results are recorded in Tables 8 through 11. The pH of the spent medium was determined by means of a Beckman pH meter. The results appear in the last column on the right in Tables 8 through 11.

In the second experiment the effect of phosphorus was studied by varying the amount of phosphorus in the form of K_2HPO_4 . These cultures were grown only in the growth chamber. Each series consisted of 14 cultures. One series, containing the original medium, was used as the control. In the second, twice the amount of available phosphorus was used and in the third, three times the amount in the control medium. The cultures were harvested on the 3, 6, 9, 12, 15, 18, and 21 days. The pH of the spent medium was determined. The results are recorded in Tables 12 through 14.

The amount of available manganese was varied in the third group of cultures. Each series was made up of 14 cultures. One series was used as the control. The amount of manganese was reduced by 1/100 in the second series and by 1/1000 in the third series. In the fourth series this element was omitted entirely. The cultures were harvested in duplicate on the 3, 6, 9, 12, 15, 18, and 21 days. The pH of the spent medium was determined. Results are recorded in Tables 15 through 18.

In the fourth series of cultures the amount of nitrogen was reduced to one-half and to one-tenth that used in the control. One series containing the control medium was also prepared. The cultures were harvested and the pH of the spent medium was determined on the 4, 7, 12, 15, and 19 days. Results are recorded in Tables 19 through 21.

The spent medium of the control cultures in each experiment were inoculated with Anabaena flos-aquae, Microcystis aeruginosa, Microcystis incerta, or Aphanizomenon flos-aquae in an effort to see if this species would inhibit other species or itself. Fresh medium was inoculated at the same time as controls. These were observed for a week but no weight determinations were made.

CHAPTER III

RESULTS

Growth of Algae

Growth of algae on agar is usually slow, but their appearance after the surface of the agar has been streaked follows a more or less distinct pattern. Diatoms develop first in a mixed culture. These are followed by representatives of the Chlorophyta. The last to appear are the cyanophyceous species. In the colored photograph (Figure 10), the agar plate on the left is two weeks old; the one on the right, six weeks old. They were prepared by streaking the agar surface with mud from the bottom of Sixth Lake. A photomicrograph of the culture on the right is shown in Figure 11. The dark brown spots are particles of soil and the lighter brown areas are patches of diatoms consisting of Navicula sp. and Fragillaria sp. The green patches are made up of Pediastrum sp., Chlorella sp. and Scenedesmus sp. The blue-green patches are Chroococcus sp. and Microcystis sp. Microscopic examination of the agar plate showed that spores of Aphanizomenon flos-aquae were present but none of them germinated.

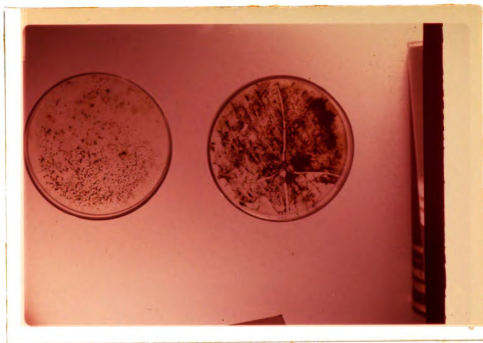


Figure 10. Agar plates inoculated with mud from the bottom of Sixth Lake. The plate on the left is 2 weeks old; the one on the right, 6 weeks old.

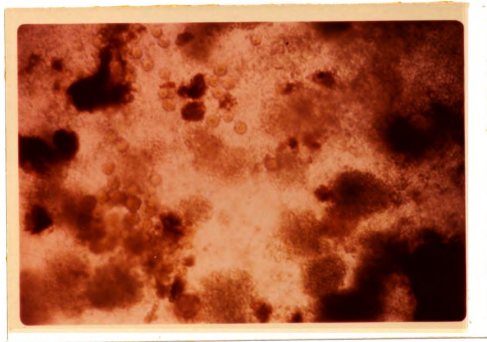


Figure 11. Photomicrograph of culture on the right in Figure 10. Dark brown spots - soil; lighter brown - diatoms; green - Pediastrum sp., Chlorella sp. and Scenedesmus sp.; blue-green - Microcystis sp. and Chroococcus sp.

Later, after this picture was taken, filaments of Oscillatoria sp., Phormidium sp. and Calothrix sp. appeared.

All attempts to culture Aphanizomenon flos-aquae from samples collected from Sixth Lake failed. Samples containing water and mud from the bottom of the lake were collected in October of 1962 and in March of 1963. These were stored at 15⁰F in a refrigerator in the dark. In April, mud from samples collected on both of these occasions was spread over the surface of agar plates which had been prepared by adding Bacto-Agar to the liquid medium. Examination of these agar plates showed that akinetes of A. flos-aquae were present but none of them germinated. Mud samples collected on both dates were frozen. Some of the samples were kept frozen for a week; others, for two weeks. After thawing mud was again streaked on agar plates. Akinetes, although present, did not germinate. Liquid cultures inoculated with this mud failed to produce Aphanizomenon flos-aquae.

Agar plates become dry and unsuitable after five or six weeks but can be restored by adding sterilized medium or sterilized distilled water to the Petri dishes. Drying occurs less rapidly if the dishes are inverted. This does not seem to be detrimental to the growth of the algae in reference to light.

Agar slants dry out less rapidly than agar plates and are, therefore, better for maintaining cultures (Figure 12). A disadvantage in using agar slants to isolate algae is that they cannot be examined under the microscope.

Streaks from the farm water hole sample failed to produce any growth until after ten days. Navicula sp. appeared first, then three days later Scenedesmus sp., Chlorella sp. and Ankistodesmus sp. were apparent. After 28 days a small patch of Microcystis incerta was found near the top of one of the agar slants. The remainder of the algae growing on this slant at this time appeared to be Chlorophyta which had grown through the agar digesting it. A portion of the Microcystis colony was streaked on fresh agar plates and slants. The newly inoculated plates and slants repeated the succession of growth described above, but at a faster rate. This species was transferred repeatedly by streaking until no other organism appeared to be present when the cultures were examined under the microscope. The complete isolation required approximately three months.

On agar this species and Microcystis aeruginosa form small clumps and spread only slightly from the inoculation points (Figure 13). After three or four weeks the cultures become yellowish because of bacterial growth (Figure 14). In liquid cultures neither of these species form colonies.



Figure 12. Maintenance of cultures on agar slants: left - Microcystis aeruginosa; right - Aphanizomenon flos-aquae. Both are 50 days old.

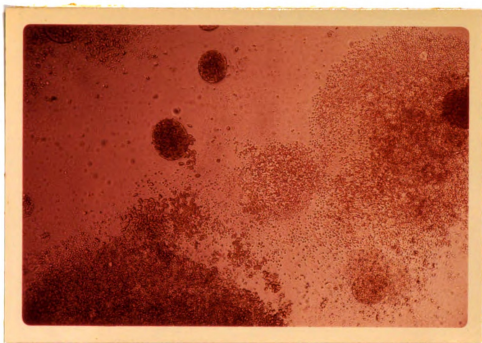


Figure 13. Photomicrograph of Microcystis aeruginosa growing on agar. Culture is 13 days old. Magnified approximately 150 times.

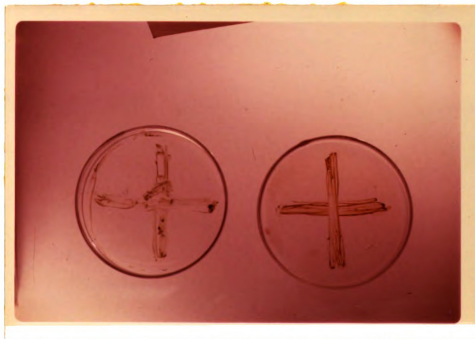


Figure 14. Microcystis aeruginosa on agar - culture on the left is 13 days old; one on the right is 50 days old. Discoloration is due to bacterial growth.

In young cultures the cells seem to be rather evenly distributed but in age they fall to the bottom of the flasks and often form thin sheets. The effect of bacterial activity, as evidenced by the change in color, becomes evident on about the 17th or 18th day. The culture eventually becomes orange in color. Liquid cultures of Microcystis incerta are shown in Figure 15. The color of the culture does not show as brightly in the photograph as in nature. Liquid cultures of Microcystis aeruginosa changed from orange to blue-green after a period of time. A whole series of cultures showing this change in color is illustrated in Figures 16 and 17.

Nostoc sp. was first observed in a three-week old culture inoculated with material from Sixth Lake. The culture had been started along with five others in soil extract medium in an attempt to isolate and culture Aphanizomenon flos-aquae. Small portions of the filaments were placed into six test tubes containing five milliliters of the medium. Growth of this species was fairly rapid and after three agar plate transfers the cultures appeared to be unialgal when examined microscopically. On agar this alga was able to spread in all directions eventually covering large areas (Figure 18). The nature of cell division in a filamentous form allows it to grow away from the point of inoculation (Figure 19). As colonies age they become tough and gelatinous and eventually produce papillae on their surfaces (Figure 18).



Figure 15. *Microcystis incerta* in liquid culture; age from right to left - 1, 2, 3, 4, and 18 weeks old. Discoloration of cultures due to bacterial activity in cultures of 3 weeks or older.

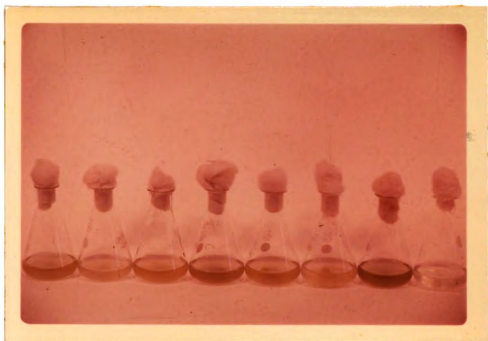


Figure 16. Microcystis aeruginosa in liquid culture; age from right to left - 1, 2, 3, 4, 5, 6, 7, and 8 weeks old. Note changes in color of this series and the series of Figure 17.

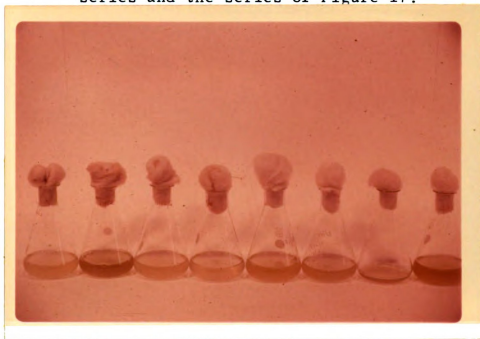


Figure 17. Microcystis aeruginosa in liquid culture; age from right to left - 9, 10, 11, 12, 13, 14, 15, 16, 17 and 18 weeks old.

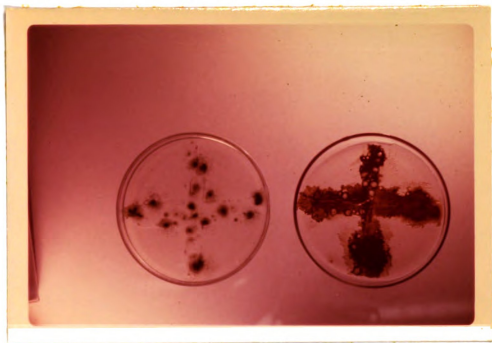


Figure 18. Nostoc sp. on agar: culture on left is 13 days old; the one on the right, 50 days old. Note papillae on the surface of the culture on right.

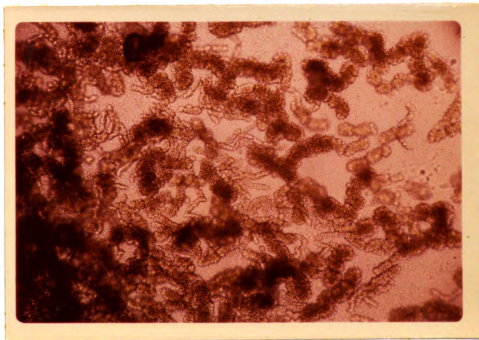


Figure 19. Photomicrograph of Nostoc sp. from agar plate on left in Figure 18. Magnified approximately 150 times.

This is undoubtedly due to the release of oxygen by the algae. Even after the agar dries this alga is able to continue living apparently being protected by the large amount of gelatinous material produced.

In liquid cultures this species forms sheet-like colonies. Growth is fairly rapid and in from eight to ten weeks the entire culture flask becomes gelatinous. Bubbles of gas, presumably oxygen, appear on the surface. Noticeable bacterial activity does not occur until after several weeks (Figure 20). One culture that has been retained over two years still shows no change in color caused by bacterial activity. All that has been done to maintain this particular culture was to add Pyrex distilled water to offset evaporation. Repeated microscopic examinations of cultures of various ages have not permitted the identification of this alga to species because no spores have developed.

Agar plates inoculated during the isolation of this species followed the same growth sequence as previously described; diatoms, green algae and finally blue-green. Chroococcus sp. was found growing on several of these agar surfaces but was never completely isolated. One filamentous form, tentatively identified as Hormogonium sp., was transferred from one of the agar plates but failed to grow.



Figure 20. Nostoc sp. in liquid culture; age from right to left - 1, 2, 21, 20 and 18 weeks. Note no discoloration due to bacterial growth.

Oscillatoria rubescens first appeared on an agar slant which had been streaked with material from the same sample that yielded Nostoc sp. Filaments, when transferred to fresh agar, grew rapidly, extending over much of the surface within ten to twelve days. The growth of this species away from the point of inoculation is related to the nature of filamentous growth and also to the fact that this species has motility. Despite the fact that growth was fairly rapid it was difficult to isolate. All material transferred was taken from growth occurring farthest from the point of inoculation. Each time transfers were made brown patches of diatoms appeared. These apparently were epiphytic forms and, because of their motility were able to move a considerable distance from the filament of Oscillatoria. Several cyanophyceous forms, including Chroococcus, appeared in the earlier transfers. After repeated transfers the cultures of O. rubescens were assumed to be unialgal because extensive microscopic examinations of samples from liquid cultures and of entire agar plates revealed no other algae. Isolation required approximately four and a half months.

In liquid cultures O. rubescens grew quite rapidly when compared with some of the other algae used in this study. The filaments grew loosely more or less throughout the medium for from four to six weeks (Figure 21). After this the filaments clumped and gas bubbles, presumably oxygen, collected at the top of the mass.



Figure 21. *Oscillatoria rubescens* in liquid culture; age from right to left - 2-1/2, 3, 5, and 7 weeks. Filamentous growth appears in culture on left.

Growth of bacterial contaminants in large enough quantities to discolor the culture usually requires five or six weeks or longer.

Oscillatoria Agardhii was first collected in January, 1963, from Alward Lake. This species was found growing under the ice in "bloom" proportions. Media inoculated with this species failed to develop growth. In early May this species was again collected. At this time the isolation was comparatively easy because the bloom seemed to be unialgal. Isolation was accomplished by streaking on agar. Figure 22 shows this species growing on agar. The culture on the right demonstrates bacterial activity. A photomicrograph of the culture on the left is shown in Figure 23. The yellowish coloration of some of the filaments is caused by bacterial growth. Growth of this species in liquid cultures follows the same pattern as that of Oscillatoria rubescens.

Discoloration of cultures of Aphanizomenon flos-aquae (Figure 24) begins about the 18th or 19th day after inoculation. This species floats near the surface of the liquid and in older cultures the filaments adhere to form the typical colony found in nature. The liquid cultures became milky in appearance, the cells became plasmolyzed and eventually lysis occurs. On agar, the filaments are able to spread from the point of inoculation (Figure 25).



Figure 22. Oscillatoria Agardhii on agar. Culture on the left is 2 weeks old; one on the right, 6 weeks old. Discoloration is the result of bacterial growth.

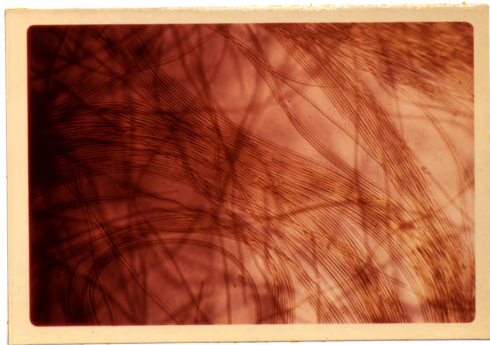


Figure 23. Photomicrograph of Oscillatoria Agardhii on agar. Magnified approximately 150 times.



Figure 24. Aphaniizomenon flos-aquae in liquid culture; age from right to left - 1, 2, 7-1/2, 8-1/2, 11 and 14 weeks old. Note tendency of alga to float at surface.

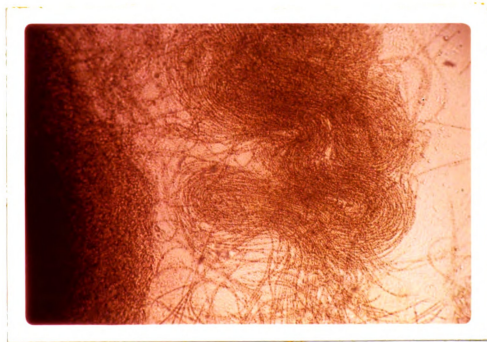


Figure 25. Photomicrograph of Aphaniizomenon flos-aquae growing on agar. Culture is 13 days old. Magnified approximately 150 times.

The growth of Anabaena flos-aquae in liquid is similar to that of Aphanizomenon flos-aquae (Figure 26). The filaments float near the surface and the cultures appear milky as they age.

Inhibitory Activity

All of the algae except Oscillatoria Agardhii were screened for the production of inhibitory extrametabolites. This species was not completely isolated until after the tests had been completed.

Anabaena flos-aquae showed no inhibition toward any of the organisms although the effect on Trichophyton rubrum is questionable (Table 2). The control grew so very slowly that it was not possible to determine whether actual inhibition was produced by the spent medium from the cultures of this alga. This is also true for the effect of the spent medium of Oscillatoria rubescens on Trichophyton rubrum (Table 3). Spent medium of O. rubescens, however, showed a slight inhibitory effect against Fusarium oxysporum.

The spent medium of Microcystis aeruginosa produced slight inhibition toward Glomerella cingulata and Trichophyton rubrum. The effect on the latter non-algal organism was in this case definitely discernible (Table 4). Microcystis incerta exhibited slight inhibition toward Glomerella cingulata, Fusarium oxysporum and Rhizopus nigricans (Table 5).



Figure 26. Anabaena flos-aquae in liquid culture; age from right to left - 1, 2, 5, 8, 11, and 14 weeks. Note similarity of growth habit to that in Figure 24.

Table 2. The effects of the spent medium of Anabaena flos-aquae on the non-algal organisms

Age of Culture (in Days)	<u>Glomerella</u> <u>cingulata</u>	<u>Fusarium</u> <u>oxysporum</u>	<u>Rhizopus</u> <u>nigricans</u>	<u>Trichophyton</u> <u>rubrum</u>	<u>Candida</u> <u>albicans</u>	<u>Staphylococcus</u> <u>aureus</u>	<u>Escherichia</u> <u>coli</u>	<u>Pseudomonas</u> <u>aeruginosa</u>
7	0	0	0	?	0	0	0	0
14	0	0	0	?	0	0	0	0
21	0	0	0	?	0	0	0	0
25	0	0	0	?	0	0	0	0
40	0	0	0	?	0	0	0	0
56	0	0	0	?	0	0	0	0
72	0	0	0	?	0	0	0	0

0 = No Inhibition

+ = Slight Inhibition

? = Indefinite

++ = More Pronounced Inhibition.

Table 3. Inhibitory effects of the spent medium of Oscillatoria rubescens on the non-algal organisms

Age of Culture (in Days)	<u>Glomerella</u> <u>cingulata</u>	<u>Fusarium</u> <u>oxysporum</u>	<u>Rhizopus</u> <u>nigricans</u>	<u>Candida</u> <u>albicans</u>	<u>Trichophyton</u> <u>rubrum</u>	<u>Staphylococcus</u> <u>aureus</u>	<u>Escherichia</u> <u>coli</u>	<u>Pseudomonas</u> <u>aeruginosa</u>
7	0	+	0	0	?	0	0	0
14	0	+	0	0	?	0	0	0
21	0	+	0	0	?	0	0	0
36	0	+	0	0	?	0	0	0
48	0	?	0	0	0	0	0	0

0 = No Inhibition

+ = Slight Inhibition

? = Indefinite

++ = More Pronounced Inhibition.

Table 4. Inhibitory effects of the spent medium of Microcystis aeruginosa on the non-algal organisms

Age of Culture (in Days)	<u>Glomerella</u> <u>cingulata</u>	<u>Fusarium</u> <u>oxysporum</u>	<u>Rhizopus</u> <u>nigrans</u>	<u>Trichophyton</u> <u>rubrum</u>	<u>Candida</u> <u>albicans</u>	<u>Staphylococcus</u> <u>aureus</u>	<u>Escherichia</u> <u>coli</u>	<u>Pseudomonas</u> <u>aeruginosa</u>
7	+	0	0	+	0	0	0	0
14	+	0	0	+	0	0	0	0
21	+	0	0	+	0	0	0	0
33	+	0	0	+	0	0	0	0
41	+	0	0	+	0	0	0	0
48	+	0	0	+	0	0	0	0
53	+	0	0	+	0	0	0	0
72	+	0	0	+	0	0	0	0

0 = No Inhibition + = Slight Inhibition

++ = More Pronounced Inhibition.

Table 5. Inhibitory effects of the spent medium of Microcystis incerta on the non-algal organisms

Age of Culture (in Days)	<u>Glomerella</u> <u>cingulata</u>	<u>Fusarium</u> <u>oxysporum</u>	<u>Rhizopus</u> <u>nigricans</u>	<u>Trichophyton</u> <u>rubrum</u>	<u>Candida</u> <u>albicans</u>	<u>Staphylococcus</u> <u>aureus</u>	<u>Escherichia</u> <u>coli</u>	<u>Pseudomonas</u> <u>aeruginosa</u>
7	+	+	+	0	0	+	0	0
14	+	+	+	0	0	+	0	0
21	+	+	+	0	0	+	0	0
36	+	+	+	0	0	+	0	0
48	+	+	+	0	0	+	0	0
60	+	+	+	0	0	+	0	0

0 = No Inhibition ++ = More Pronounced Inhibition

+ = Slight Inhibition.

Table 6. Inhibitory effects of the spent medium of Nostoc sp. on the non-algal organisms

Age in Culture (in Days)	<u>Glomerella</u> <u>cingulata</u>	<u>Fusarium</u> <u>oxysporum</u>	<u>Rhizopus</u> <u>nigricans</u>	<u>Trichophyton</u> <u>rubrum</u>	<u>Candida</u> <u>albicans</u>	<u>Staphylococcus</u> <u>aureus</u>	<u>Escherichia</u> <u>coli</u>	<u>Pseudomonas</u> <u>aeruginosa</u>
7	0	++	0	0	0	0	0	0
14	0	++	0	0	0	0	0	0
28	0	++	0	0	0	0	0	0
63	0	++	0	0	0	0	0	0
75	0	++	0	0	0	0	0	0
102	0	++	0	0	0	0	0	0

0 = Inhibition

++ = More Pronounced Inhibition

+ = Slight Inhibition.

*Crushed.

Nostoc sp. showed no inhibitory activity toward any of the non-algal organisms until after the alga was crushed by means of a pestle and mortar. After this there was a slight inhibitory effect against Fusarium oxysporum.

The spent medium of Aphanizomenon flos-aquae showed the greatest inhibitory activity against the non-algal organisms (Table 7). Slight inhibition of Rhizopus nigricans and Pseudomonas aeruginosa occurred. In the case of the former, the growth and especially the retardation of sporulation was evident when compared with the growth and sporulation of the control. Stronger inhibition of Fusarium oxysporum and Staphylococcus aureus was demonstrated. Figure 27 shows the effect produced on S. aureus. No inhibition was demonstrated against Escherichia coli, Trichophyton rubrum, Candida albicans (Figure 28), or Glomerella cingulata (Figure 29).

The results of testing the bacterial contaminants of the algae against the organisms which were inhibited by the spent medium were all negative. It can be assumed, then that the inhibition produced was not a product of the bacterial symbiont. The possibility of the inhibitory activity being a result of the combined activity of the alga and bacteria, however, cannot be ignored.

Table 7. Inhibitory effects of the spent medium of Aphanizomenon flos-aquae on the non-algal organisms

Age of Culture (in Days)	<u>Glomerella</u> <u>cingulata</u>	<u>Fusarium</u> <u>oxysporum</u>	<u>Rhizopus</u> <u>nigricans</u>	<u>Trichophyton</u> <u>rubrum</u>	<u>Candida</u> <u>albicans</u>	<u>Staphylococcus</u> <u>aureus</u>	<u>Escherichia</u> <u>coli</u>	<u>Pseudomonas</u> <u>aeruginosa</u>
7	0	++	+	0	0	++	0	+
9	0	++	+	0	0	++	0	+
14	0	++	+	0	0	++	0	+
21	0	++	+	0	0	++	0	+
28	0	++	+	0	0	++	0	+
36	0	++	+	0	0	++	0	+
41	0	++	+	0	0	++	0	0
62	0	++	+	0	0	++	0	0
70	0	++	+	0	0	++	0	0

0 = No Inhibition ++ = More Pronounced Inhibition

+ = Slight Inhibition.

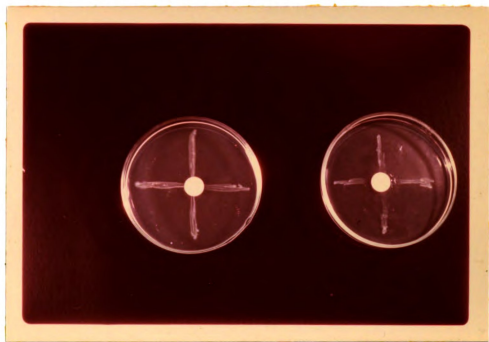


Figure 27. Spent medium of *Aphanizomenon flos-aquae* against *Staphylococcus aureus* 2 days after inoculation. Control is on the left. Note area of inhibition on plate on the right.

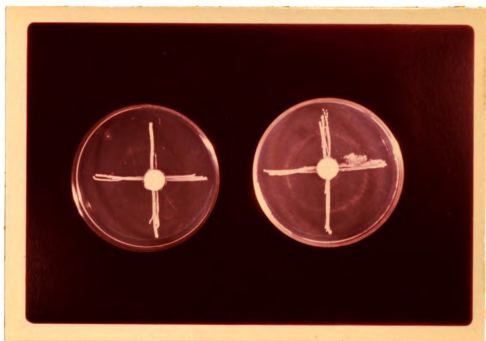


Figure 28. Spent medium of Aphanizomenon flos-aquae against Candida albicans 2 days after inoculation. Control is on the left. No inhibition demonstrated.

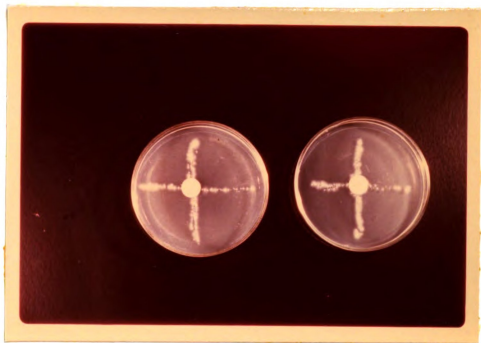


Figure 29. Spent medium of Aphanizomenon flos-aquae against Glomerella cingulata 2 days after inoculation. Control is on the left. No inhibition demonstrated.

Identification of the Bacteria

One of the bacteria isolated was identified as Bacillus cereus var. mycoides Flüggé comb. nov. Bacillus mycoides Flüggé. This is a spore-forming rod which lives as an aerobe but can also grow in the absence of oxygen (facultative anaerobe). It is widespread, especially in soil. The physiological characteristics of this variety are identical with those of B. cereus. Gelatin is rapidly liquified and milk is rapidly peptonized. Nitrates are reduced to nitrites. Both of these bacteria produce acids from glucose, fructose, dextrin, glycerol, and salicin. Both bacteria hydrolyze starch. No acid is produced by either of these from arabinose, rhamnose, xylose, raffinose, inulin and mannitol and usually none is produced from mannose and lactose.

These two species differ in manner of growth on agar. Colonies of B. cereus are large, flat, entire or irregular and whitish in color. Colonies may also be thin, spreading, very rough and arborescent, or smooth and dense. Agar slants produce an abundant growth of non-adherent, spreading, dense whitish to yellowish colonies. B. cereus var. mycoides produces grayish colonies and thin colonies. Long twisted chains are often produced which enable the colony to spread over the surface of the agar. Thin, grayish rhizoidal colonies are produced on agar slants. These spread over the

surface adhering to it or growing into the agar. On older slants growth is thicker and softer. A second difference involves motility; B. cereus is motile while B. cereus var. mycoides is usually non-motile.

B. cereus var. mycoides produces few spores on agar. The rhizoid form of colony is lost when this species is grown in flasks containing 100 milliliters of broth. Gordon (1940: cited in Bergey's Manual) says that the resulting forms cannot be differentiated from B. cereus. It is possible that B. cereus var. mycoides is a morphotype of B. cereus. Holzmüller (1909: cited in Bergey's Manual) identified four varieties of B. mycoides which he designated by Greek letters. He also discovered four new species, B. effusus, B. olfactorius, B. nanus and B. dendroides. These were possibly only morphotypes of B. cereus var. mycoides. Many reports of new varieties may be reports of morphotypes of some previously described species.

The other organism was found to be a gram-negative rod which was able to grow singly, in pairs, or in short chains. This organism produced no change in litmus milk indicating that no acid was formed from lactose and that casein was peptidized. No indol reaction resulted in tryptophane broth indicating that this substance was not usable in the metabolism of the organism. Gelatin was liquified and nitrates were reduced indicating that this bacterium is proteolytic and denitrifying.

The optimum temperature for growth was found to be 25 to 30°C but growth did occur at 37°C. When this organism was grown at 25°C it was motile. If grown at 37°C it was nonmotile, but became motile again at 25°C. If it was grown at 25°C and then placed in an environment at 37°C it became nonmotile. A yellow pigment is produced by this organism and the best pigment production occurs when it is grown at 25°C.

The bacterium is sensitive to chloromycetin, polymyxin, tetracycline and dihydrostreptomycin but resistant to penicillin and erythromycin. The organism belongs to the genus Flavobacterium (Bergey, 1957). The members of this genus are aerobes or facultative anaerobes and have feeble powers of attacking carbohydrates. This particular species isolated had peritrichous flagella. Because of the above characteristics plus the fact that it occurs in fresh water it appears to be the species diffusum. It is very similar to F. aquatile but the latter does not reduce nitrates.

When the algal filament is stained the bacteria appear to be growing on the outside of the sheath. It is possible that one end may be slightly embedded in the mucilage of the sheath. The fact that the bacteria do not grow symbiotically within the sheath seems to be substantiated by the ease with which they can be grown on agar.

Effects of Various Concentration of Minerals
on the Growth of Aphanizomenon flos-aquae

Although the medium produced satisfactory growth there is always the possibility that a particular medium might contain too much of one element or not enough of another and that growth could be increased. In growing Aphanizomenon flos-aquae it was found that increase in growth of cultures placed on the shaker was more rapid than that in cultures placed in the growth chamber (Tables 8 and 9). The remainder of the experiments were carried on in the growth chamber because bacterial growth proceeded at a faster rate in those cultures grown on the shaker. This faster growth may have been the result of higher temperature or increased aeration on the shaker.

The growth of Aphanizomenon flos-aquae in the control medium in each experiment was similar (Tables 9, 12, 15, and 19) (Figures 30, 41, 50, 61). It can be assumed safely then that the growth pattern obtained for the other series in these experiments were reasonably accurate even if the experiments were only conducted in duplicate. The pH of the controls did not agree as well as the growth curves but a definite pattern can also be seen when the curves in Figures 31, 42, 51, 62 are compared. An increase in pH to a maximum of 8.5 to 8.95 occurred. The highest pH was recorded for the 15th day. The increase to the maximum was followed by a rather rapid decrease to 7.75 in some and 7.85 in others.

Table 8. Experiment I - Growth of Aphanizomenon flos-aquae in control medium on shaker (wt. in mg.)

Age of Culture (Days)	Wt. of Disk	Wt. of Disk and Filter	Wt. of Filter	Wt. of Disk	Wt. of Disk, Filter and Alga	Wt. of Filter and Alga	Wt. of Alga	pH
5	117.8	200.9	83.1	119.3	203.2	83.9	0.9	7.20
5	117.8	200.6	82.8	119.3	203.0	83.7	0.9	7.25
7	117.8	199.9	82.1	119.3	202.6	83.3	1.2	7.60
7	117.8	199.7	81.9	119.3	202.5	83.2	1.3	7.50
12	117.4	205.6	88.2	119.3	209.0	89.7	1.5	8.00
12	117.4	205.2	87.8	119.3	208.7	89.4	1.6	8.20
16	119.3	195.8	76.5	119.3	198.1	78.8	2.3	8.45
16	119.3	195.8	76.5	119.3	198.3	79.0	2.5	8.50
19	124.9	209.6	84.7	119.3	209.1	89.8	5.1	8.00
19	124.9	209.5	84.6	119.3	208.8	89.5	4.9	8.05

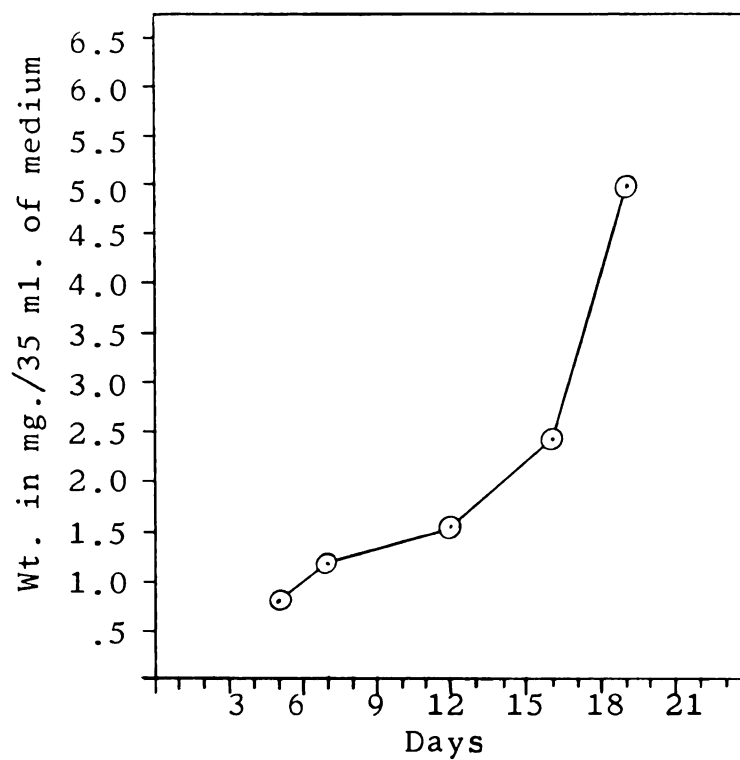


Figure 30. Growth increments of *Aphanizomenon flos-aquae* in mg./35 ml. of medium. Exp. I - Control medium on shaker.

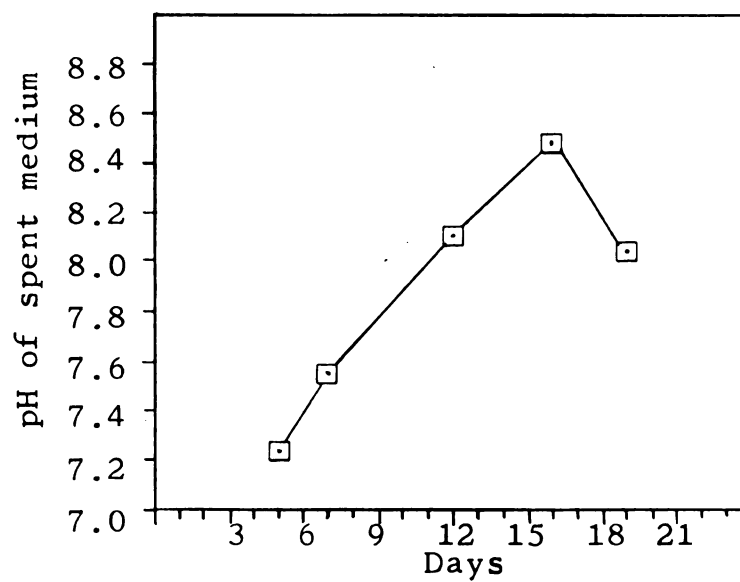


Figure 31. pH of spent medium at time of harvesting. Exp. I - Control medium on shaker.

Table 9. Experiment I - Growth of Aphanizomenon flos-aquae in mg. (control medium in growth chamber) (wt. in mg.)

Age of Culture (Days)	Wt. of Disk	Wt. of Disk and Filter	Wt. of Filter	Wt. of Disk	Wt. of Disk, Filter and Alga	Wt. of Filter and Alga	Wt. of Alga	pH
5	117.8	199.3	81.5	119.3	201.7	82.4	0.9	7.15
5	117.8	199.6	81.8	119.3	201.9	82.6	0.8	7.10
7	117.8	199.9	82.1	119.3	203.1	83.8	1.7	7.60
7	117.8	200.1	82.3	119.3	203.1	83.8	1.5	7.50
12	117.4	206.4	89.0	119.3	210.1	90.8	1.8	8.10
12	117.4	205.9	88.5	119.3	209.7	90.4	1.9	8.15
16	119.3	206.4	87.1	119.3	209.2	89.9	2.8	8.40
16	119.3	205.8	86.5	119.3	208.9	89.6	3.1	8.45
19	124.9	199.1	74.2	119.3	197.7	78.4	4.2	8.10
19	124.9	200.4	75.5	119.3	198.8	79.5	4.0	8.05

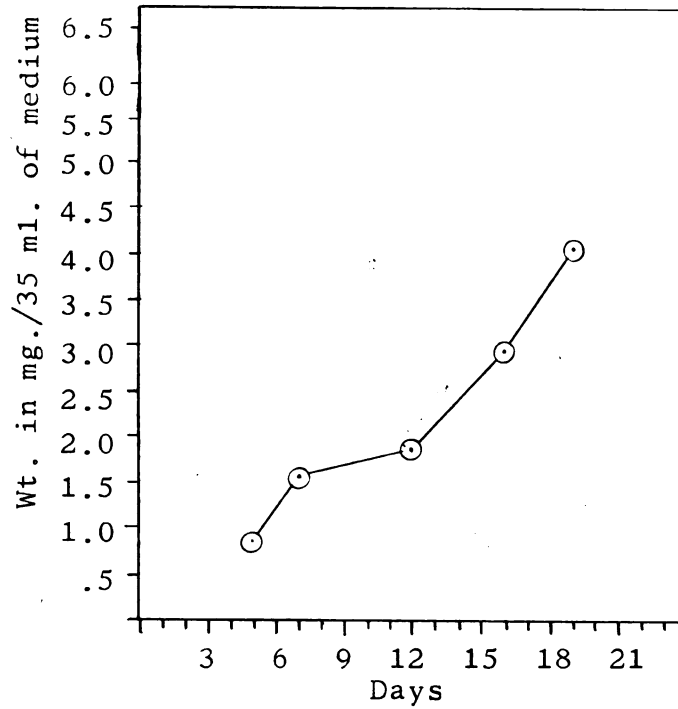


Figure 32. Growth increments of *Aphanizomenon flos-aquae* in mg./35 ml. of medium. Exp. I - Control medium in growth chamber.

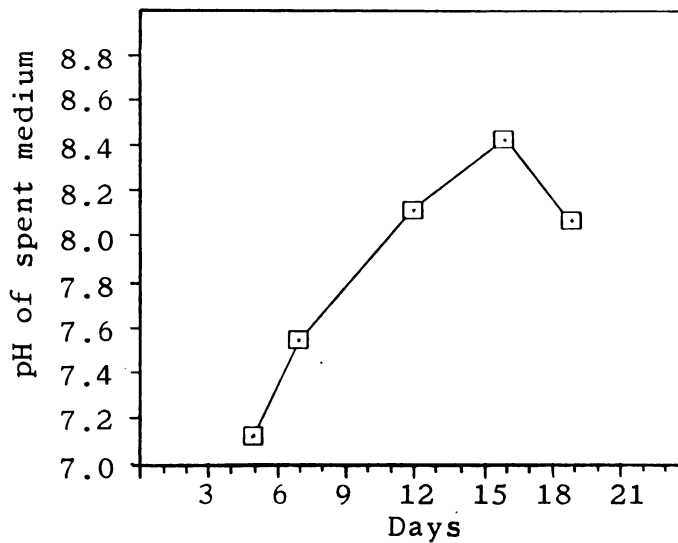


Figure 33. pH of spent medium at time of harvesting. Exp. I - Control medium in growth chamber.

Table 10. Experiment I - Growth of Aphanizomenon flos-aquae in medium containing twice the available nitrogen (wt. in mg.)

Age of Culture (Days)	Wt. of Disk	Wt. of Disk and Filter	Wt. of Filter	Wt. of Disk	Wt. of Disk, Filter and Alga	Wt. of Filter and Alga	Wt. of Alga	pH
5	117.8	199.7	81.9	119.3	201.5	82.2	0.3	7.10
5	117.8	199.6	81.8	119.3	201.6	82.3	0.5	7.15
7	117.8	199.6	81.8	119.3	202.4	83.1	1.3	7.10
7	117.8	199.5	81.7	119.3	202.2	82.9	1.2	7.10
12	117.4	205.8	88.4	119.3	209.2	89.9	1.5	8.20
12	117.4	205.7	88.3	119.3	209.2	89.9	1.6	8.25
16	119.3	197.6	78.3	119.3	199.5	80.2	1.9	8.10
16	119.3	197.7	78.4	119.3	199.5	80.2	1.8	8.05
19	124.9	202.2	77.3	119.3	198.3	79.0	1.7	7.70
19	124.9	209.6	84.7	119.3	205.7	86.4	1.7	7.75

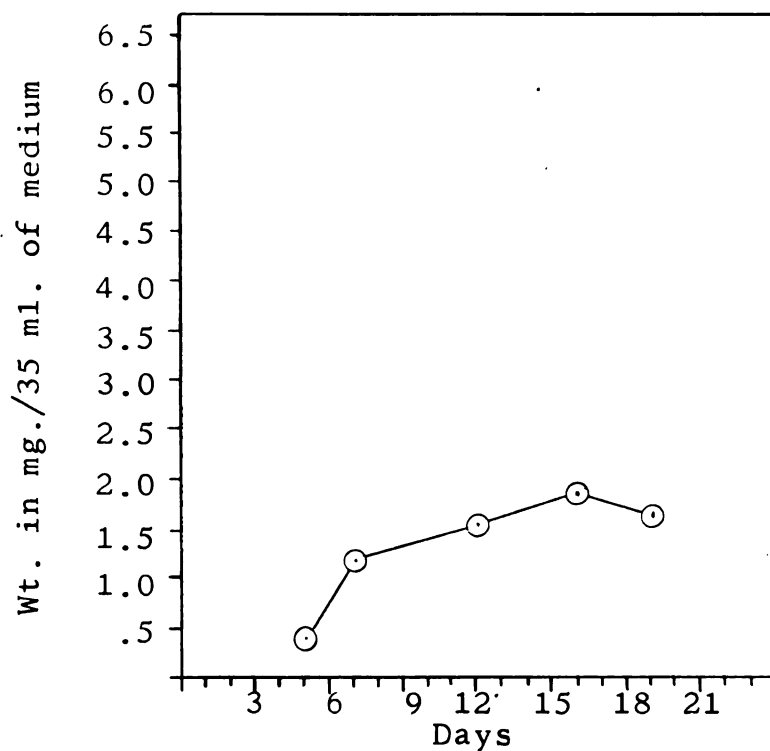


Figure 34. Growth increments of *Aphanizomenon flos-aquae* in mg./35 ml. of medium. Exp. I - Medium containing twice the amount of nitrogen as in the control.

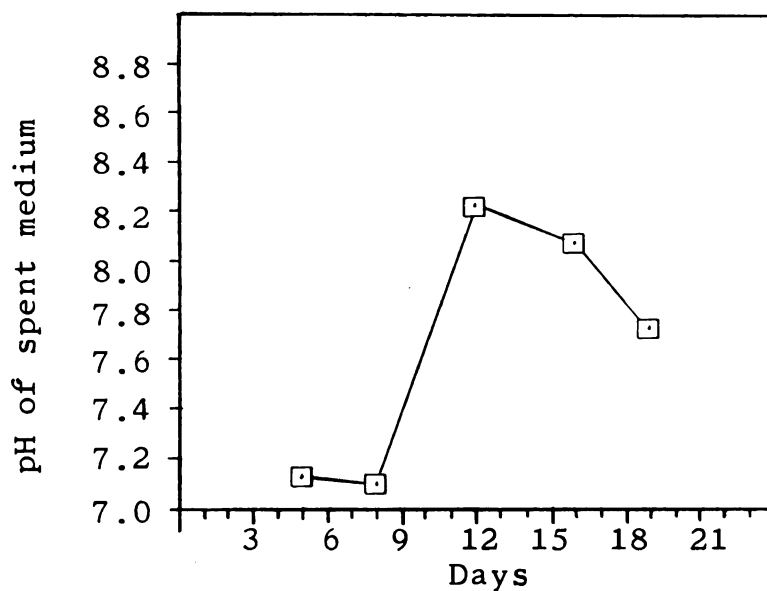


Figure 35. pH of spent medium at time of harvesting. Exp. I - Medium containing twice the amount of nitrogen as in the control.

Table 11. Experiment I - Growth of Aphanizomenon flos-aquae in medium containing three times the available nitrogen (wt. in mg.)

Age of Culture (Days)	Wt. of Disk	Wt. of Disk and Filter	Wt. of Filter	Wt. of Disk	Wt. of Disk and Filter and Alga	Wt. of Filter and Alga	Wt. of Alga	pH
5	117.8	199.4	81.6	119.3	201.0	81.7	0.1	7.00
5	117.8	199.5	81.7	119.3	201.2	81.9	0.2	7.00
7	117.8	199.2	81.4	119.3	201.2	81.9	0.5	7.15
7	117.8	199.3	81.5	119.3	201.3	82.0	0.5	7.15
12	117.4	205.7	88.3	119.3	208.1	88.1	0.5	7.70
12	117.4	204.5	87.1	119.3	206.9	87.5	0.5	7.75
16	119.3	206.9	87.6	119.3	207.4	88.1	0.5	7.80
16	119.3	206.4	87.1	119.3	206.8	87.5	0.4	7.80
19	124.9	202.7	77.8	119.3	197.6	78.3	0.5	7.40
19	124.9	202.8	77.9	119.3	197.7	78.4	0.5	7.35

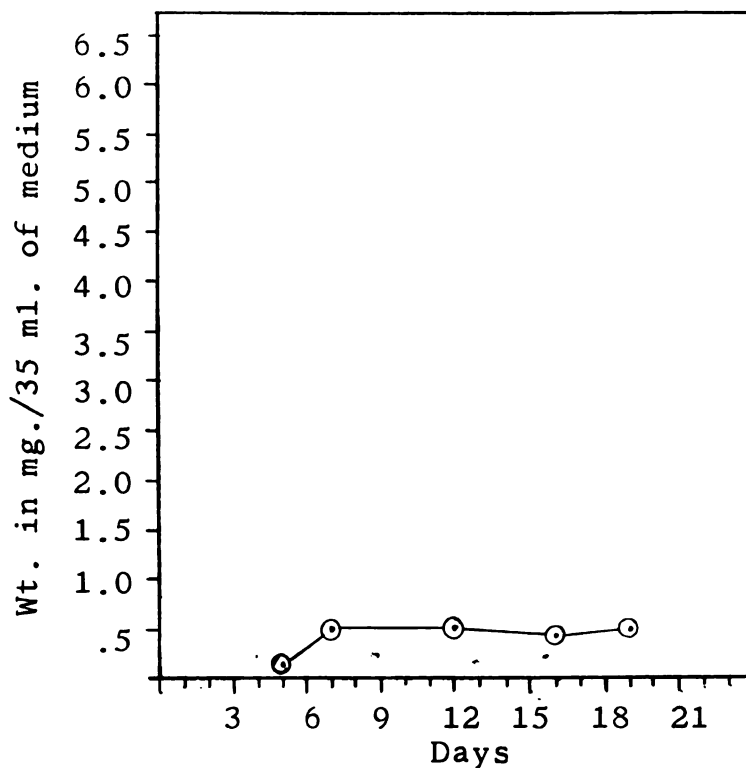


Figure 36. Growth increments of Aphanizomenon flos-aquae in mg./35 ml. of medium. Exp. I - Medium containing three times the amount of nitrogen as in the control.

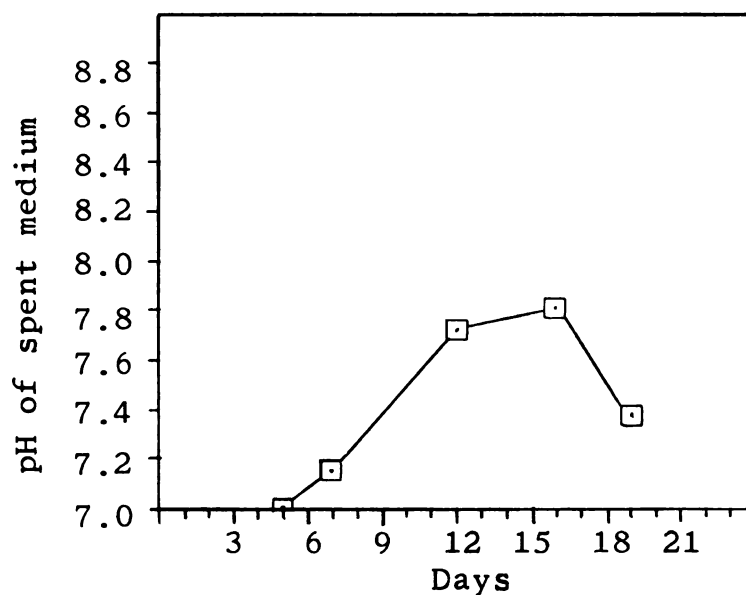


Figure 37. pH of spent medium at time of harvesting. Exp. I - Medium containing three times the amount of nitrogen as in the control.

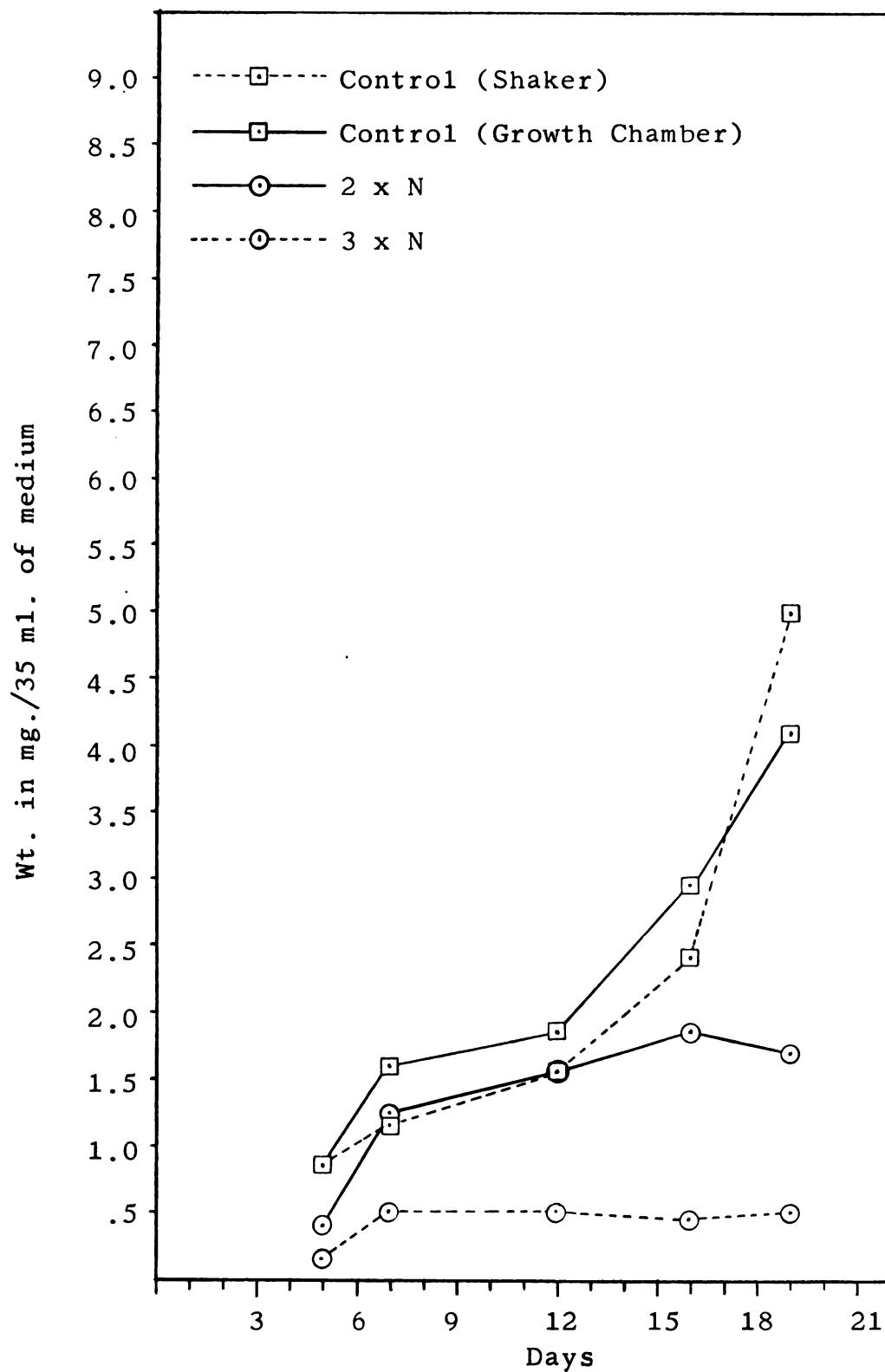


Figure 38. Comparison of growth increments of Aphanizomenon flos-aquae. Exp. I - Variations in nitrogen concentration.

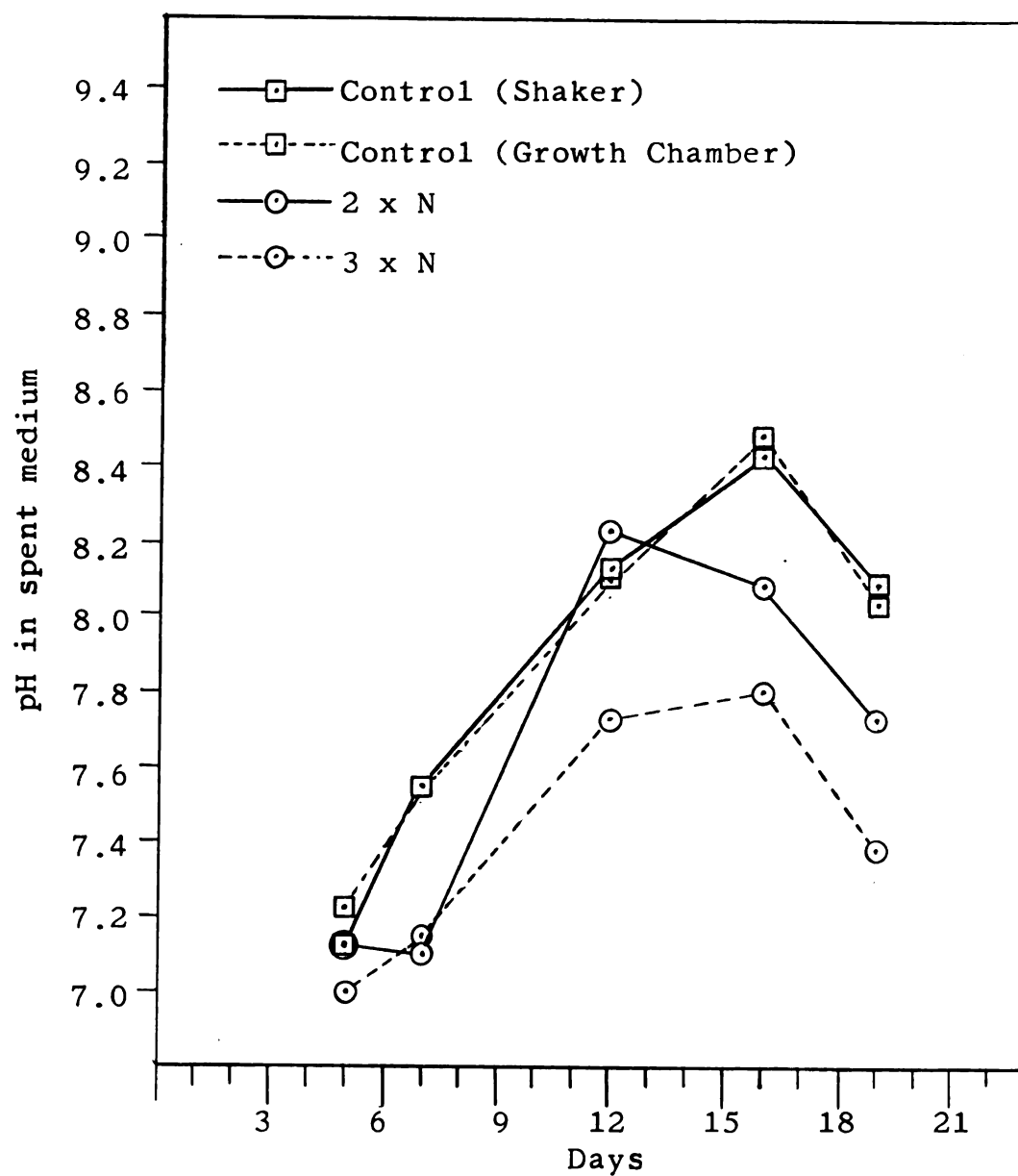


Figure 39. Comparison of pH of spent medium at the time of harvesting. Exp. I - Variations in nitrogen concentration.

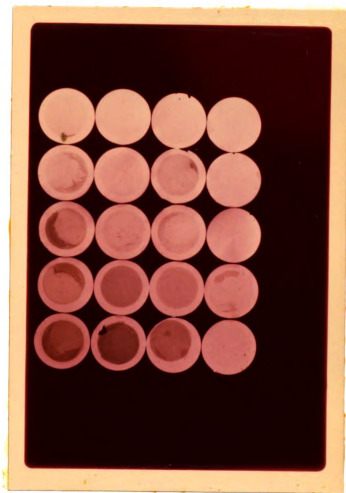


Figure 40. Millipore filters from Experiment I; age from top to bottom, 5, 7, 12, 16, and 19 days; from left to right, control on shaker, control in growth chamber, 2 x nitrogen, and 3 x nitrogen. Note absence of growth in series on right which contained 3 x nitrogen.

When the amount of nitrogen source in the medium was doubled the maximum growth was 1.7 milligrams. The maximum growth in the control was 5.1 milligrams (Table 8). The pH of this series reached a maximum of 8.25 on the 12th day. This compares with a maximum of 8.45 in the control (Figure 33).

Increasing the amount of available nitrogen to three times the amount in the control inhibited growth. The maximum of 0.5 milligrams was recorded on the 6th day. The pH for this series was less than 8.0 (Figure 35). The millipore filters, one for each day of harvesting, can be seen in Figure 40. The maximum growth in this series can be seen on the last filter in the second row from the left.

The effects on the growth of A. flos-aquae of increased concentrations of available phosphorus are recorded in Tables 13 and 14. The growth of the alga decreased when the concentration of this element was increased. The reduction in growth was not as great as in the cultures in which the nitrogen was increased. The maximum growth in the series in which the phosphorus was doubled was 3.2 milligrams and in the medium which contained three times the amount of this element the maximum was 2.9 milligrams (Figure 45). The difference in growth in these two series was very small but the growth was only about half as much as in the control (Figures 43 and 45).

Table 12. Experiment II - Growth of Aphanizomenon flos-aquae in control medium (wt. in mg.)

Age of Culture (Days)	Wt. of Disk	Wt. of Disk and Filter	Wt. of Filter	Wt. of Disk	Wt. of Disk and Filter and Alga	Wt. of Filter and Alga	Wt. of Alga	pH
3	154.7	241.1	86.4	150.8	238.2	87.4	1.0	7.60
3	154.7	241.8	87.1	150.8	238.8	88.0	0.9	7.55
6	154.7	241.8	87.1	150.8	239.5	88.7	1.6	7.85
6	154.7	241.8	87.1	150.8	239.4	88.6	1.5	7.80
9	153.9	244.3	90.4	150.8	243.4	92.6	2.2	7.85
9	153.9	244.6	90.7	150.8	243.9	93.1	2.4	7.95
12	151.7	242.5	90.8	150.8	243.9	93.1	2.3	8.05
12	151.7	240.3	88.6	150.8	241.6	90.8	2.2	8.10
15	154.7	244.7	90.0	150.8	244.1	93.3	3.3	8.50
15	154.7	245.7	91.0	150.8	245.2	94.4	3.4	8.55
18	156.7	243.3	86.6	150.8	242.1	91.3	4.7	8.35
18	156.7	244.3	87.6	150.8	242.9	92.1	4.5	8.30
21	156.8	245.5	88.7	150.8	245.0	94.2	5.5	7.80
21	156.8	243.7	86.9	150.8	242.8	92.0	5.1	7.85

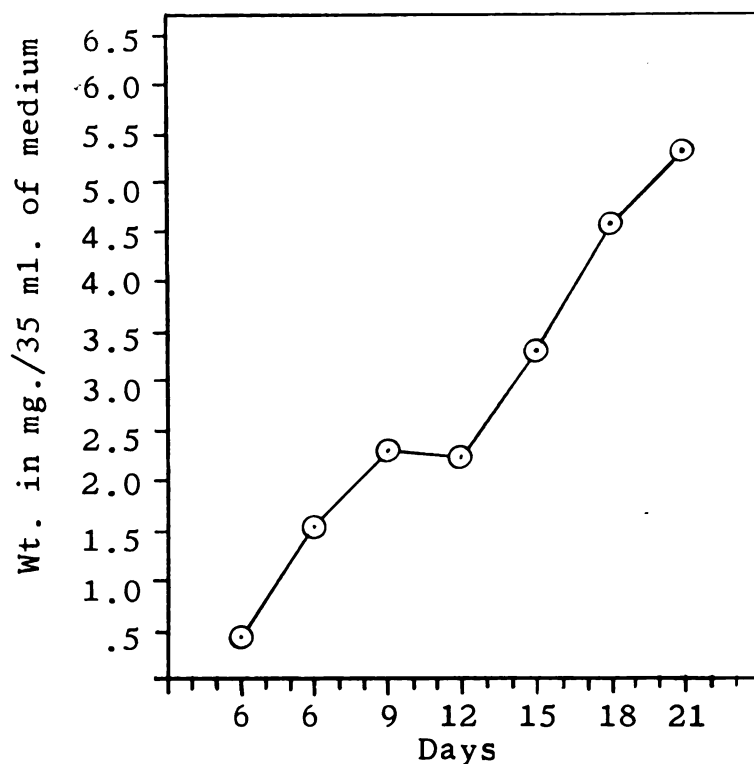


Figure 41. Growth increments of *Aphanizomenon flos-aquae* in mg./35 ml. of medium. Exp. II - Control medium.

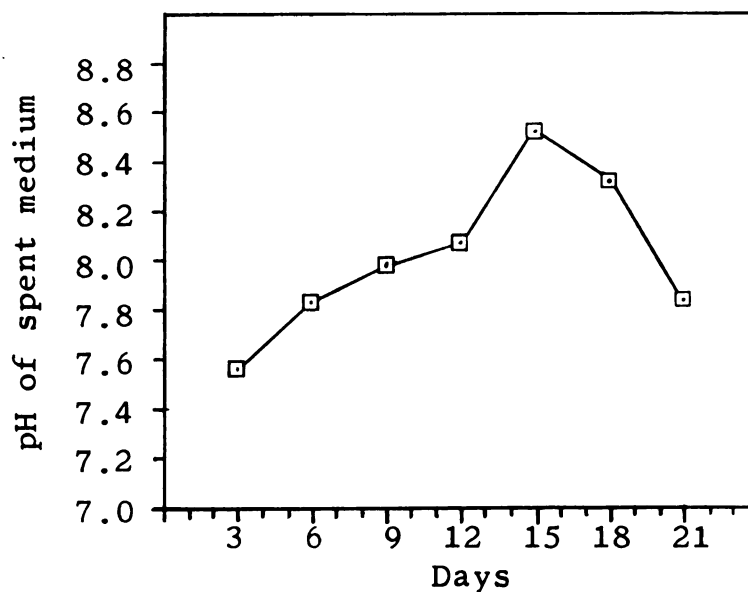


Figure 42. pH of spent medium at time of harvesting. Exp. II - Control medium.

Table 13. Experiment II - Growth of Aphanizomenon flos-aquae in medium containing twice the amount of available phosphorus (wt. in mg.)

Age of Culture (Days)	Wt. of Disk	Wt. of Disk and Filter	Wt. of Filter	Wt. of Disk	Wt. of Disk Filter and Alga	Wt. of Filter and Alga	Wt. of Alga	pH
3	154.7	241.8	87.1	150.8	238.9	88.1	1.0	7.65
3	154.7	243.5	88.8	150.8	240.6	89.8	1.0	7.60
6	154.7	241.9	87.2	150.8	239.2	88.4	1.2	7.60
6	154.7	241.7	87.0	150.8	239.2	88.4	1.4	7.70
9	153.9	244.2	90.3	150.8	242.6	91.8	1.5	7.80
9	153.9	244.6	90.7	150.8	242.9	92.1	1.4	7.85
12	151.7	240.4	88.7	150.8	241.8	91.0	2.3	8.00
12	151.7	240.4	88.7	150.8	241.7	90.9	2.2	8.15
15	154.7	244.8	90.1	150.8	243.8	93.0	2.9	8.00
15	154.7	244.9	90.2	150.8	243.8	93.0	2.8	8.05
18	156.7	243.2	86.5	150.8	240.5	89.7	3.2	8.05
18	156.7	243.2	86.5	150.8	240.2	89.4	2.9	8.05
21	156.8	243.6	86.8	150.8	240.5	89.7	2.9	8.25
21	156.8	242.8	86.0	150.8	239.9	89.1	3.1	8.25

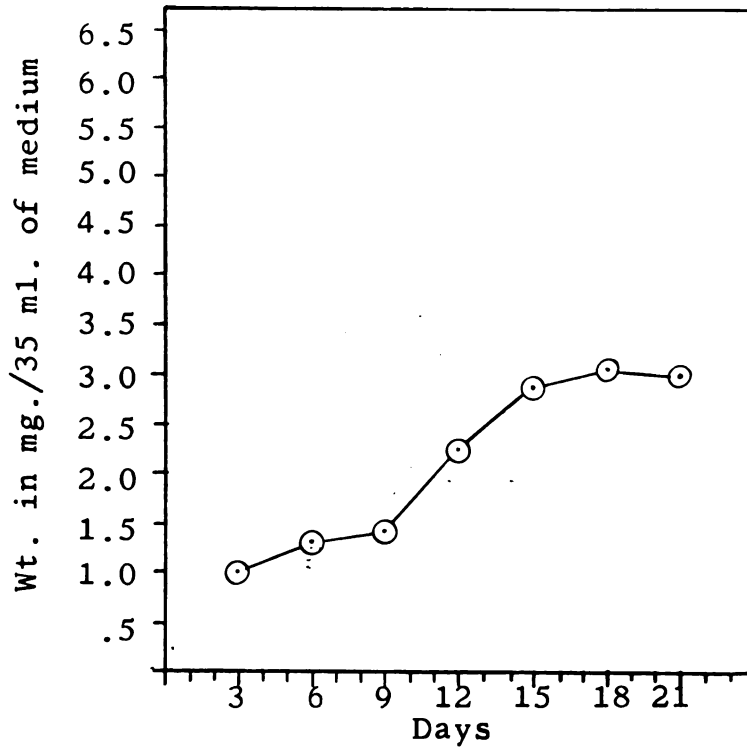


Figure 43. Growth increments of *Aphanizomenon flos-aquae* in mg./35 ml. of medium. Exp. II - Medium containing twice the amount of phosphorus as in the control.

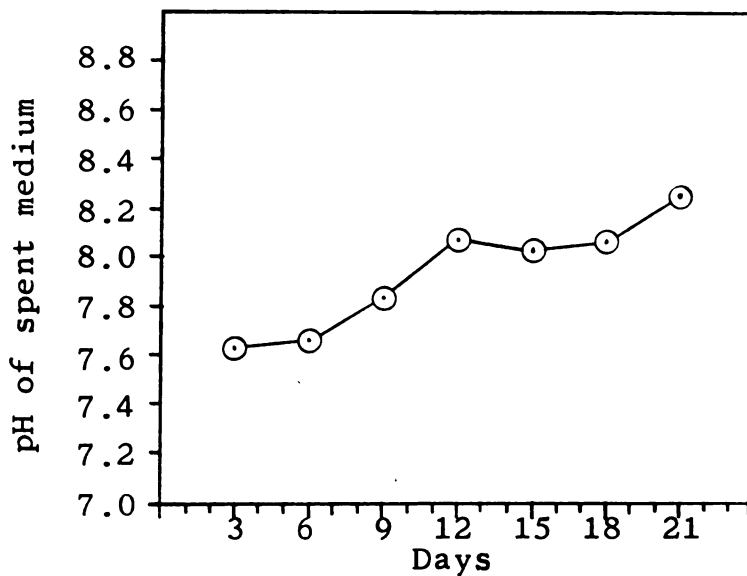


Figure 44. pH of spent medium at time of harvesting. Exp. II - Medium containing twice the amount of phosphorus as in the control.

Table 14. Experiment II - Growth of Aphanizomenon flos-aquae in medium containing three times the amount of available phosphorus (wt. in mg.)

Age of Culture (Days)	Wt. of Disk	Wt. of Disk and Filter	Wt. of Filter	Wt. of Disk	Wt. of Disk and Alga	Wt. of Filter and Alga	Wt. of Alga	pH
3	154.7	242.9	88.2	150.8	240.0	89.2	1.0	7.65
3	154.7	242.9	88.2	150.8	239.9	89.1	0.9	7.60
6	154.7	242.3	87.6	150.8	239.7	88.9	1.3	7.75
6	154.7	242.6	87.9	150.8	240.1	89.3	1.4	7.75
9	153.9	245.1	91.2	150.8	243.2	92.4	1.2	7.70
9	153.9	244.6	90.7	150.8	242.7	91.9	1.2	7.80
12	151.7	240.7	89.0	150.8	241.5	90.7	1.7	8.10
12	151.7	240.9	89.2	150.8	241.7	90.9	1.7	8.10
15	154.7	243.5	88.8	150.8	242.2	91.4	2.6	7.95
15	154.7	244.0	89.3	150.8	242.3	91.5	2.2	8.05
18	156.7	242.9	86.2	150.8	239.5	88.7	2.5	8.15
18	156.7	246.1	89.4	150.8	242.5	91.7	2.3	8.15
21	156.8	246.6	89.8	150.8	243.4	92.8	2.8	8.35
21	156.8	246.8	90.0	150.8	243.3	92.5	2.5	8.25

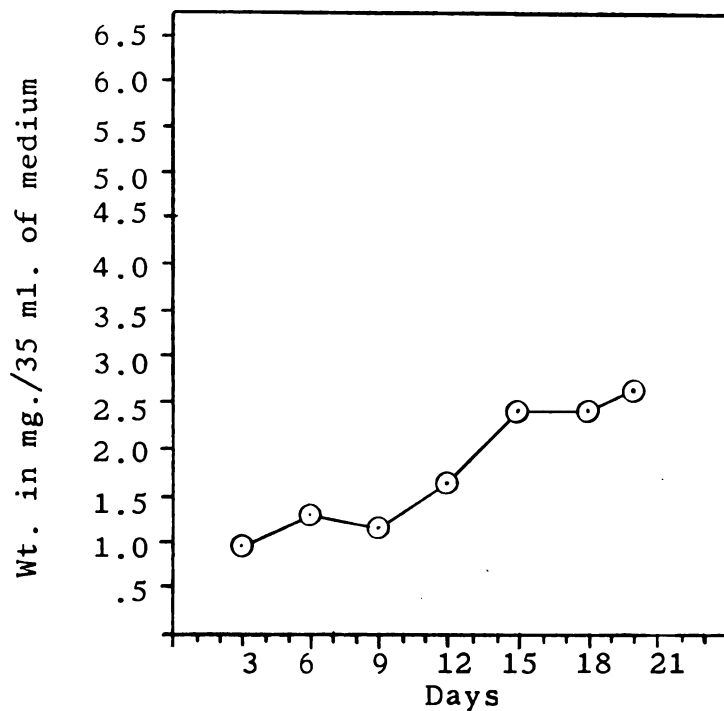


Figure 45. Growth increments of Aphanizomenon flos-aquae in mg./35 ml. of medium. Exp. II - Medium containing three times the amount of phosphorus as in the control.

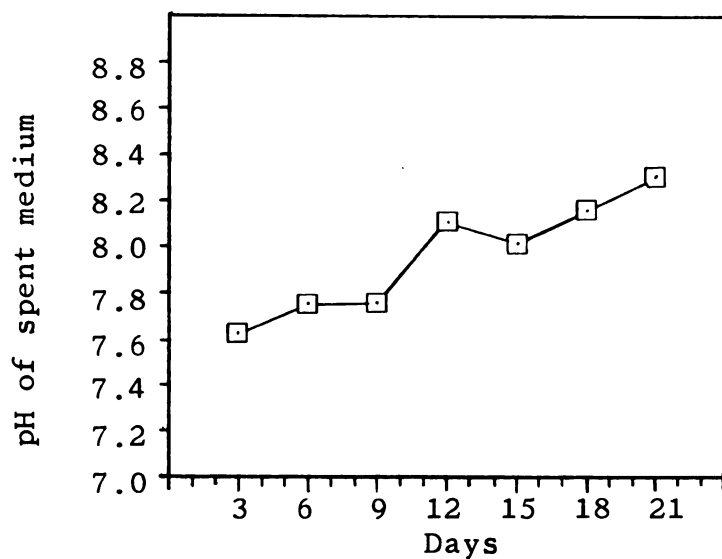


Figure 46. pH of spent medium at time of harvesting. Exp. II - Medium containing three times the amount of phosphorus as in the control.

It is interesting to compare the pH curves for these series. The control exhibited a rise to a maximum followed by a rather rapid drop. In the other two series the pH increased more slowly but continued to increase until the experiment was terminated (Figure 44 and 46). The maximum pH for the experimental series was 8.35 on the 21st day compared with the maximum of 8.55 for the control on the 15th day. The millipore filters shown in Figure 49 are from these three series. The maximum growth can be seen on the last filter in the first row on the left.

Decreasing the amount of manganese increased the growth of A. flos-aquae. Decreasing the manganese to 1/100th the amount in the control produced a maximum growth of 8.4 milligrams (Figure 52) compared with a maximum of 6.1 for the control (Figure 50). This is an increase of almost 25 per cent. Reduction in the concentration of this element to 1/1000th of the concentration found in the control further increased the growth; the maximum being 9.1 milligrams (Figure 54), an increase of almost 50 per cent compared with the growth in the control (Tables 16 and 17). Omission of this element entirely produced better growth than the control medium; a maximum of 8.3 milligrams (Figure 56). The pH of the cultures in which manganese was reduced rose higher than the pH of the control. A maximum of 8.95 occurred in the medium containing 1/100th the amount of manganese in the control (Figure 53).

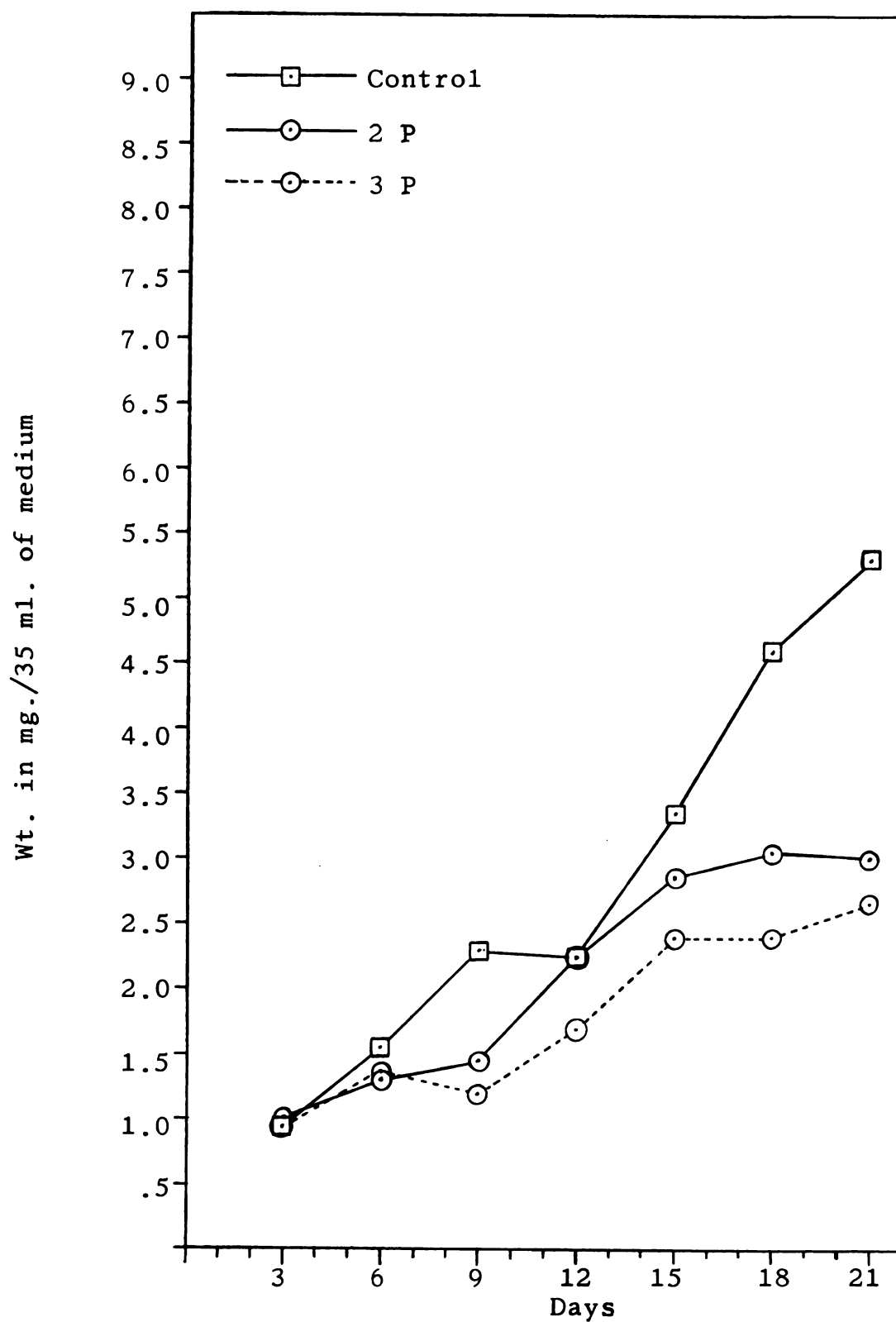


Figure 47. Comparison of growth increments of *Aphanizomenon flos-aquae*. Exp. II - Variations in phosphorus concentration.

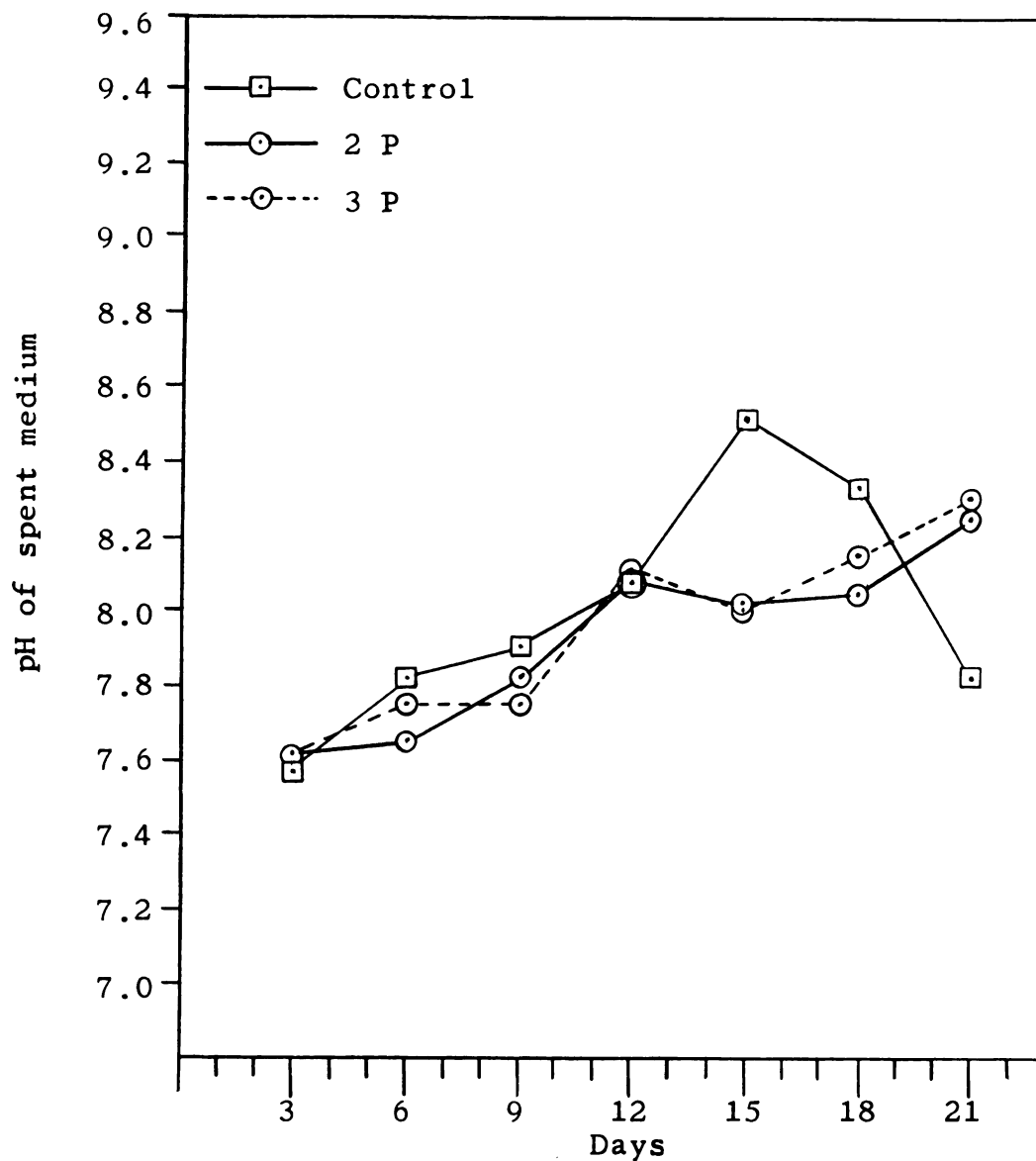


Figure 48. Comparison of pH of spent medium at time of harvesting. Exp. II - Variation in phosphorus concentration.

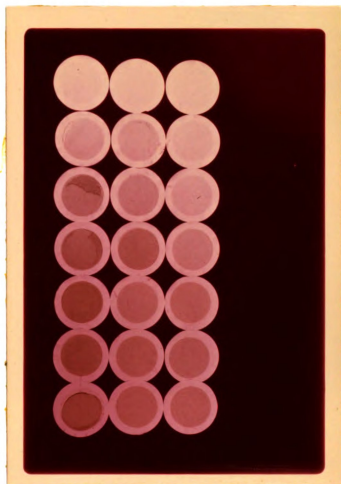


Figure 49. Millipore filters from Experiment II; age from top to bottom, 3, 6, 9, 12, 15, 18, and 21 days; from left to right, control, 2 x phosphorus, and 3 x phosphorus. Greatest growth occurred in control.

Table 15. Experiment III - Growth of Aphanizomenon flos-aquae in control medium (wt. in mg.)

Age of Culture (Days)	Wt. of Disk	Wt. of Disk and Filter	Wt. of Filter	Wt. of Disk	Wt. of Disk Filter and Alga	Wt. of Filter and Alga	Wt. of Alga	pH
3	149.7	236.9	87.2	144.4	232.7	88.3	1.1	7.60
3	149.7	236.9	87.2	144.4	232.8	88.4	1.2	7.60
6	149.7	236.9	87.2	150.1	238.9	88.8	1.6	8.25
6	149.7	237.5	87.8	150.1	239.5	89.4	1.6	8.15
9	144.4	230.5	86.1	150.1	238.1	88.0	1.9	8.05
9	144.4	230.5	86.1	150.1	238.2	88.1	2.0	8.25
12	150.5	242.9	92.4	150.1	244.7	94.6	2.2	8.10
12	150.5	242.9	92.4	150.1	244.7	94.6	2.2	8.25
15	148.2	236.9	88.7	150.1	242.4	92.3	3.6	8.35
15	148.2	237.3	89.1	150.1	243.0	92.9	3.8	8.65
18	149.5	239.2	89.7	154.8	248.9	94.1	4.4	8.55
18	149.5	239.5	90.0	154.8	248.9	94.1	4.1	8.35
21	149.5	236.5	87.0	154.8	247.4	92.6	5.6	7.75
21	149.5	236.1	86.6	154.8	247.5	92.7	6.1	7.85

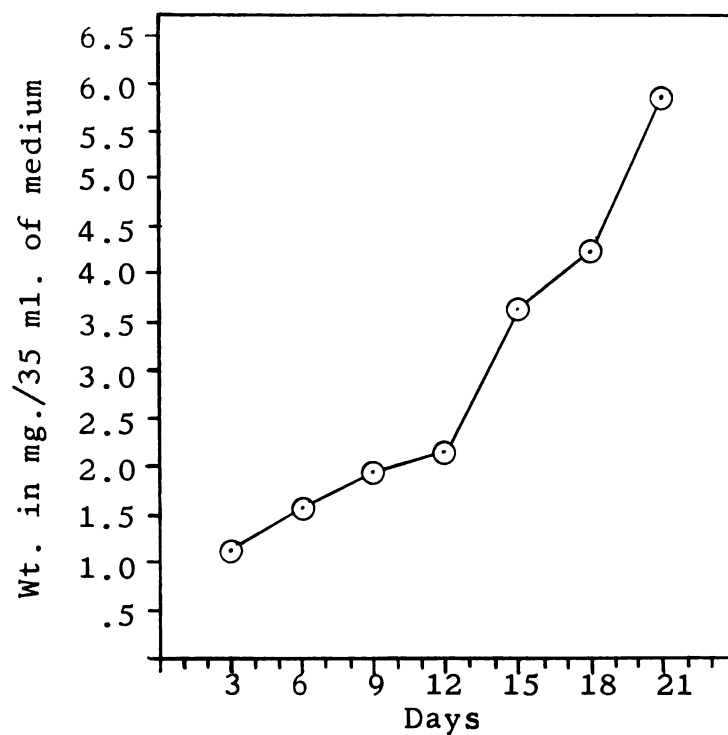


Figure 50. Growth increments of *Aphanizomenon flos-aquae* in mg./35 ml. of medium. Exp. III - Control medium.

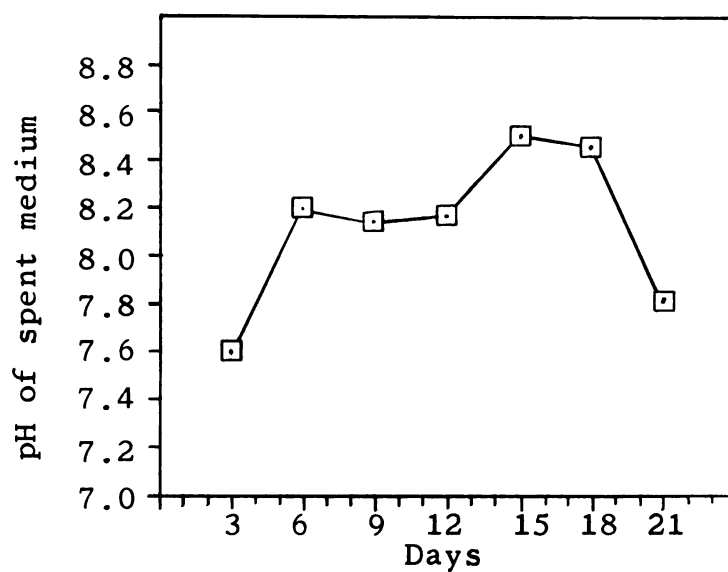


Figure 51. pH of spent medium at time of harvesting. Exp. III - Control medium.

Table 16. Experiment III - Growth of Aphanizomenon flos-aquae in medium containing 0.01 the amount of available manganese (wt. in mg.)

Age of Culture (Days)	Wt. of Disk	Wt. of Disk and Filter	Wt. of Filter	Wt. of Disk	Wt. of Disk, Filter and Alga	Wt. of Filter and Alga	Wt. of Alga	pH
3	149.7	236.2	86.5	144.4	232.0	87.6	1.1	7.65
3	149.7	236.2	86.5	144.4	231.9	87.5	1.0	7.60
6	149.7	239.3	89.6	150.1	240.8	90.7	1.1	8.00
6	149.7	238.5	88.8	150.1	240.1	90.0	1.2	7.90
9	144.4	231.4	87.0	150.1	238.6	88.5	1.5	8.00
9	144.4	229.9	85.5	150.1	237.3	87.2	1.7	8.00
12	150.5	243.5	93.0	150.1	234.7	94.6	1.6	8.95
12	150.5	242.8	92.3	150.1	244.1	94.0	1.7	8.75
15	148.2	237.4	89.2	150.1	245.5	95.4	6.2	8.65
15	148.2	237.3	89.1	150.1	244.9	94.8	5.7	8.70
18	149.5	239.6	90.1	154.8	252.2	97.4	7.3	8.55
18	149.5	235.4	85.9	154.8	248.3	93.5	7.6	8.95
21	149.5	236.0	86.5	154.8	249.4	94.6	8.1	7.90
21	149.5	239.7	90.2	154.8	253.4	98.6	8.4	8.10

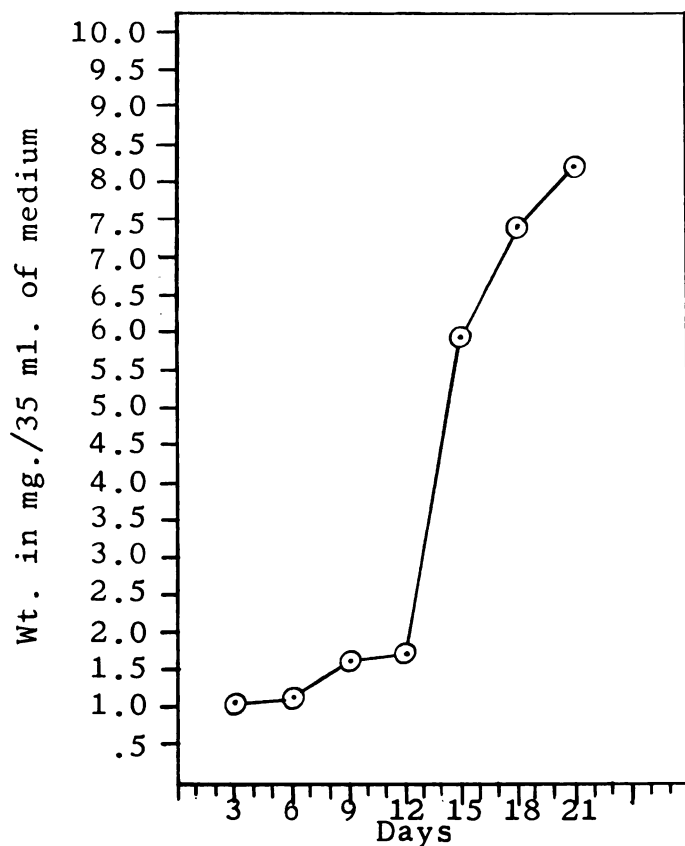


Figure 52. Growth increments of Aphanizomenon flos-aquae in mg./35 ml. of medium. Exp. III - Medium containing 0.01 the amount of manganese as in the control.

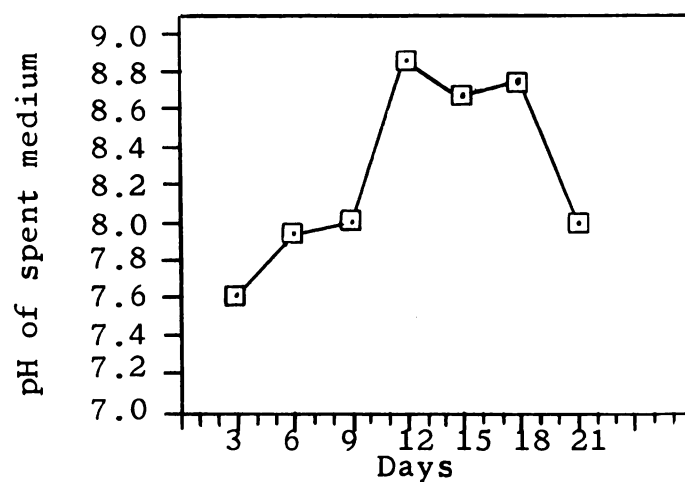


Figure 53. pH of spent medium at time of harvesting. Exp. III - Medium containing 0.01 the amount of manganese as in the control.

Table 17. Experiment III - Growth of *Aphanizomenon flos-aquae* in medium containing
 Limit of available manganese (wt. in mg.)

Table 17. Experiment III - Growth of *Aphanizomenon flos-aquae* in medium containing 0.001 the amount of available manganese (wt. in mg.)

Age of Culture (Days)	Wt. of Disk	Wt. of Disk and Filter	Wt. of Filter	Wt. of Disk	Wt. of Disk, Filter and Alga	Wt. of Filter and Alga	Wt. of Alga	pH
3	149.7	236.9	87.2	144.4	233.4	89.0	1.8	7.65
3	149.7	236.5	86.8	144.4	233.1	88.7	1.9	7.60
6	149.7	237.4	87.7	150.1	239.6	89.5	1.8	8.15
6	149.7	238.7	89.0	150.1	240.2	90.1	1.1	8.10
9	144.4	232.1	87.7	150.1	239.5	89.4	1.7	8.15
9	144.4	229.6	85.2	150.1	237.1	87.0	1.8	8.20
12	150.5	242.5	92.0	150.1	246.4	96.3	4.3	8.80
12	150.5	242.7	92.2	150.1	246.2	96.1	3.9	8.60
15	148.2	237.3	89.1	150.1	246.7	96.6	7.5	8.60
15	148.2	237.3	89.0	150.1	245.5	95.4	6.4	8.60
18	149.5	235.6	86.1	154.8	248.3	93.5	7.4	8.45
18	149.5	234.9	85.4	154.8	247.6	92.8	7.4	8.40
21	149.5	235.6	86.1	154.8	250.1	95.3	9.2	7.85
21	149.5	235.6	86.1	154.8	250.0	95.2	9.1	7.80

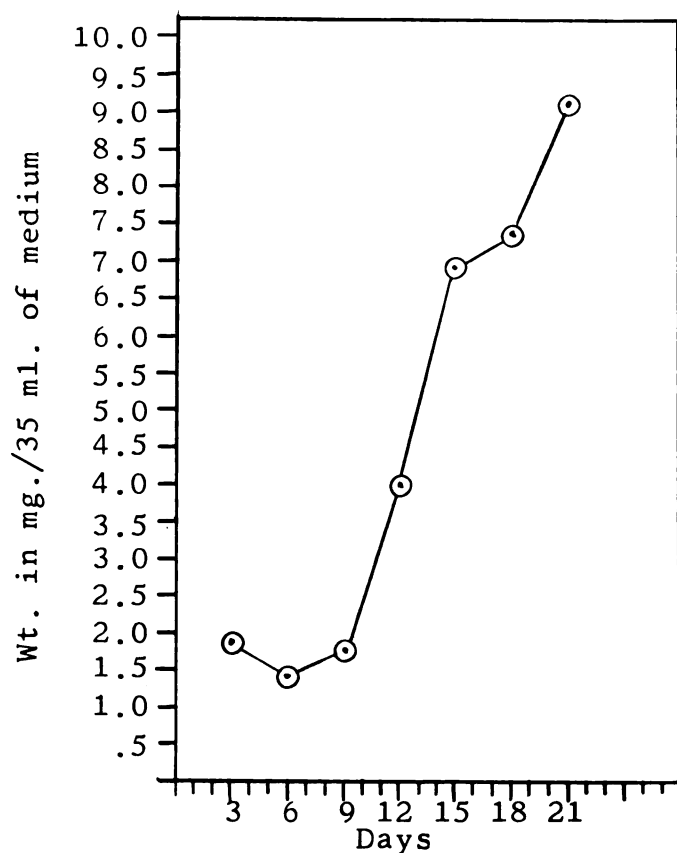


Figure 54. Growth increments of Aphanizomenon flos-aquae in mg./35 ml. of medium. Exp. III - Medium containing 0.001 the amount of manganese as in the control.

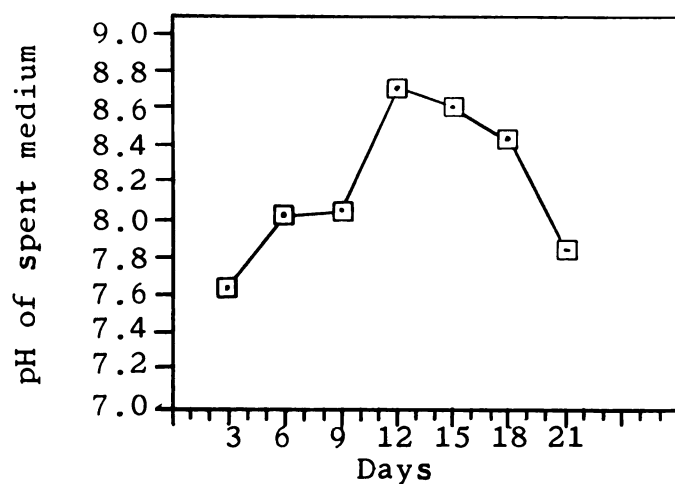


Figure 55. pH of spent medium at time of harvesting. Exp. III - Medium containing 0.001 the amount of manganese as the control.

Table 18. Experiment III - Growth of Aphanizomenon flos-aquae in medium containing no added manganese (wt. in mg.)

Age of Culture (Days)	Wt. of Disk	Wt. of Disk and Filter	Wt. of Filter	Wt. of Disk	Wt. of Disk, Filter and Alga	Wt. of Filter and Alga	Wt. of Alga	pH
3	149.7	236.1	86.4	144.4	231.2	86.8	0.4	7.60
3	149.7	235.9	86.0	144.4	230.7	86.3	0.3	7.50
6	149.7	238.2	88.5	150.1	239.1	89.0	0.5	7.85
6	149.7	238.2	88.5	150.1	239.0	88.9	0.4	7.80
9	144.4	230.6	86.2	150.1	237.8	87.7	1.5	8.05
9	144.4	230.6	86.2	150.1	237.6	87.5	1.3	8.00
12	150.5	243.4	92.9	150.1	246.5	96.4	3.5	8.55
12	150.5	243.7	93.2	150.1	246.1	96.0	3.8	8.55
15	148.2	237.6	89.4	150.1	245.9	95.8	6.4	8.55
15	148.2	237.6	89.4	150.1	245.7	95.6	6.2	8.50
18	149.5	235.1	85.6	154.8	248.5	93.7	8.1	8.45
18	149.5	235.1	85.6	154.8	248.1	93.3	7.7	8.40
21	149.5	235.4	85.9	154.8	248.7	93.9	8.0	7.70
21	149.5	235.4	85.6	154.8	248.7	93.9	8.0	7.75

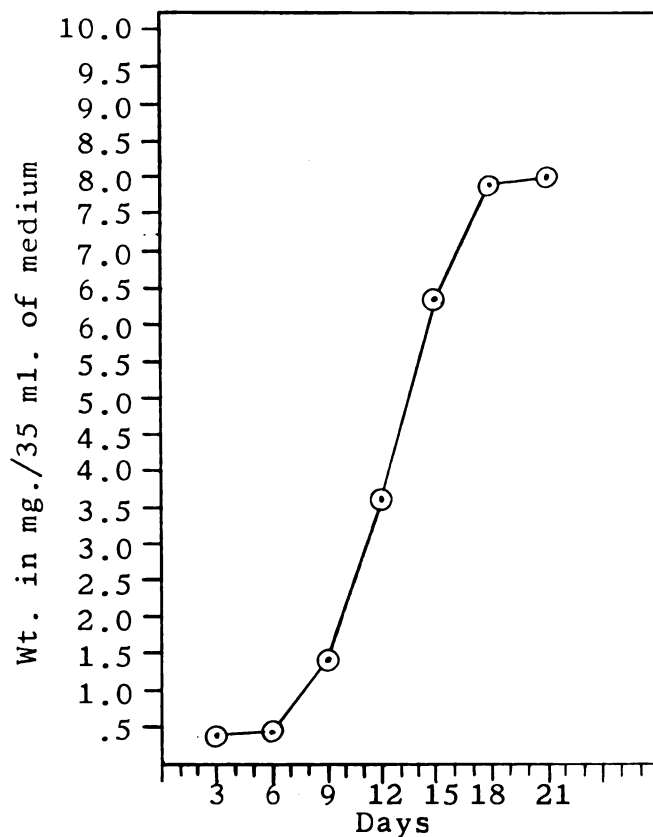


Figure 56. Growth increments of Aphanizomenon flos-aquae in mg./35 ml. of medium. Exp. III - Medium with no manganese added.

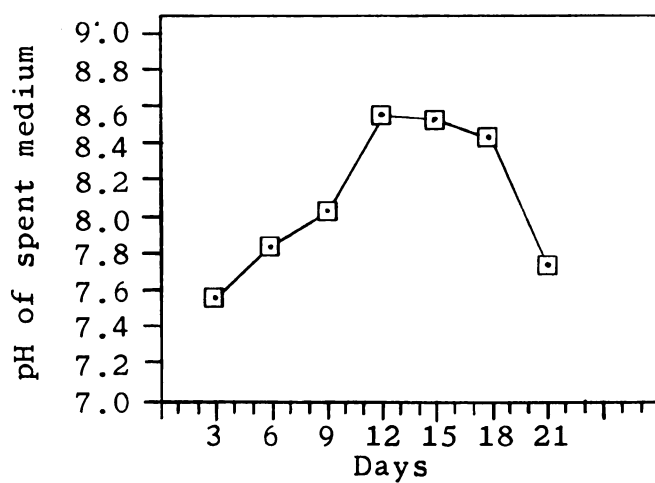


Figure 57. pH of spent medium at time of harvesting. Exp. III - Medium with no manganese added.

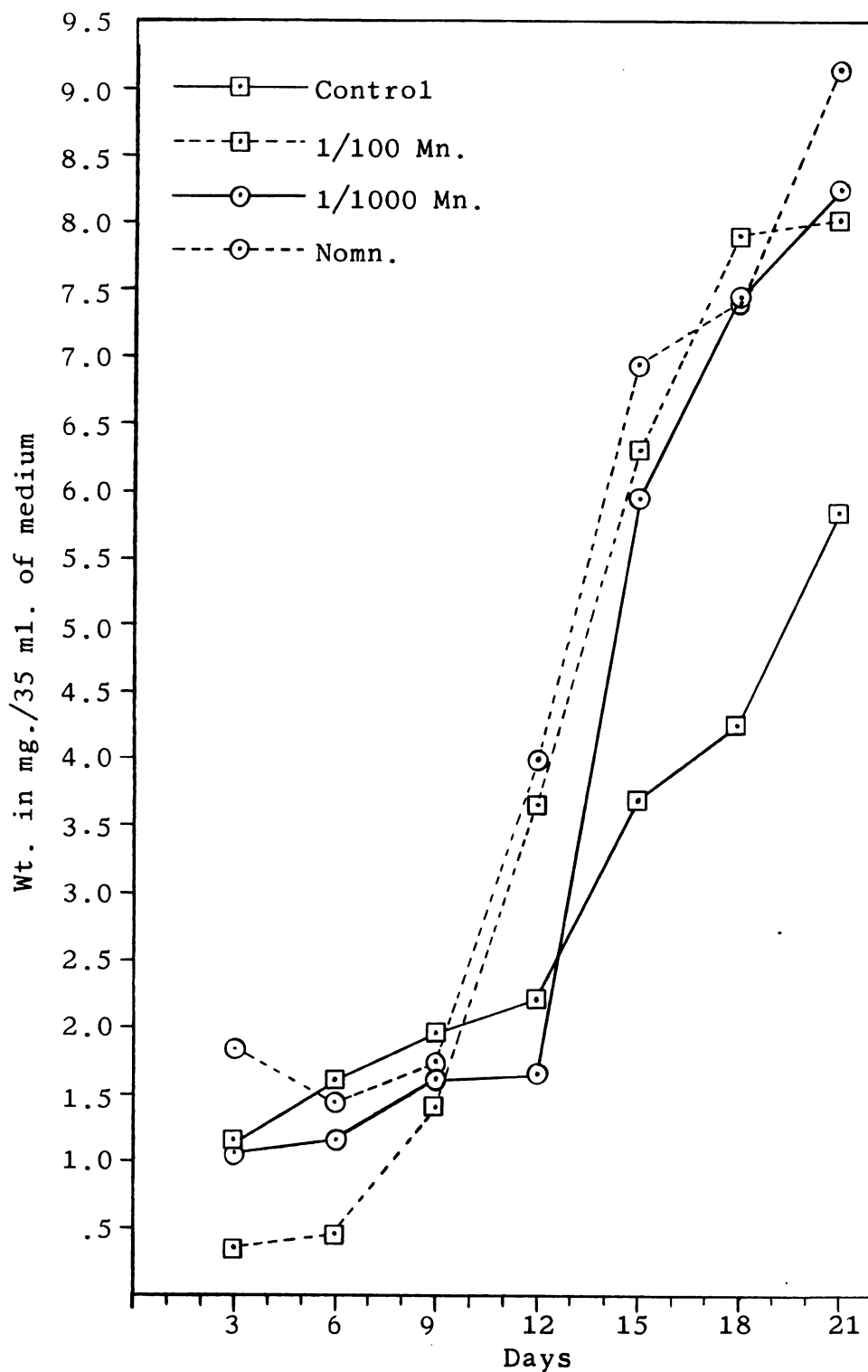


Figure 58. Comparison of growth increments of *Aphanizomenon flos-aquae*. Exp. III - Decrease in manganese concentration.

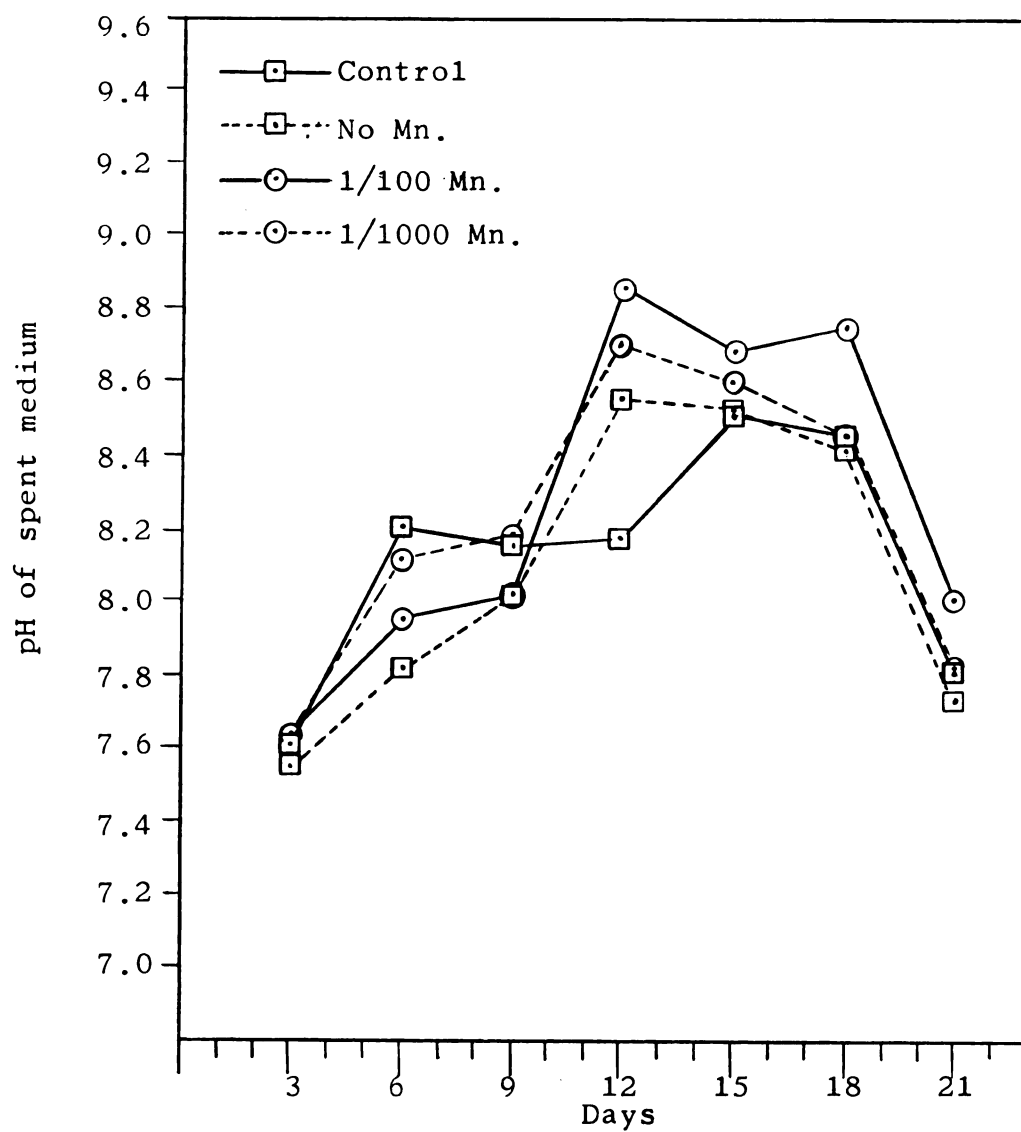


Figure 59. Comparison of pH of spent medium at time of harvesting. Exp. III - Decrease in manganese concentration.

The millipore filters in Figure 60 shows the results of varying the amounts of this element.

Reducing the amount of available nitrogen to one-half the amount in the control did not effect the growth appreciably (Table 20). The decrease to one-tenth the amount in the control increased the maximum growth to 5.7 milligrams, only one milligram greater than the maximum in the control (Figures 61, 63, 65).

Of all the pH determinations made those for this series were the most peculiar (Figures 62, 64, 66). The pH rose to a maximum of 8.0 in the cultures in which the nitrogen was reduced to one-tenth (Figure 66). The pH of these two series did not exhibit the pattern of the controls. The pH remained fairly steady between 7.3 and 8.0.

Microcystis aeruginosa, M. incerta and Anabaena flos-aquae inoculated into the spent medium from the cultures of Aphanizomenon flos-aquae produced what appeared to be normal growth when compared with cultures of these same species inoculated into fresh medium. The increase in bacterial growth occurred at about the same time as in the controls. This was not true when the spent medium was reinoculated with Aphanizomenon flos-aquae. The alga did not grow. The culture became milky and microscopic examination showed that the cells were plasmolyzed. This species evidently produces an autoinhibitor.

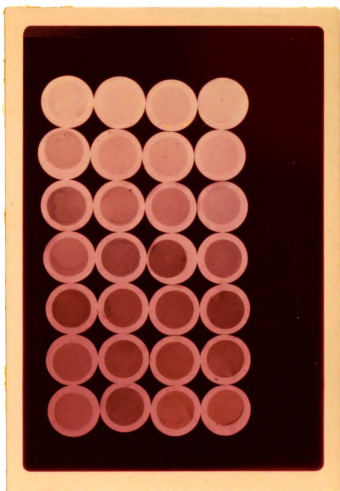


Figure 60. Millipore filters from Experiment III; age from top to bottom, 3, 6, 9, 12, 15, 18, and 21 days; from left to right, control, 1/100 x manganese, 1/1000 x manganese, and no added manganese. Note best growth in second series from right.

Table 19. Experiment IV. Growth of Aphanizomenon flos-aquae in control medium
(wt. in mg.)

Age of Culture (Days)	Wt. of Disk	Wt. of Disk and Filter	Wt. of Filter	Wt. of Disk	Wt. of Filter and Alga	Wt. of Filter and Alga	Wt. of Alga	pH
4	154.4	243.4	89.0	152.8	242.8	90.0	1.0	7.20
4	154.4	243.0	88.6	152.8	242.3	89.5	0.9	7.25
7	154.4	243.1	88.7	152.8	243.0	90.2	1.5	7.60
7	154.4	240.2	85.8	152.8	240.2	87.4	1.6	7.65
12	154.4	241.7	87.3	152.8	242.0	89.2	1.9	7.95
12	154.4	240.1	85.7	152.8	240.6	87.8	2.1	7.90
15	154.4	242.6	88.2	152.8	244.2	91.4	3.2	8.40
15	154.4	242.5	88.1	152.8	244.2	91.4	3.2	8.45
19	154.4	242.4	88.0	152.8	245.1	92.3	4.3	8.00
19	154.4	242.5	88.1	152.8	245.4	92.6	4.5	8.00

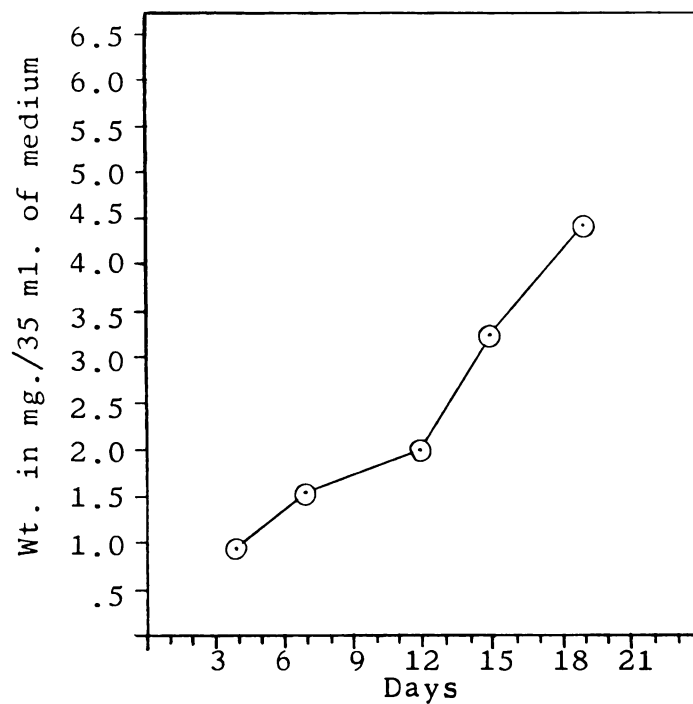


Figure 61. Growth increments of *Aphanizomenon flos-aquae* in mg./35 ml. of medium. Exp. IV - Control medium.

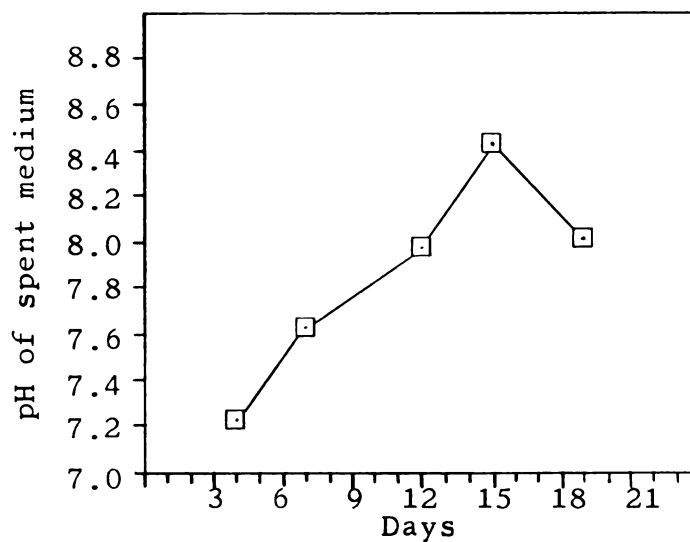


Figure 62. pH of spent medium at time of harvesting. Exp. IV - Control medium.

Table 20. Experiment IV - Growth of Aphanizomenon flos-aquae in medium containing 0.5 the amount of available nitrogen (wt. in mg.)

Age of Culture (Days)	Wt. of Disk	Wt. of Disk and Filter	Wt. of Filter	Wt. of Disk	Wt. of Disk and Filter and Alga	Wt. of Filter and Alga	Wt. of Alga	pH
4	154.4	243.4	89.0	152.8	242.5	89.7	0.7	7.30
4	154.4	243.0	88.6	152.8	242.2	89.4	0.8	7.35
7	154.4	242.5	88.1	152.8	242.6	89.8	1.7	7.45
7	154.4	242.8	88.4	152.8	242.8	90.0	1.6	7.60
12	154.4	243.7	89.3	152.8	244.9	92.1	2.8	7.65
12	154.4	239.7	85.3	152.8	240.8	88.0	2.7	7.65
15	154.4	241.7	87.3	152.8	243.4	90.6	3.3	7.70
15	154.4	243.2	88.8	152.8	244.7	91.9	3.1	7.65
19	154.4	243.0	88.6	152.8	245.3	92.5	3.9	7.70
19	154.4	243.4	89.0	152.8	245.9	93.1	4.1	7.70

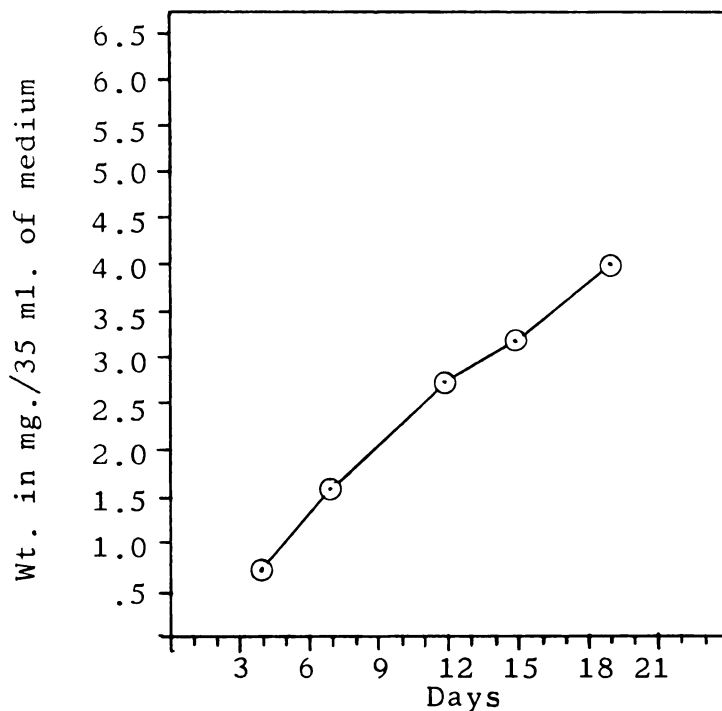


Figure 63. Growth increments of Aphanizomenon flos-aquae in mg./35 ml. of medium. Exp. IV - Medium containing one-half the amount of nitrogen as in the control.

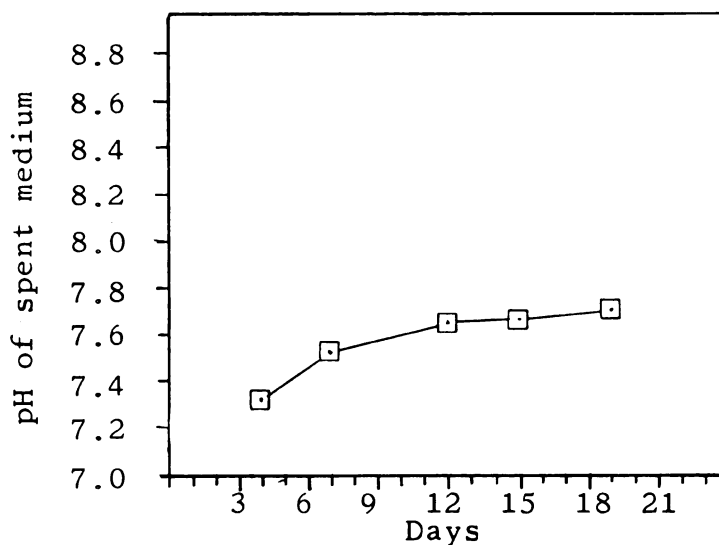


Figure 64. pH of spent medium at time of harvesting. Exp. IV - Medium containing one-half the amount of nitrogen as in the control.

Table 21. Experiment IV - Growth of *Aphanizomenon flos-aquae* in medium containing 0.1 the amount of available nitrogen (wt. in mg.)

Age of Culture (Days)	Wt. of Disk	Wt. of Disk and Filter	Wt. of Filter	Wt. of Disk	Wt. of Filter and Alga	Wt. of Filter and Alga	Wt. of Alga	pH
4	154.4	243.0	88.6	152.8	242.7	89.9	1.3	7.35
4	154.4	243.4	89.0	152.8	242.9	90.1	1.1	7.35
7	154.4	240.2	85.8	152.8	240.5	87.7	1.9	7.60
7	154.4	240.0	85.6	152.8	240.9	88.1	2.5	7.55
12	154.4	239.8	85.4	152.8	240.8	88.0	2.6	8.00
12	154.4	239.7	85.3	152.8	240.8	88.0	2.7	7.85
15	154.4	243.9	89.5	152.8	246.4	93.6	4.1	7.85
15	154.4	239.4	85.0	152.8	242.2	89.4	4.4	7.90
19	154.4	239.7	85.3	152.8	243.6	90.8	5.5	7.90
19	154.4	239.6	85.2	152.8	243.7	90.9	5.7	7.85

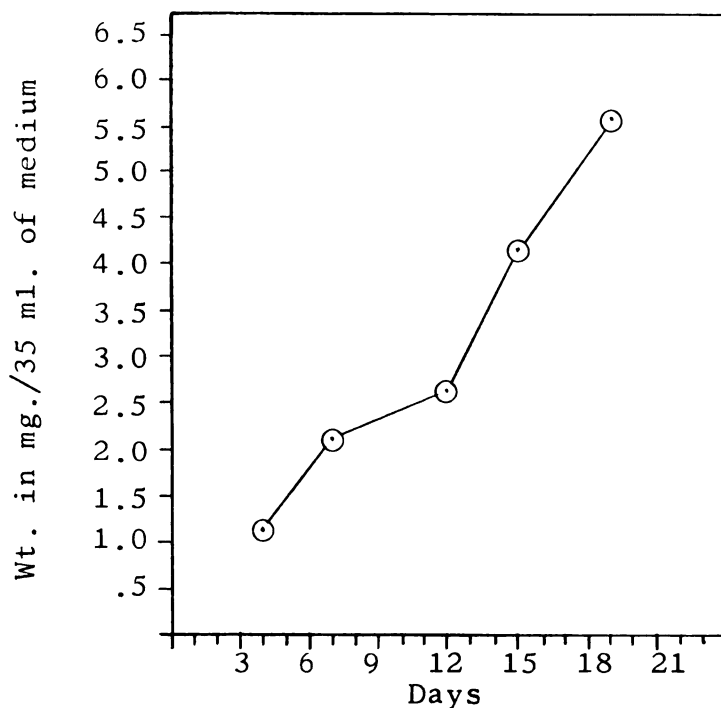


Figure 65. Growth increments of *Aphanizomenon flos-aquae* in mg./35 ml. of medium. Exp. IV - Medium containing 0.1 the amount of nitrogen as in the control.

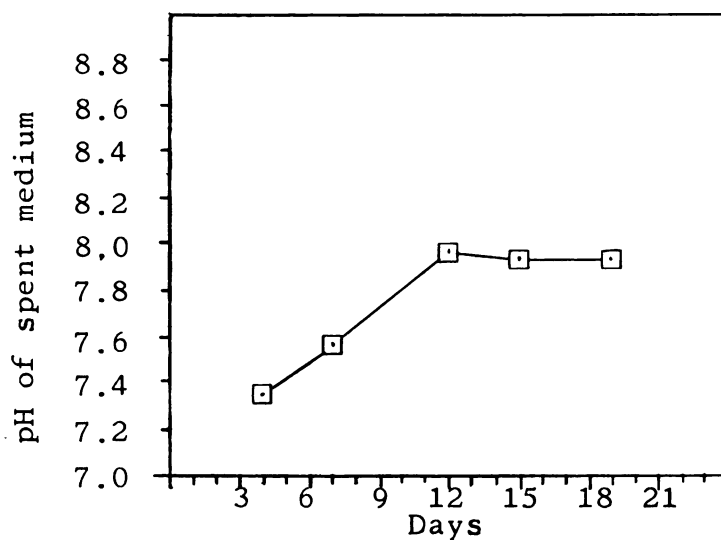


Figure 66. pH of spent medium at time of harvesting. Exp. IV - Medium containing 0.1 the amount of nitrogen as in the control.

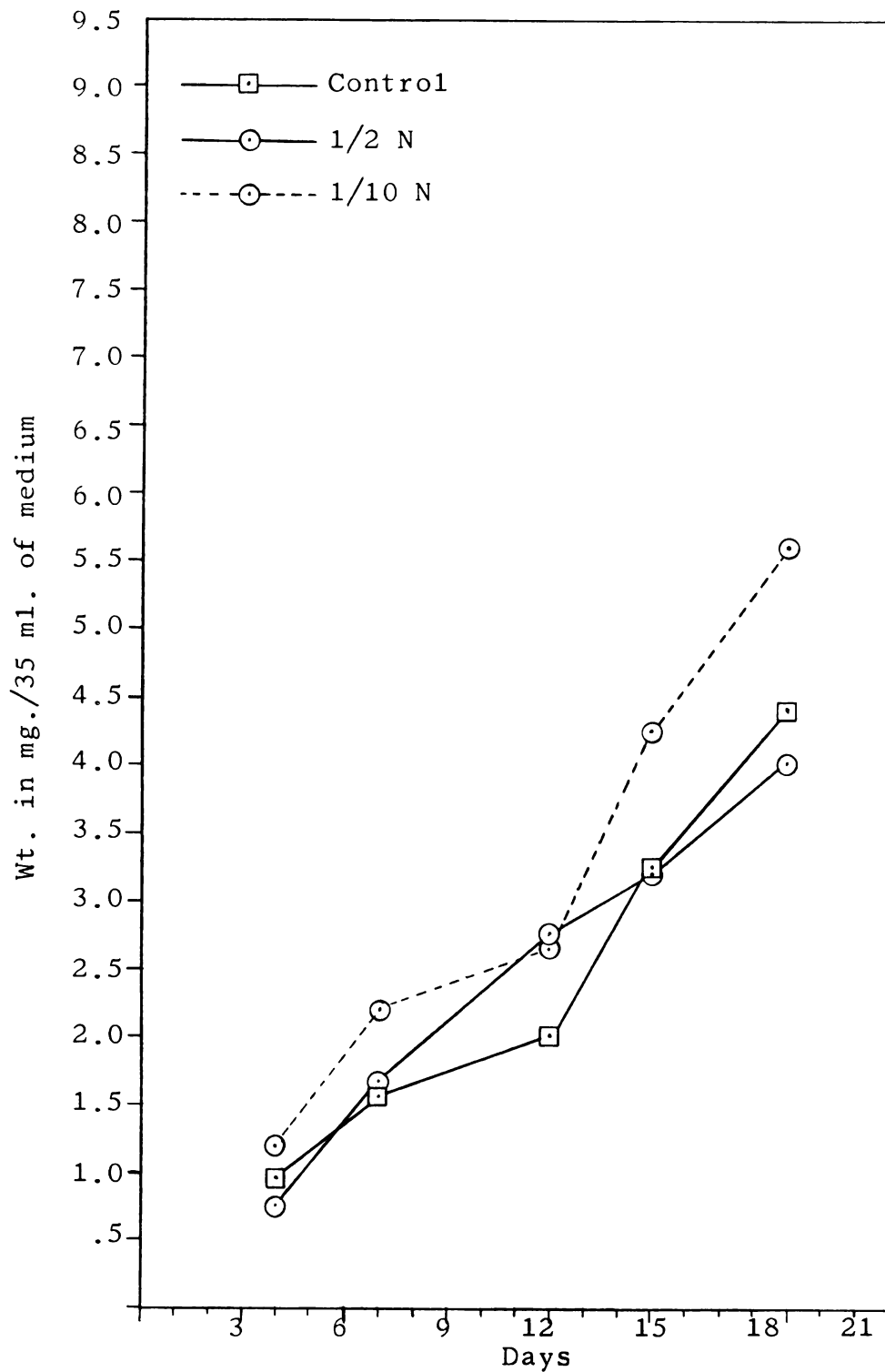


Figure 67. Comparison of growth increments of Aphanizomenon flos-aquae. Exp. IV - Decrease in nitrogen concentration.

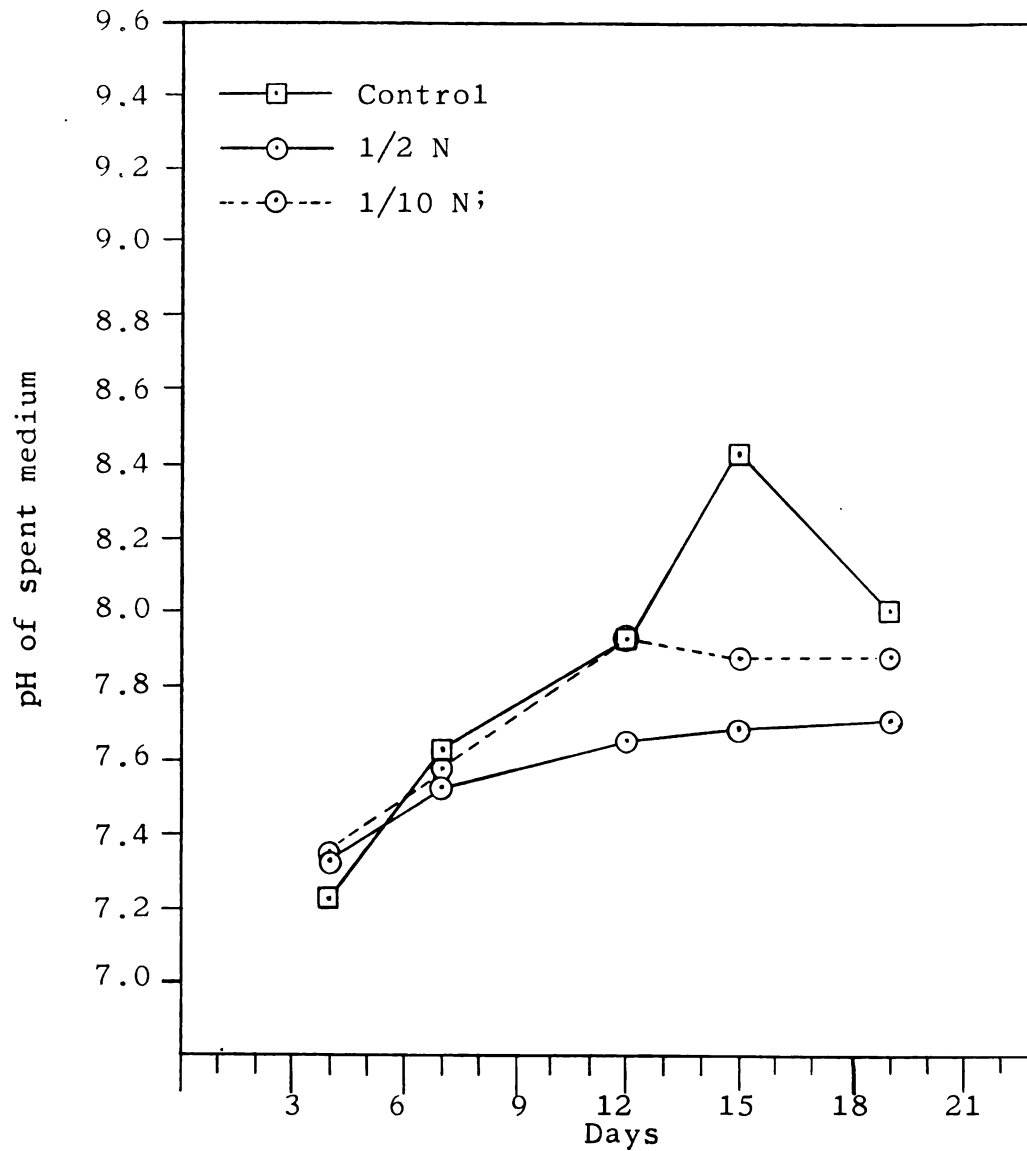


Figure 68. Comparison of pH of spent medium at time of harvesting. Exp. IV - Decrease in nitrogen concentration.

CHAPTER IV

DISCUSSION

Ecological Considerations in the Production of Water-Blooms

Although several blue-green species occur in the same bloom, a number of collections have been made which have consisted almost entirely of one species. This is true of the samples collected from Alward Lake which consisted of Oscillatoria Agardhii. Several factors may be involved in the determination of dominance of one species. The recurrence of the same species in a particular habitat year after year would certainly be related in part to the manner in which over-wintering was accomplished. Further, certain growth requirements are known to be more species-specific than was formerly thought and these could certainly explain dominance. Of equal, or perhaps of more, importance is the production of extrametabolites by one species which inhibit the growth of others. The study of the production and an understanding of the effects of extrametabolites necessitates the acquisition of more knowledge of the physiology and ecology of blue-green algae and their bacterial symbionts.

The conditions for bloom-production were similar in all three locations from which the algae used in this work were isolated. The water hole from which Microcystis incerta was isolated was located in a depression and received run-off from several directions. The water was undoubtedly rich in nitrogen and phosphorus from the manure deposited by the cattle. In addition the water was shallow and consequently warm. These conditions are conducive to the development of some kinds of blooms. M. incerta is a colonial species which may be tychoplanktonic or may occur in blue-green granulated masses on the bottom of hard and soft water lakes (Prescott, 1962).

The conditions in this pasture, described in the introduction, were favorable for the development of black leg in the cattle pastured here. The symptoms of staggering and fast death certainly agree with those described in the literature in situations where death was attributed to algal toxins. The presence of M. incerta in a lethal water-bloom was recorded by Stewart et al. in 1950. Black leg and several other diseases might produce similar symptoms. It cannot be said with any certainty that the deaths were the result of the alga or of some undiagnosed disease. The possibility of algal poisoning, however, cannot be eliminated.

Sixth Lake, which had been investigated several times prior to the time collections of Aphanizomenon flos-aquae

were made, was also rich in phosphorus and nitrogen from the wastes received from the village of Edmore. There were only three summer cottages on this lake which eliminated the possibility of pollution from residents in the immediate area. Fifth Lake evidently received enough pollution through the channel to permit the growth of blue-green algae. The polluting substances evidently were diluted sufficiently in Fourth Lake to make this lake unsuitable for blue-green blooms.

Alward Lake, being shallow, is also susceptible to water-blooms. It is likely that run-off from the surrounding farm land carried some waste material and that overflow from the septic tanks serving the homes and the resort on the lake adds to the nutrients available for algal growth.

As pointed out in the introduction, many investigators have considered the phosphorus and nitrogen concentrations the most important factors in blue-green bloom production. Hammer (1964) recently studied 23 lakes in southern Saskatchewan, Canada. Some were fresh-water, others extremely saline. All were known to produce blooms. The bloom species included Aphanizomenon flos-aquae, Anabaena flos-aquae, Microcystis aeruginosa, Oscillatoria rubescens, Oscillatoria prolifica (Grev.) Gomont, and Lyngbya birgei G. M. Smith. The first three produced the largest number of blooms. The blooms were unialgal at times and consisted of several species at other times. Other species, including

several species of diatoms were present occasionally but never reached bloom proportions. He found that orthophosphate was important in the production of blooms. The orthophosphate was accumulated and stored by the algae until utilized in bloom production. It was then released from the algae. The blooms occurred one or two weeks after the highest concentration of this substance was present in the water. The optimal concentration varied considerably for the species investigated being 0.15 to 0.30 ppm for Anabaena flos-aquae and 2.61 ppm for Aphanizomenon flos-aquae. The concentrations of orthophosphate which produced the heaviest blooms of Microcystis aeruginosa in various lakes varied so much that no figure could be quoted. The concentrations for this species were, however, lower than those for Anabaena flos-aquae and Aphanizomenon flos-aquae. Greater concentrations produced lighter blooms of Microcystis aeruginosa while greater concentrations up to 2.61 ppm produced heavier blooms of Aphanizomenon flos-aquae than lesser concentrations. There is, then, definitely some correlation between the orthophosphate concentration and the growth to bloom proportions of certain species of blue-green algae.

Hrbáček (1964) worked for several years on the ecology of blooms of Aphanizomenon flos-aquae and Microcystis aeruginosa in water bodies in Czechoslovakia. He studied ten backwaters of the Elbe River, six fish ponds, and three water reservoirs. He found that these blooms occurred more

frequently in the fish ponds where the phosphate and nitrogen concentrations were higher but found little evidence to indicate that these concentrations were responsible entirely for these blooms. Although algae were present in the stomach of some fish there was no direct evidence that the fish exerted any great influence on the production of blooms. Neither could he correlate the blooms with the depth of the water or the production of antibiotics by the algae. He did find, however, that the species of Daphnia present in blooms of these two algae were different. Daphnia pulicaria Forbes was associated with blooms of A. flos-aquae and D. cucullata Sars and D. hyalina Leydig with M. aeruginosa. These Daphnia, by selective grazing, evidently removed competitive phytoplankton; in one case permitting A. flos-aquae to flourish and in the other, M. aeruginosa. He concluded that water blooms were a result of the activities of the body of water as a whole.

Hammer (1964) correlated the succession of bloom species in Saskatchewan lakes with the temperature of the water. Anabaena flos-aquae appeared when the temperature of the water reached 5°C and the population remained fairly constant until the water reached 14°C. Maximum blooms of this species were produced at a temperature of 20°C. Aphanizomenon flos-aquae was rarely present until after a temperature of 20°C was reached. Maximum blooms were produced between 23.5 and 26.5°C; temperatures usually prevalent in mid-July.

Microcystis aeruginosa produced blooms within a range of 14.5 to 26°C with the heaviest blooms occurring above 20°C. The fall bloom of Anabaena flos-aquae occurred at temperatures of 21 down to 3.5°C. Often both Aphanizomenon and Microcystis reached bloom proportions at the same time but Aphanizomenon declined first. Hammer suggested that this was the result of competition for nutrients with M. aeruginosa becoming victorious.

Guseva, as discussed in the introduction, correlated algal blooms in the Ucha Reservoir with the concentrations of manganese and iron. Hrbáček (1964), although not concerned with the concentrations of these two elements, found that fish ponds contained 0.05 ppm of manganese in the spring and 0.54 ppm in the fall. The concentration of iron varied from 0.4 ppm in the spring to 2.7 ppm in the fall. These concentrations were much higher than those used by Guseva. The fish ponds that produced blooms of M. aeruginosa contained three times the amount of manganese and twice the amount of iron as those which supported A. flos-aquae blooms. He could make no general statement with regard to the effect of these two minerals but it is evident that the requirements of the two algae are not the same.

It is evident that the statement made in the first paragraph of this discussion is true; namely, that several factors which are closely interrelated are responsible for water blooms. The interrelationships existing in a community

are so interwoven that one cannot be separated and studied alone. Hrbáček recognized that the community was the result of the interaction of many factors but, in the opinion of this writer, he under-emphasized the importance of nitrogen and phosphorus concentrations and the production of extra-metabolites.

Isolation of Algae

Pure cultures of algae are more easily obtained by streaking material from samples containing the desired species on agar than by isolating single filaments or cells by means of micropipettes. It is difficult to segregate a single species and very often more than one is taken by the pipette. Many cultures inoculated with material removed in this way had to be discarded because of the rapid growth of undesired species. This necessitated many inoculations. Another disadvantage of this method is the fact that liquid cultures cannot be examined under the microscope.

Succession of Algae on Agar

It is interesting to note that succession of algal species on agar follows the same pattern which has been previously described for natural bodies of water. In nature this was attributed to the concentrations of minerals and to the production of extrametabolites by preceding species. As

in nature, the medium was probably better suited for the growth of diatoms when the cultures were started. These reached a maximum and declined perhaps because of the production of autoinhibitors or because of the decrease in concentration of some particular element. The extrametabolites evidently modified the environment so that other species could grow more easily. Once a species was isolated it was able to grow fairly well on agar. It is easier to maintain cultures on agar because of facility in handling. Another advantage is that growth is slower and interfering bacteria do not develop as rapidly.

Growth in Liquid Cultures

Observations over a period of from two to two and a half months indicated that most of the cultures reached their peak in three to four weeks after inoculation and then began to decline. Simultaneously bacterial growth, becoming evident by discoloration of the cultures, began on the 18th to 21st day after inoculation. The decline in growth may be the result of mineral depletion in the medium. Algae growing in nature are constantly exposed to environmental changes which are capable of replenishing materials in the water. In culture this is not possible. There is no replenishing of minerals making depletion of one or more necessary elements a very important factor to consider.

The decline in growth also may be the result of shading of the alga either by the alga itself or by the bacterial symbionts. The lack of light undoubtedly is a factor to be considered. The present work indicated that the production of auto-inhibitory extrametabolites is responsible for the decline in growth. Extrametabolites also play an important role in nature where depletion of minerals and shading are of less importance than they are in culture.

Temperature and Light

The growth of cultures at room temperature and higher light intensity was greater than growth of cultures in the growth chamber. Most culturing of algae has been conducted at temperatures ranging from 18°C to 25°C, temperatures which are within the range of those occurring in natural habitats. Chu (1942) found that the growth of the algal species he studied was not appreciably effected when the temperatures ranged between 8 and 24°C. Growth is retarded in some species at temperatures of 35°C or higher and at temperatures of 5 to 10°C (Allison et al., 1937; Kratz and Myers, 1955). The cultures of Aphanizomenon flos-aquae in the control medium (Experiment I) which were placed on the shaker at room temperature produced slightly more growth. Bacteria in the cultures grown on the shaker increased more rapidly than in the cultures grown in the chamber. The more rapid

increase in bacterial growth of the cultures grown at room temperature is the result of higher temperature. In nature it is difficult to correlate temperature with population because light and other environmental factors vary with the temperature (Prescott, 1939).

Hammer, as discussed above, correlated the production of blooms with the concentration of orthophosphate and with the temperature. These two factors are important but so are other factors, such as pH, light intensity, available nitrogen, and the concentration of minor elements, including magnesium, manganese, and iron. This correlation with temperature does not always apply. Aphanizomenon flos-aquae was collected from Sixth Lake on October 31, 1962, when the temperature of the water was 9.8°C. He neglected almost completely the effects of extrametabolites in the community.

The amount of light received by cultures, except when extremely intense, seems to be of little significance. Cultures have been found to grow well at intensities of 160 foot candles up to 1480 foot candles (Allen, 1952; Myers, 1951; Allen and Arnon, 1955). Natural light is not adequate for quantitative culture experiments because it is so variable in intensity. Most researchers have used fluorescent lights in preference to tungsten because the latter produce too much heat. Continuous illumination of cultures has not been studied thoroughly enough to justify any general statement in respect to its value. In comparing the control

series of Aphanizomenon flos-aquae (Experiment I) illuminated continuously, with the series on the shaker, subjected to 16 hours of illumination followed by 8 hours of darkness, no adverse effects are noted. This is a relationship which needs to be explored further.

Inhibitory Activity

In the present study, antibacterial activity was exhibited by Microcystis incerta and Aphanizomenon flos-aquae against Staphylococcus aureus. The latter alga also exhibited slight activity toward Pseudomonas aeruginosa. In this work no attempt to extract the inhibitory substance was made because the primary interest was to obtain information concerning these species as they grow in nature. Water taken from Sixth Lake during a bloom of Aphanizomenon inhibited the growth of Staphylococcus aureus. Although growth of bacteria from the water was evident around the filter paper disc, there was a rather extensive area of inhibition of S. aureus beyond this region. This bacterium appears to be very sensitive to extrametabolites produced by the blue-green algae because several investigators have reported inhibition of this species (Wurtz, 1949; Lefevre et al., 1952; Simonds, 1954; Davidson, 1961, 1962).

Antifungal activity was demonstrated by several of the algae. The most pronounced activity was that of

Aphanizomenon flos-aquae against Fusarium oxysporum. Further work is necessary on the antifungal properties of the blue-green algae with emphasis on those fungi which are normally aquatic. It may be that this group is effective in natural bodies of water against saprophytic and parasitic fungi. This fact is substantiated by the work of Gaukham and Ryabov conducted in the Dnepr Reservoir (1962).

Not all blue-green blooms occurring in nature are toxic. Gorham et al. (1962) found that the ability of Microcystis aeruginosa to produce a toxin was genetically controlled. The same was found to be true for Anabaena flos-aquae (Gorham et al., 1964). Of fourteen strains isolated, eight were toxic and six were non-toxic. The toxin appeared to be different chemically from that produced by M. aeruginosa in that it acted more rapidly and was secreted readily into the surrounding medium.

Shilo (1964) has studied the blooms produced by Prymnesium parvum Carter in fish ponds in Israel. In culture three active principles were demonstrated; an ichthyotoxin, a hemolysin, and a cytotoxin. The formation of toxin was greatest during the stationary phase of growth. He concluded that all blooms of P. parvum were potentially toxic but the formation of toxins, their release, and their subsequent activity were the result of factors in the environment. Activity depended to a great extent on the presence

of co-factors, such as the cations calcium and magnesium, streptomycin, and spermine, that evidently increased the stability of the molecule of toxin. The toxins were inactivated in culture by weak electrolytes such as ammonium sulfate. This inactivation could readily occur in natural bodies of water where many weak electrolytes are available. Changes in pH in the culture medium produced reversible changes in activity of the toxins. All of these conditions must be optimal for the production of toxins in nature. In addition, the toxins in nature can be inactivated by adsorption on colloidal particles suspended in the water or on bottom soil. All of these factors are important in the production of blue-green blooms and should be included in studies on the toxicity of these blooms.

Bacterial Symbionts

Bacteria are classified on the basis of their physico-chemical requirements and little is known of the ecology of these organisms in natural environments. Particular ecological conditions, as for other living organisms, will cause an increase in the numbers of the species best adapted to these conditions. It is possible, although not completely established, that there are distinct terrestrial, marine and fresh water species.

There are many unanswered questions concerning the relationship between the bacteria and the algae. The bacteria could be parasitic. If parasitic they certainly would be classified as facultative parasites because they are able to grow extremely well on agar media. The filaments of the alga do not disintegrate which would indicate that the cells are not utilized for food by the bacteria. The decline in growth and eventual plasmolysis and death of the alga is caused by the production of auto-inhibitors. In culture the accumulation of this material brings about a more rapid decline in growth than in natural environments where dilution and dispersion take place. Reduction in light brought about by the growth of large numbers of bacteria is probably a contributing factor to the decline of the algal growth in culture.

The protoplasts of the Cyanophyta are surrounded by an inner layer of unknown composition and an outer layer, or cell-sheath, considered to be a polysaccharide by most investigators. Fritsch (1959) is of the opinion that the mucilage is secreted by or through the inner layer and that the layer last secreted functions as the cell-sheath at that particular time. Experimental work by Lemaire in 1901 showed that the sheaths of some species of Cyanophyta are composed of an acid, such as pectic acid, combined with an organic base. This compound he called "schizophycose" (cited by Fritsch, 1959).

From the scant amount of research done on the chemical composition of the sheath, it is possible that its composition is as varied as the nutritional requirements. Acid hydrolysis of the mucilage of several species yielded arabinose and glucose from Rivularia bullata (Poir.) Berkeley, galactose and mannose from Calothrix pluvinata Kg. and arabinose from Nostoc commune Vauch. (Payen, 1938). The hydrolysis of the sheath of Calothrix scopulorum (W. et M.) Ag. yielded galactose, a pentose, and sulfuric acid. The mucilage was considered to be a calcium or magnesium salt of a polysaccharide sulfuric acid (Kylin, 1943b). The sheath of Nostoc commune has been found recently to consist of 30 per cent galacturonic and glucuronic acid, 10 per cent rhamnose, 25 per cent D-xylose and 35 per cent galactose, glucose and an unknown sugar (Jones et al., 1952). The polysaccharide produced by Nostoc muscorum contained two unidentified components, arabinose, glucose, galactose, rhamnose, xylose and ribose (Biswas, 1959). Prolonged hydrolysis of the mucilage of Anabaena cylindrica yielded glucose, xylose, glucuronic acid, galactose, rhamnose and arabinose in the molar ratios of 5:4:4:1:1:1 (Bishop et al., 1954).

Both of the bacteria isolated appeared consistently when Aphanizomenon flos-aquae was streaked on agar. It is not known whether both are associated with the alga in nature. The alga is one that had been isolated several

years ago and conceivably, the bacteria could have been added during transfer of the alga to fresh culture medium. Once a bacterium has entered a culture it is conceivable that, although not normally found in association with the alga, it could adapt. Of the two bacteria isolated, Flavobacterium diffusum is a weak hydrolyzer of carbohydrate and would, therefore, have little effect on the sheath of A. flos-aquae if it is truly a polysaccharide. Bacillus cereus var. mycoides, on the other hand, is able to utilize a wide range of carbohydrates, and, if it is a symbiont or parasite in nature, could well attack the mucilaginous sheath. The sheath is not destroyed, however, because it is being replaced constantly through the inner layer. Although both bacteria isolated are proteolytic they may not be able to utilize the protoplasts of this alga. It also seems feasible that the protoplasts are not attacked because of the protection afforded by the mucilage.

The relationship is, therefore, one of mutualism or commensalism. The bacteria which live in the water grow as epiphytes on other living organisms or on pieces of debris suspended in water. They utilize organic compounds in their immediate surroundings as sources of energy. In nature the organic materials present in the water are used rapidly so that little evidence is found of their presence. In cultures these materials can accumulate and can be characterized

chemically. Extracellular nitrogenous compounds, mostly peptides but also amino acids, are liberated by nitrogen-fixing blue-green algae in culture (Fogg, 1952; Magee and Burris, 1954). Other blue-greens liberate extrametabolites. These are not products of autolysis but are liberated by actively growing cells especially in older cultures. The release is thought to be the result of some mineral deficiency. It has been demonstrated that an iron deficiency causes the release of extrametabolites by Nostoc sp. and Anabaena cylindrica in a culture (Fogg, 1952; Venkataraman, 1961). Continuous shaking of Nostoc sp. decreased the amount of extrametabolites released (Hendrickson, 1957). Other investigations have shown that 20 to 30 per cent of the total material synthesized is secreted by some algae (Lewin, 1955, 1956). Lewin found that several bacteria could utilize glycollic acid as a source of carbon. Glycollic acid, derived from the intermediates in the photosynthetic process, is undoubtedly produced by all plants able to carry on photosynthesis (cited by Fogg and Nalewajko, 1964). It is possible then that the bacteria can live entirely on the extrametabolites that are liberated from the algae. If the bacteria do utilize these materials and contribute nothing to the relationship the symbiosis is commensualism.

Existing evidence, however, suggests that the relationship is mutualistic. Bacteria are known to release

extrametabolites into their environment. One very important substance released is Vitamin B₁₂ or its analogues. This vitamin or one of its analogues may be important as a growth factor or stimulator for algae. If the alga can or does depend on these substances for growth while at the same time releasing extrametabolites that are usable by the bacteria both organisms benefit from this association. The symbiotic relationship may account for the fact that efforts to establish bacterial-free cultures of many cyanophytes have not been successful.

Effects of Varying the Mineral Concentrations

The medium used as a control contained 14 ppm of nitrogen, just under the optimum concentration of 15 ppm reported by McLachlan for this species (McLachlan, 1957). Increasing the concentration decreased the growth. A concentration of 42 ppm inhibited growth. Decreasing the concentration to 7 ppm had little effect on the growth while a decrease to 1.4 ppm produced a slight increase in growth. This suggests that the actual requirement of this species for nitrogen is less than the amount in the control medium. The results support the suggestions of Guseva (1937), namely, that conditions for the development of a bloom of Aphanizomenon flos-aquae developed only after the concentration of nitrogen decreased. In all reports the amounts of nitrogen

occurring in natural waters is considerably less than the amount used in synthetic media.

The control medium used in this experiment contained 0.178 ppm of phosphorus as K_2HPO_4 which is less than the amount in the media used by Guseva and by McLachlan. Increasing the concentration decreased the growth slightly. One possible explanation for the inconsistencies in the results of these works may be the differences in the media with respect to other minerals and in the form in which the minerals were supplied. For example, Guseva used $Ca(NO_3)_2$ as a source of nitrogen and McLachlan used $NaNO_3$. This latter compound was also used in the preparation of the medium used in the present study.

Another possibility for differences is the ratio of the amount of nitrogen to the amount of phosphorus. In the work of Gerloff et al. (1950, 1952), the ratio of these two elements that produced the best growth was 30 to 1 in the instance of Coccochloris Peniocyctis and 75 to 1 for Anacystis marina. McLachlan (1957) found that 40 times more nitrogen than phosphorus was necessary for maximum growth of Aphanizomenon flos-aquae. In this work the ratio of these two elements that produced the best growth was between 7 to 1 and 14 to 1.

The concentration of manganese may be the most important factor in the growth of Aphanizomenon flos-aquae.

Guseva reported that Anabaena Lemmermannii was apparently insensitive to the concentration he used. On the other hand he also concluded that a concentration of 0.005 ppm in the form of MnSO_4 is conducive to better growth of Aphanizomenon flos-aquae. Death of Aphanizomenon occurred when the concentration of manganese reached 0.2 to 0.3 ppm in the reservoir.

The control medium used in this experiment contained 0.383 ppm of manganese in the form of $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$. When manganese was omitted entirely from the medium the growth was greater than in the control. This element, however, does have a stimulatory effect when present in a concentration of 0.00383 ppm. This stimulatory effect is more evident at a concentration of 0.000383 ppm. The concentration of 0.00383 is comparable to the concentration of 0.005 ppm which Guseva reported as being stimulatory to Aphanizomenon flos-aquae.

In this series of cultures the ratio of nitrogen to phosphorus was 78 to 1. In the other experiments in which the nitrogen and phosphorus were varied it was undoubtedly the manganese that effected the growth adversely. A group of experiments, therefore, should be conducted using the medium with the amount of manganese reduced to one of these lower concentrations and in which the concentrations of nitrogen and phosphorus are varied.

Harvey (1949) thought that the manganese content of lakes and streams would not be great enough to limit the growth of algae. Gerloff and Skoog (1958) showed that there is an antagonism between calcium and manganese. Although this antagonism is not sufficient to effect the availability in natural waters it is probably responsible for limiting the level of availability of this element so that it does not become toxic. It is also probable that a high level of calcium could bring about a deficiency of manganese although this has never been demonstrated in natural waters. Fogg (1955) demonstrated that algae are able to release extra-metabolites in the form of polypeptides. He suggested that these are important in maintaining the balance of essential elements including manganese. This maintenance of balance is accomplished in the medium used here by EDTA. This substance, a chelating agent, combines with ions in the medium and tends to release them as they are needed. It is inactive biologically.

Although Aphanizomenon flos-aquae was collected many times, this alga never survived in culture. Rose (1934) and Rodhe (1948) were never able to maintain this species in culture very long. Gerloff et al. (1951) were the first workers to succeed in culturing this species. Since this alga was collected so many times throughout the growing season, the inability to maintain this alga does not seem to be

related to making collections during a stage in the life cycle at which growth could not be expected. The akinetes were also identified in mud samples from the lake, completing the reproductive cycle of this alga. It is difficult to speculate on the reason for this inability to culture this alga. A reasonable speculation might be that the concentration of manganese was too high to permit it to adapt to the culture medium. Perhaps a necessary growth factor was lacking in the culture medium.

Changes in pH

In nature the pH of bodies of fresh-water varies considerably depending primarily on the geology of the region and modified by the activities of organisms in the lake and in the vicinity of the lake. Some lakes have a pH as low as 4.4 while others have a pH as high as 10. A particular lake may exhibit a wide variation in a single day. Welch (1935) reported a variation of 6.6 to 9.2 in a single lake. Swingle (1947) found that the pH of one lake varied from 7.0 before dawn to 9.5 in the middle of the afternoon. Few studies have been made of the effects of pH on algal species in particular ecosystems but existing evidence indicates that there is some correlation between the two.

In the present work all of the cultures except the two series in which nitrogen was reduced (Experiment IV) exhibited a gradual increase in pH up to a maximum. This

was followed by a rather rapid decline. In the two containing less nitrogen there was little variation. It would appear that pH changes (in cultures) are related to the concentration of nitrate in some way. In actively growing cultures the nitrate is removed and the removal of this ion results in a higher pH. This explanation seems very plausible until the results of tripling the amount of nitrogen are examined (Experiment I). In these series, despite the fact that practically no growth occurred, the pH followed the general pattern, a gradual increase followed by an abrupt decrease.

The decrease in pH is related in part to an increase in carbon dioxide from respiration. The bacteria do not begin to multiply rapidly in a pH above 8 (Thimann, 1955). The bacteria, therefore, would not contribute much carbon dioxide until approximately the 18th day. This is the time noted previously when discoloration due to bacterial growth first becomes apparent.

The changes in pH are most likely associated with the complex interreactions of the constituents in the medium. These need further study. Undoubtedly in such a mixture of chemicals there are some that will precipitate.

In this study the pH of actively growing cultures never rose higher than 9.0. The maximum growth produced in this medium at this pH compares favorably with the maximum

growth obtained by McLachlan at a pH of 10.00.

Further work at a higher pH should be undertaken to determine whether better growth would occur in this medium if the pH were higher. Comparison of the growth reported by McLachlan indicated that this medium is superior to the one used for Aphanizomenon flos-aquae.

CHAPTER V

CONCLUSIONS

1. The growth of algae on agar plates and slants inoculated by streaking material from samples containing the desired species is slow and follows the pattern of seasonal distribution described for natural bodies of water. Other investigators should bear this in mind if a problem is undertaken which is similar in scope to the present work.

2. It has been demonstrated that several of the blue-green algae used in this study exhibit antibacterial and antifungal activity. Aphanizomenon flos-aquae demonstrated the greatest inhibitory activity. This inhibition should be studied further in efforts to extract and identify the inhibiting substance or substances.

3. Inhibitory activity of these algae toward aquatic animals, bacteria and fungi was not investigated but could be very important in the natural ecology of the blue-green algae. Studies along these lines should be undertaken to determine whether extrametabolites produced by these algae are responsible for the distribution of other organisms in an aquatic environment.

4. The production of an auto-inhibitor was demonstrated for Aphanizomenon flos-aquae. This auto-inhibitor was responsible for the lack of growth of Aphanizomenon flos-aquae in the spent medium of cultures from which the growth had been harvested. Studies of the production of auto-inhibitors and their effects on the natural ecology of the algae should be undertaken to determine whether they are effective in bringing about the seasonal fluctuations in numbers and species of algae.

5. Two bacteria were isolated from cultures of Aphanizomenon flos-aquae. Further investigations into the ecology and physiology of the blue-green algae should include more extensive studies involving the relationship between the alga and the bacteria. It would be interesting to isolate bacteria from other species of blue-green algae and determine whether the same species were found in association with other algae or whether bacteria are species-specific.

6. The results of this study and comparison with the results of similar studies suggest that mineral requirements are species-specific. Of the minerals varied in this study the concentration of manganese was the most significant, the best growth occurring at a concentration of 1/1000th of that used in the control.

BIBLIOGRAPHY

- Abbott, B. C., and Ballantine, D. 1957. The toxin from Gymnodinium veneficum Ballantine. Jour. Mar. Biol. Assoc. U. K., 36:169-189.
- Adolph, E. F. 1931. The size of the body and the size of the environment in the growth of tadpoles. Biol. Bull., 61:350-375.
- Ahlgren, H. L., and Aamodt, O.S. 1939. Harmful root extracts as a possible explanation for effects noted between various species of grasses and legumes. Jour. Amer. Soc. Agron., 31:982-985.
- Akehurst, S. C. 1931. Observations on pond life, with special reference to the possible causation of swarming of phytoplankton. Roy. Micro. Soc. Jour., 51:237-265.
- Alexander, G. W., and Sieminska, J. 1963. Winter water bloom of Oscillatoria Agardhii. Unpublished.
- Aleyev, B. S. 1934. Secretion of organic substances by algae into the surrounding medium. Mikrobiol., 3:506-508.
- Algeus, S. 1951. Studies on the cultivation of algae in artificial light. Physiol. Plant., 4:742-753.
- Allee, W. C., Bowen, E. S., Welty, J. C., and Oesting, R. 1934. The effect of homotypic conditioning of water on the growth of fishes, and chemical studies of the factors involved. Jour. Exp. Zool., 68:182-213.
- Allen, M. B. 1952. The cultivation of Myxophyceae. Arch. Mikrobiol., 17:34-53.
- Allen, M. B. 1956. Excretion of organic compounds by Chlamydomonas. Arch. Mikrobiol., 24:163-168.
- Allen, M. B., and Dawson, E. Y. 1960. Production of antibacterial substances by benthic tropical marine algae. Jour. Bact., 79:459-460.

- Allen, W. E. 1928. Review of five years of studies on phytoplankton at Southern California piers, 1920-1924 inclusive. Bull. Scripps Inst. Oceanogr., Tech. Series, 1:357-401.
- Allen, W. E. 1936. Surface plankton diatoms in the North Pacific Ocean in 1934. Modrono, 3:250-252.
- Allison, F. E., and Hoover, S. R. 1935. Conditions which favour nitrogen fixation by a blue-green alga. Transactions of the Third International Congress of Soil Science, 1:145-147.
- Allison, F. E., Hoover, S. R., and Morris, H. J. 1937. Physiological studies with the nitrogen-fixing alga, Nostoc muscorum. Bot. Gaz., 98:433-463.
- Anderson, G. C. 1961. Recent changes in the trophic nature of Lake Washington - a review. IN: Algae and Metropolitan Wastes. Rob't A. Taft. San. Eng. Cent. Tech. Rep't. W61-3. Pp. 27-33.
- Anderson, L. S. 1960. Toxic shellfish in British Columbia. Amer. Jour. Public Health, 50:71-83.
- Antia, N. J. 1963. A microbiological assay for biotin in sea water. Can. Jour. Microbiol., 9:403.
- Armstrong, F. A. J., and Boalch, G. T. 1960. Volatile organic matter in algal culture media and sea water. Nature, 185:761-762.
- Arnon, D. E. 1956. Phosphorus metabolism and photosynthesis. Ann. Rev. Plant Physiol., 7:325-354.
- Arthur, J. C. 1883. Some algae of Minnesota supposed to be poisonous. Bull. Minn. Acad. Sci., 2:1-12.
- Arthur, J. C. 1886a. Some algae of Minnesota supposed to be poisonous. Fourth Biennial Rep't., Board of Regents, Univ. Minn. Suppl. 1, Dept. of Agri., 97-103.
- Arthur, J. C. 1886b. Second report on some algae of Minnesota supposed to be poisonous. Fourth Biennial Rep't., Board of Regents, Univ. Minn., Suppl. 1, Dept. of Agri., 109-112.
- Ashworth, C. T., and Mason, M. F. 1946. Observations on the pathological changes produced by a toxic substance present in blue-green algae. (Microcystis aeruginosa.) Amer. Jour. Path., 22:369-383.

- Astakhova, T. V., Kun, M. S., and Teplyi, D. L. 1960. Cause of carp disease in the Lower Volga. Doklady Akad. Nauk SSSR. 133:1205-1208. A. I. B. S. Transl. of Doklady (Biol. Sci. Sec.), 133:579-581. 1961.
- Atkins, W. R. G. 1923. The phosphate content of fresh-water and salt water in its relation to the growth of algal plankton. Jour. Mar. Biol. Assoc. U. K., 13: 119-150.
- Atkins, W. R. G. 1925. Seasonal changes in the phosphate content of sea water in relation to the growth of the algal plankton during 1923-1924. Jour. Mar. Biol. Assoc. U. K., 13:700-720.
- Atkins, W. R. G. 1926. The phosphate content of sea water in relation to the growth of algal plankton. Jour. Mar. Biol. Assoc. U. K., 14:447-467.
- Atkins, W. R. G. 1928. Seasonal variation in the phosphate and silicate content of sea water during 1926 and 1927 in relation to the phytoplankton crop. Jour. Mar. Biol. Assoc. U. K., 15:191-205.
- Atkins, W. R. G., and Harris, G. T. 1924. Seasonal changes in the water and heleo plankton of fresh-water ponds. Proc. Roy. Dublin Soc., 18 N. S.:13-19.
- Andus, L. J., and Quastel, J. H. 1947. Coumarin as a selective phytocidal agent. Nature, 159:320-324.
- Avers, C. J., and Goodwin, R. H. 1956. Studies on roots. IV. Effects of coumarin and scopoletin on the standard root growth pattern of Phleum pratense. Amer. Jour. Bot., 43:612-620.
- Ayers, W. A., and Papavizas, G. C. 1963. Violet-pigmented pseudomonads with antifungal activity from the rhizosphere of beans. Appl. Microbiol., 11:533-538.
- Baer, H., Holden, M., and Seigel, B. 1946. The nature of the antibacterial agents from Anemone pulsatilla. Jour. Biol. Chem., 162:65.
- Baker, M. N. 1948. The Quest for Pure Water. Chapter XVII. Algae troubles and their conquest. The American Water Works Assoc., Inc. New York. Pp. 391-414.
- Ballantine, D., and Abbott, B. C. 1957. Toxic marine flagellates: their occurrence and physiological effects on animals. Jour. Gen. Microbiol., 16:274-281.

- Banner, A. H. 1959. A dermatitis-producing alga in Hawaii. *Hawaii Med. Jour.*, 19:33-36.
- Bargellini, Y., Pianto, E. del -, and Marine, B. 1946. Sur l'activité antibactérienne de deux acides lichéniques: l'acide usnique et l'acide vulpinique. *Atti. Act. Nacion. Linechi Rendic. Cl. Sc. Fis. Nat.*, 1: 1252-1255.
- Barnum, D. A., Henderson, J. A., and Stewart, A. G. 1950. Algae poisoning in Ontario. *Ontario Milk Producer*, 25:312.
- Bartsch, A. F. 1961. Induced eutrophication - a growing water resource problem. *Rob't. A. Taft San. Eng. Cent. Tech. Rep't. W61-3. Pp. 6-9.*
- Becker, Y., Guillement, J., Guyot, L., and Lefievre, D. 1951. Sur un aspect phytopathologique du problème des substances racinaires toxiques. *Compt. Rend. Acad. Sci. Paris*, 233:198.
- Bedford, Duke of, and Pickering, S. U. 1914. The effect of one crop on another. *Jour. Agr. Sci.*, 6:136-151.
- Beers, C. D. 1933. The relation of density of population to rate of reproduction in the ciliates Didinium nasutum and Stylonychia pustulata. *Arch. Protistenk.*, 80:36-64.
- Beers, C. D. 1945. Some factors affecting excystment in the ciliate Tillina magna. *Physiol. Zool.*, 18:82.
- Belcher, J. H., and Fogg, G. E. 1958. Studies on the growth of Xanthophyceae in pure culture. III. Tribonema aequalle Pascher. *Arch. Mikrobiol.*, 30:17-22.
- Bell, T., Aurand, L., and Etchells, J. 1960. Cellulase inhibitors in grape leaves. *Bot. Gaz.*, 122:143-148.
- Benedict, H. M. 1941. The inhibitory effect of dead roots on growth of brome grass. *Jour. Amer. Soc. Agron.*, 33:1108-1109.
- Benedict, R. G., and Langlykke, A. R. 1947. Antibiotics. *Ann. Rev. Microbiol.*, 1:193-236.
- Bennet, E. L., and Bonner, J. 1953. Isolation of plant growth inhibitors from Thamnosma montana. *Amer. Jour. Bot.*, 40:29-33.

- Benoit, R. J. 1957. Preliminary observations on calcium and vitamin B₁₂ in fresh water. *Limnol. and Oceanogr.*, 2:233-240.
- Benoit, R. J., and Curry, J. J. 1961. Algae blooms in Lake Zoar, Connecticut. IN: *Algae and Metropolitan Wastes*. Rob't. A. Taft San. Eng. Cent. Tech. Rep't. W61-3. Pp. 18-22.
- Bentley, J. C. 1958. Role of plant hormones in algal metabolism and ecology. *Nature*, 181:1499-1502.
- Bentley, J. C. 1959. Plant hormones in marine phytoplankton, zooplankton and sea water. *Int. Oceanogr. Congr. Prepr.*, 910-912.
- Berdnikow, A. 1924. Les milieux de culture dits "vacunes" et l'antagonisme des microbes in vitro. *Compt. Rend. Soc. Biol.*, 91:859-861.
- Bergersen, F. J. 1960. Biochemical pathways of legume root nodule nitrogen fixation. *Bacteriol. Rev.*, 24:246-250.
- Bergman, F., Parnos, I., and Reich, K. 1963. Observations on the mechanism of action and on the quantitative assay of ichthyotoxin from Prymnesium parvum Carter. *Toxicol. and Appl. Pharmacol.*, 5:637-649.
- Bigelow, H. B. 1926. Plankton of the offshore waters of the Gulf of Maine. *Bull. Bur. Fisheries*, 40, Part II: 1-509.
- Bigelow, H., Lillick, L., and Sears, M. 1940. Phytoplankton and planktonic protozoa of the offshore waters of the Gulf of Maine. *Trans. Amer. Phil. Soc.*, 31:149-191.
- Birge, E. A., and Juday, C. 1911. The inland lakes of Wisconsin. The dissolved gases of the water and their biological significance. *Wisconsin Geol. and Nat. Hist. Survey, Bull. 22, Sci. Ser.* Pp. 259.
- Birkeland, Jorgen. 1949. *Microbiology and Man*. Appleton-Century-Crofts, Inc. New York. Pp. xii + 525.
- Bishop, C. T., Adams, G. A., and Hughes, E. O. 1954. A polysaccharide from the blue-green alga, Anabaena cylindrica. *Can. Jour. Chem.*, 32:999-1004.

- Bishop, C. T., Anet, E. F. L. J., and Gorham, P. R. 1959. Isolation and identification of the fast-death factor in Microcystis aeruginosa NRC-1. Can. Jour. Biochem. Physiol., 37:453-471.
- Biswas, B. B. 1957. A polysaccharide from Nostoc muscorum. Sci. and Culture (Calcutta), 22:696-697.
- Blinks, L. R. 1951. Physiology and biochemistry of algae. IN: Manual of Phycology. Edited by G. M. Smith. Chronica Botanica Co. Waltham, Massachusetts. Pp. 263-291.
- Blum, J. L. 1954. Evidence for a diurnal pulse in stream phytoplankton. Science, 119:732-734.
- Blum, J. L. 1956. The ecology of river algae. Bot. Rev., 22:291-341.
- Blum, J. L. 1960. Algal populations in flowing water. IN: The Ecology of Algae. Univ. of Pittsburgh Spec. Publ., 2:11-21.
- Bode, N. R. 1939. Ueber die Blattanssscheidung des Wermuts und ihre Wirkung auf andere Pflanzen. Planta, 30:566.
- Bold, H. C. 1942. The cultivation of algae. Bot. Rev., 8:69-138.
- Bonner, J. 1944. An inhibitor of plant growth obtained from cultures of guayule plants. Amer. Jour. Bot., 31:8.
- Bonner, J. 1946. Relation of toxic substances to growth of guayule in soil. Bot. Gaz., 107:343-351.
- Bonner, J. 1950. The role of toxic substances in the interactions of higher plants. Bot. Rev., 16:51-65.
- Bonner, J., and Galston, A. W. 1944. Toxic substances from the culture media of guayule which may inhibit growth. Bot. Gaz., 106:185-198.
- Börner, H. 1959. The apple replant problem. I. The excretion of phlorizin from apple root residues. Contr. Boyce Thompson Inst., 20:39-56.
- Börner, H. 1960. Liberation of organic substances from higher plants and their role in soil sickness problems. Bot. Rev., 26:393-424.

- Bossenmaier, E. F., Olson, T. A., Rueger, M. E., and Marshall, W. H. 1954. Some field and laboratory aspects of duck sickness at Whitewater Lake, Manitoba. Trans. of the 19th North American Wildlife Conference. Pp. 153-175.
- Braarud, T. 1945a. A phytoplankton survey of the polluted waters of Oslo Fjord. Hvalrad. Skr., 29:1-142.
- Braarud, T. 1945b. Experimental studies on marine plankton diatoms. Avh. Norske Vidensk. Akad., 10:3-142.
- Braarud, T., Gaarder, K. R., and Grontved, J. 1953. Phytoplankton of the North Sea and adjacent waters in May, 1948. Rapp. Cons. Explor. Mer., 133:5-89.
- Brandenberg, T. O., and Shigley, F. M. 1947. "Water Bloom" as a cause of poisoning of livestock in North Dakota. Jour. Amer. Vet. Med. Assoc., 110:18-21.
- Branham, J. M., and Metz, C. B. 1959. Inhibition of fertilization and agglutination in Arbacia by an extract of Fucus. Biol. Bull., 117:392-393.
- Breed, R. S., Murray, E. G. D., and Smith, N. R. 1957. Bergey's Manual of Determinative Bacteriology. Seventh edition. The Williams and Wilkins Company. Baltimore.
- Brian, P. W. 1957. The ecological significance of anti-biotic production. IN: Microbial Ecology. Edited by R. E. O. Williams and Spicer, C. C. Cambridge University Press. London. Pp. 168-186.
- Brock, T. D. 1961. Chloramphenicol. Bacteriol. Rev., 25: 32-45.
- Brooks, A. J. 1952. Some observations on the feeding of protozoa on fresh-water algae. Hydrobiologia, 4:281-293.
- Brooks, A. J. 1955. The aquatic fauna as an ecological factor in studies of the occurrence of fresh-water algae. Rev. Algalog., 1:141-145.
- Brooks, A. J. 1957. Water-blooms. New Biology, 23:1-16.
- Brooks, A. J. 1959. The waterbloom problem. Proc. Soc. Water Treatment and Examination, 8:133-137.

- Brooks, A. J., and Rozoska, J. 1954. The influence on the development of the Nile plankton. *Jour. Animal Ecol.*, 23:101-114.
- Brooks, M. G. 1951. Effects of black walnut trees and their products on other vegetation. *West Virginia Univ., Agr. Sta. Bull.* 347:1-31.
- Brown, A. H., and Webster, G. C. 1953. The influence of light on the rate of respiration of the blue-green alga Anabaena. *Amer. Jour. Bot.*, 40:753-756.
- Brown, M. E. 1946. The growth of the brown trout (Salmo trutta Linn.) I. Factors influencing the growth of trout fry. *Jour. Exp. Biol.*, 22:118-144.
- Brown, T. E., and Wilson, D. E. 1958. $C^{14}O$ fixation in normal and manganese deficient Chlorella pyrenoidosa. *Plant Physiol.*, 33:Suppl. xxv.
- Bryan, A. H., and Bryan, C. C. 1956. *Bacteriology Principles and Practice*. Barnes and Noble, Inc. New York. Pp. xv + 422.
- Buell, H. F. 1938. A community of blue-green algae in a Minnesota pond. *Ecology*, 19:224-232.
- Bunt, J. S., Tchon, Y. T., and Gould, J. 1961. Blue-green algae. *Nature*, 192:1274-1276.
- Burke, J. M., Marchisotto, J., McLaughlin, J. J. A., and Provasoli, L. 1960. Analysis of the toxin produced by Gonyaulax catenula in axenic culture. *Ann. N. Y. Acad. Sci.*, 90:837-847.
- Burkholder, P. R., and Burkholder, L. M. 1953. Vitamin B_{12} in suspended solids and marsh muds along the coast of Georgia. *Limnol. and Oceanogr.*, 1:202-208.
- Burkholder, P. R., Evans, A. W., McVeigh, I., and Thornton, H. K. 1944. Antibiotic activity of lichens. *Proc. Nat. Acad. Sci. Wash.*, 30:250-255.
- Burrows, William. 1963. *Textbook of Microbiology*. W. B. Saunders Company. Philadelphia and London. Pp. xvi + 1155.
- Buxton, E. W. 1960. Effects of pea root exudates on the antagonism of some rhizosphere microorganisms toward Fusarium oxysporum f. lisi. *Jour. Gen. Microbiol.*, 22:678-689.

- Canter, H. M., and Lund, J. W. G. 1948. Studies on plankton parasites. I. Fluctuations in the numbers of Asterionella formosa Hass. in relation to fungal epidemics. New Phytol., 47:238-261.
- Carlson, Y. A. 1908. Brilliant Gulf waters. Monthly Weather Review, 36:371-372.
- Castenholz, R. W. 1958. Seasonal changes in the algae of fresh water and saline lakes in Lower Grand Coulee, Washington. Diss. Abst., 18:33-34.
- Chalupa, J., and Stepanek, M. 1960. Limnological study of the Reservoir Sedice near Zeliv. XIII. Permanganate, BOD and plankton. Institute of Chem. Tech., Prague Faculty of Tech. of Fuel and Water, 4:293-323.
- Chan, E. C. S., Katznelson, H., and Westlake, D. W. S. 1963. The influence of soil and root extracts on the associative growth of selected soil bacteria. Can. Jour. Microbiol., 9:187-197.
- Chandler, D. C. 1940. Limnological studies of western Lake Erie. I. Plankton and certain physical-chemical data on the Bass Island region from September, 1938 to November, 1939. Ohio Jour. Sci., 40:291-336.
- Chandler, D. C. 1942. Limnological studies of western Lake Erie. II. Light penetration and its relation to turbidity. Ecology, 23:41-52.
- Chandler, D. C. 1944. Limnological studies of western Lake Erie. IV. Relation of limnological and climatic factors to the phytoplankton of 1941. Trans. Amer. Microsc. Soc., 63:203-236.
- Chandler, D. C., and Weeks, O. B. 1945. Limnological studies of western Lake Erie. V. Relation to limnological and meteorological conditions to the production of phytoplankton in 1942. Ecol. Monogr., 15:435-456.
- Chaput, M., and Grant, G. A. 1958. Toxic algae. III. Screening of a number of species. Defence Res. Chem. Lab., Ottawa, Report 279. Defence Res. Board of Canada.
- Chen, T. T. 1945. Induction of conjugation in Paramecium bursaria. Proc. Nat. Acad. Sci., 31:404.

- Chu, S. P. 1942. The influence of the mineral composition of the medium on the growth of planktonic algae. Part I. Methods and culture media. Jour. Ecol., 30:284-325.
- Chu, S. P. 1943. The influence of the mineral composition of the medium on the growth of planktonic algae. Part II. The influence of the concentration of inorganic nitrogen and phosphate and phosphorus. Jour. Ecol., 31:109-148.
- Chu, S. P. 1946. The utilization of organic phosphorus by phytoplankton. Jour. Mar. Biol. Assoc. U. K., 26:285-295.
- Clark, F. E. 1957. Nodulation responses of two near isogenic lines of the soybean. Can. Jour. Microbiol., 3:113-123.
- Clarke, G. L. 1939. The relation between diatoms and copepods as a factor in the productivity of the sea. Quart. Rev. Biol., 14:60-64.
- Cohen, S. G., and Rief, C. B. 1953. Cutaneous sensitization to blue-green algae. Jour. Allergy, 24:452-457.
- Coleman, A. W. 1959. Sexual isolation in Pandorina morum. Jour. Protozool., 6:249-264.
- Coleman, A. W. 1962. Sexuality. IN: Physiology and Biochemistry of Algae. Edited by R. A. Lewin. Academic Press, Inc. New York. Pp. 711-729.
- Collier, A. 1958. Some biochemical aspects of red tides and related oceanographic problems. Limnol. and Oceanogr., 3:33-39.
- Collons, V. G. 1963. The distribution and ecology of bacteria in fresh-water. Proc. Soc. Water Treatment and Examination, 12:40-73.
- Connell, C. H., and Cross, J. B. 1950. Mass mortality of fish associated with the protozoan Gonyaulax in the Gulf of Mexico. Science, 112:359-363.
- Conrad, H. M., and Saltman, P. 1962. Growth substances. IN: Physiology and Biochemistry of Algae. Edited by R. A. Lewin. Academic Press, Inc. New York. Pp. 663-671.

- Cook, A. H., and Evidge, J. A. 1951. Fertilization in the Fucaceae: investigations on the nature of the chemical substances produced by eggs of Fucus serratus and F. vesiculosus. Proc. Roy. Soc. B, 138:97-114.
- Cook, M. T. 1921. Wilting caused by walnut trees. Phytopath., 11:346.
- Cooley, Donald G. 1954. The Science Book of Wonder Drugs. Pocket Books, Inc. New York. Pp. 247.
- Cooper, L. H. N. 1933. Chemical constituents of biological importance in the English Channel. Jour. Mar. Biol. Assoc. U. K., 18:677-728.
- Cooper, L. H. N. 1935a. Iron in the sea and marine plankton. Proc. Roy. Soc. Lond., Ser. B., 118:419-438.
- Cooper, L. H. N. 1935b. The rate of liberation of phosphate in sea water by the breakdown of plankton organisms. Jour. Mar. Biol. Assoc. U. K., 20:197-202.
- Cooper, L. H. N. 1937. On the ratio of nitrogen to phosphorus in the sea. Jour. Mar. Biol. Assoc. U. K., 22:177-182.
- Cooper, L. H. N. 1938. Phosphate in the English Channel. Jour. Mar. Biol. Assoc. U. K., 23:181-195.
- Cooper, L. H. N. 1948. The distribution of iron in the waters of the western English Channel. Jour. Mar. Biol. Assoc. U. K., 27:279-313.
- Cornman, I. 1946. Alteration of mitosis by coumarin and parasorbic acid. Amer. Jour. Bot., 33:217.
- Correll, David L. 1961. A study of the ribonucleic acid--polyphosphate complexes isolated from Anabaena variabilis and synchronized Chlorella pyrenoidosa. Doctoral Dissertation. Michigan State University.
- Cotton, H. L. 1914. Algal poisoning. Jour. Amer. Vet. Med. Assoc., 9:903-904.
- Cowey, C. B. 1956. A preliminary investigation of the variation of vitamin B₁₂ in oceanic and coastal waters. Jour. Mar. Biol. Assoc. U. K., 35:609-620.

- Crump, Lettice M. 1950. The influence of bacterial environment on the excystment of amoeba from soil. Jour. Gen. Microbiol., 4:16.
- Cubbon, M. H. 1925. Effect of a rye crop on the growth of grapes. Jour. Amer. Soc. Agron., 17:568-577.
- Cullimore, D. R., and Woodbine, M. 1963. A rhizosphere effect of the pea root on algae. (Stimulatory effect on growth under autotrophic conditions.) Nature, 198: 304-305.
- Cupp, Easter. 1937. Seasonal distribution and occurrence of marine diatoms and dinoflagellates at Scotch Cap, Alaska. Bull. Scripps Inst. Ocean. Tech., 4:71-100.
- Daisley, K. W., and Fisher, L. R. 1958. Vertical distribution of vitamin B₁₂ in the sea. Jour. Mar. Biol. Assoc. U. K., 37:683-686.
- Darken, M. A. 1953. Production of vitamin B₁₂ by micro-organisms and its occurrence in plant tissues. Bot. Rev., 19:99-130.
- Davidson, F. F. 1959a. Effects of extracts of blue-green algae on pigment production by Serratia marcescens. Jour. Gen. Microbiol., 20:605-611.
- Davidson, F. F. 1959b. Poisoning of wild and domestic animals by a toxic water-bloom of Nostoc rivulare Kütz. Jour. Amer. Water Works Assoc., 51:1277-1288.
- Davidson, F. F. 1961. Antibacterial activity of Oscillatoria formosa Bory extract. Water and Sewage Works, 108:417-420.
- Davidson, F. F. 1962. Antibacterial activity of extracts obtained from blue-green algae. Paper presented at 17th Annual Purdue Industrial Waste Conference. 9 pp.
- Davidson, V. M., and Huntsman, A. G. 1926. The causation of diatom maxima. Trans. Roy. Soc. Canada, 20:119-125.
- Davis, C. C. 1948. Gymnodinium brevis sp. nov., a cause of discolored water and animal mortality in the Gulf of Mexico. Bot. Gaz., 109:358-360.
- Davis, C. C. 1954. A preliminary study of the plankton of the Cleveland Harbor area, Ohio. II. The distribution and quantity of phytoplankton. Ecol. Monogr., 24:321-347.

- Davis, C. C. 1956. Notes on a bloom of Euglena haematodes in Ohio. Ecology, 37:192-193.
- Davis, E. A. 1952. Quantitative studies of factors influencing the growth of Chlorella pyrenoidosa. Carnegie Inst., Washington. Yearbook No. 51:135-138.
- Davis, R. F. 1928. The toxic principle of Juglans nigra as identified with synthetic juglone and its toxic effects on tomato and alfalfa plants. Amer. Jour. Bot., 15:620.
- Deem, A. W., and Thorp, F., Jr. 1939. Toxic algae in Colorado. Jour. Amer. Vet. Med. Assoc., 95:542-544.
- Dillenberg, H. O., and Dehnel, M. K. 1960. Toxic waterbloom in Saskatchewan, 1959. Can. Med. Assoc. Jour., 83:1151-1154.
- Diwald, K. 1938. Die ungeschlechtliche und geschlechtliche Fortpflanzung von Glenodinium lubiniensiforme sp. nov. Flora. (Jena), 132:174-192.
- Dobbs, C. G., and Henson, W. H. 1953. A widespread fungistasis in soils. Nature, 172:197-199.
- Domogalla, B. P., Juday, C., and Petersen, W. H. 1925. The forms of nitrogen found in certain lake waters. Jour. Biol. Chem., 63:269-285.
- Dragovich, Alexander, Finucane, John H., Kelly, John A., Jr., and May, Billie Z. 1963. Counts of red tide organisms, Gyrodinium breve, and associated oceanographic data from Florida West Coast. U. S. Fisheries and Wildlife Serv. Spec. Sci. Rept. Fish., 455:1-40.
- Droop, M. R. 1953. On the ecology of flagellates from brackish and fresh water rockpools of Finland. Acta Botanica Fennica, 51:1-52.
- Droop, M. R. 1955a. A pelagic diatom requiring cobalamin. Jour. Mar. Biol. Assoc. U. K., 34:229-231.
- Droop, M. R. 1955b. Some factors governing encystment in Haematococcus pluvialis. Arch. Mikrobiol., 21:267-272.
- Droop, M. R. 1957a. Auxotrophy and organic compounds in the nutrition of marine phytoplankton. Jour. Gen. Microbiol., 16:286-293.

- Droop, M. R. 1957b. Vitamin B₁₂ in marine ecology. *Nature*, 180:1041-1042.
- Droop, M. R. 1958. Requirements for thiamine among some marine supra-littoral Protista. *Jour. Mar. Biol. Assoc. U. K.*, 37:323-330.
- Droop, M. R. 1961. Vitamin B₁₂ and marine ecology: the response of Monochrysis lutheri. *Jour. Mar. Biol. Assoc. U. K.*, 41:69-76.
- Droulet, F., and Daily, W. A. 1939. The planktonic freshwater species of Microcystis. *Field Mus. Nat. Hist. Bot. Serv.*, 20:67-83.
- Edmonson, W. T. 1957. Trophic relations of the zooplankton. *Trans. Amer. Micr. Soc.*, 76:225-245.
- Einsele, W., and Grim, J. 1938. Über den Kieselsäuregehalt planktischer Diatomeen und dessen Bedeutung für einige Fragen ihrer Ökologie. *Z. Bot.*, 32:545-590.
- Elkan, G. H. 1961. A nodulation-inhibiting root excretion from a non-nodulating soybean strain. *Can. Jour. Microbiol.*, 7:851-856.
- Elkan, G. H. 1962. Comparison of rhizosphere microorganisms of genetically related nodulating and non-nodulating soybeans. *Can. Jour. Microbiol.*, 8:79-87.
- Enomoto, Y. 1959. Studies on the plankton off the west coast of Kyushu. II. On the conditions for the vernal blooming of phytoplankton. *Bull. Jap. Soc. Fish.*, 25:172-182.
- Epstein, S., and Williams, B. 1946. *Miracles from Microbes*. Rutgers University Press. New Brunswick. Pp. xi + 155.
- Erdmann, M. H., and Harrison, C. M. 1947. The influence of domestic rye-grass and redtop upon the growth of Kentucky bluegrass and Chewing's fescue in lawn and turf mixture. *Jour. Amer. Soc. Agron.*, 39:682-689.
- Eyster, C. 1952. Necessity of boron for Nostoc muscorum. *Nature*, 170:755.
- Eyster, C. 1958. The micro-element nutrition of Nostoc muscorum. *Ohio Jour. Sci.*, 58:25-33.

- Fahreus, G., and Ljunggren, H. 1959. The possible significance of pectic enzymes in root hair infection by nodule bacteria. *Physiol. Plant.*, 12:145-154.
- Fetteroff, C. M., Jr., and Carr, R. F. 1962. Stream and lake survey, Edmore through the Six Lakes Chain, Montcalm County. Michigan State Water Resources Commission.
- Firkins, G. S. 1953. Toxic algae poisoning. *Iowa State Coll. Vet.*, 24:30-39.
- Fish, C. J. 1925. Seasonal distribution of the plankton of the Woods Hole region. *Bull. U. S. Bur. Fish.*, 41:91-179.
- Fitch, C. P., Bishop, L. M., Boyd, W. L., Gortner, R. A., Rogers, C. F., and Tilden, J. E. 1934. "Waterbloom" as a cause of poisoning in domestic animals. *Cornell Veter.*, 24:30-39.
- Fleming, R. H. 1939. The control of diatom populations by grazing. *Jour. Const. Int. Explor. Mer.*, 14:1-20.
- Flint, L. H., and Moreland, C. F. 1946. Antibiosis in the blue-green algae. *Amer. Jour. Bot.*, 33:218. (Abstr.)
- Fogg, G. E. 1941. The gas-vacuoles of the Myxophyceae (Cyanophyceae). *Cambridge Biol. Rev.*, 16:205-217.
- Fogg, G. E. 1951. Studies on nitrogen fixation by blue-green algae. II. Nitrogen fixation by Mastigocladus laminosus Cohn. *Jour. Exp. Bot.*, 2:117-120.
- Fogg, G. E. 1952. The production of extracellular nitrogenous substances by a blue-green alga. *Proc. Roy. Soc. London B*, 139:373-397.
- Fogg, G. E. 1953. *The Metabolism of Algae*. John Wiley & Sons, Inc. New York. Pp. ix + 149.
- Fogg, G. E. 1954. Algal physiology and biochemistry. *Nature*, 173:434-435.
- Fogg, G. E. 1956. The comparative physiology and biochemistry of the blue-green algae. *Bacteriol. Rev.*, 20:148-165.
- Fogg, G. E. 1958. Extracellular products of phytoplankton and the estimation of primary production. *Cons. Internat. Explor. Mer., Rapp. et Proc. Verb.*, 144:56-60.

- Fogg, G. E. 1962. Extracellular products. IN: Physiology and Biochemistry of Algae. Ralph A. Lewin, Ed. Academic Press, Inc. New York. Pp. 475-489.
- Fogg, G. E., and Boalch, G. T. 1958. Extracellular products in pure cultures of brown algae. *Nature*, 181:789-790.
- Fogg, G. E., and Nalewajko, C. 1964. Glycollic acid as an extracellular product of phytoplankton. *Verh. Internat. Verein. Limnol.*, 15:806-810.
- Fogg, G. E., and Westlake, D. F. 1955. The importance of extracellular products of algae in freshwater. *Inter. Assoc. Theor. Appl. Limnol.*, 12:219-232.
- Ford, J. E. 1959. The influence of certain derivatives of vitamin B₁₂ upon the growth of microorganisms. *Jour. Gen. Microbiol.*, 21:693-701.
- Ford, J. E., and Goulden, J. D. S. 1959. The influence of vitamin B₁₂ on growth rate and cell composition of the flagellate Ochromonas malhamensis. *Jour. Gen. Microbiol.*, 20:267-276.
- Förster, H. 1957. Das Wirkungsspektrum der Kopulation von Chlamydomonas eugametos. *Z. Naturforsch.*, 12b:765-770.
- Förster, H. 1959. Die Wirkungsstärken einiger Wellenlängen zum Auslösen der Kopulation von Chlamydomonas moewusii. *Z. Naturforsch.*, 14b:479-480.
- Förster, H., and Wiese, L. 1954a. Untersuchungen zur Kopulationsfähigkeit von Chlamydomonas eugametos. *Z. Naturforsch.*, 9b:470-471.
- Förster, H., and Wiese, L. 1954b. Gamonwirkungen bei Chlamydomonas eugametos. *Z. Naturforsch.*, 9b:548-550.
- Förster, H., and Wiese, L. 1955. Gamonwirkung bei Chlamydomonas reinhardtii. *Z. Naturforsch.*, 10b:91-92.
- Förster, H., Wiese, L., and Braunitzer, G. 1957. Über das agglutinierend wirkende Gynogamon von Chlamydomonas eugametos. *Z. Naturforsch.*, 11b:315-317.
- Foster, J. W. 1949. Chemical activities of fungi. Academic Press, Inc. New York.

- Fowden, L. 1951. Amino acids of certain algae. *Nature*, 167:1030.
- Francis, G. 1878. Poisonous Australian lake. *Nature*, 18:11.
- Fred, E. B., Baldwin, I. L., and McCoy, E. 1932. Root nodule bacteria and leguminous plants. Univ. of Wisc. Studies in Science. Madison.
- Fritsch, F. E. 1959. The Structure and Reproduction of the Algae. Vol. II. Phaeophyceae, Rhodophyceae, Myxophyceae, and a Foreword. The Syndics of the Cambridge University Press. London. Pp. xiv + 939.
- Fuller, J. L. 1937. Feeding rates of Calanus finmarchicus in relation to environmental conditions. *Biol. Bull.* Woods Hole, 72:233-246.
- Funke, G. L. 1943. The influence of Artemisia absinthium on neighboring plants. *Blumea*, 5:281-293.
- Gaarder, T., and Gran, H. H. 1935. Investigations of the production of plankton in the Oslo Fjord. *Rapp. Cons. Explor. Mer.*, 40:1-48.
- Galtsoff, P. S. 1949. The mystery of the red tide. *Scientific Monthly*, 68:108-117.
- Garb, S. 1961. Differential growth-inhibitors produced by plants. *Bot. Rev.*, 27:422-443.
- Gaukhman, Z. S., and Ryabov, F. P. 1962. (The interrelationships between the blue-green algae and the bacterioflora of the middle Dnepr and the Dnepr Reservoir. IN: Vysshaya Shkolo (Higher School), 33-35.) *Biol. Abstr.*, 44, No. 22042, 1963.
- Gauld, D. T. 1951. The grazing rate of planktonic copepods. *Jour. Mar. Biol. Assoc. U. K.*, 29:695-706.
- Gerschon, D. 1961. Genetic studies of effective nodulation in Lotus spp. *Can. Jour. Microbiol.*, 7:961-963.
- Gerloff, G. C., Fitzgerald, G. P., and Skoog, F. 1950a. The isolation, purification, and culture of blue-green algae. *Amer. Jour. Bot.*, 37:216-218.

- Gerloff, G. C., Fitzgerald, G. P., and Skoog, F. 1950b. The isolation, purification, and nutrient requirements of blue-green algae. IN: The Culturing of Algae. The Charles F. Kettering Foundation. Pp. 27-44.
- Gerloff, G. C., Fitzgerald, G. P., and Skoog, F. 1950c. The mineral nutrition of Coccochloris peniocyctis. Amer. Jour. Bot., 37:835-840.
- Gerloff, G. C., Fitzgerald, G. P., and Skoog, F. 1952. The mineral nutrition of Microcystis aeruginosa. Amer. Jour. Bot., 39:26-32.
- Gerloff, G. C., and Skoog, F. 1954. Cell contents of nitrogen and phosphorous as a measure of their availability for growth of Microcystis aeruginosa. Ecology, 35:348-353.
- Gerloff, G. C., and Skoog, F. 1957a. Availability of iron and manganese in southern Wisconsin Lakes for the growth of Microcystis aeruginosa. Ecology, 38:551-556.
- Gerloff, G. C., and Skoog, F. 1957b. Nitrogen as a limiting factor for the growth of Microcystis aeruginosa in a southern Wisconsin Lake. Ecology, 38:556-561.
- Geyer, R. A. 1948. Mass mortality of aquatic life from natural causes. Humble Oil and Refining Co. Exploration Dept. Pub. Pp. 15.
- Gillam, W. G. 1925. The effect on livestock of water contaminated with fresh-water algae. Jour. Amer. Vet. Med. Assoc., 67:780-784.
- Goldman, C. R. 1960. Primary productivity and limiting factors of the Alaska peninsula. Ecol. Monogr., 30: 207-230.
- Golueke, C. G. 1960. The ecology of a biotic community consisting of algae and bacteria. Ecology, 40:65-72.
- Goodwin, R. H., and Taves, C. 1950. The effect of coumarin derivatives on the growth of Avena roots. Amer. Jour. Bot., 37:224-231.
- Gorham, P. R. 1960. Toxic waterblooms of blue-green algae. Can. Vet. Jour., 1:235-245.

- Gorham, P. R. 1962. Laboratory studies on the toxins produced by waterblooms of blue-green algae. Amer. Jour. Pub. Health, 52:2100-2105.
- Gorham, P. R., McLachlan, J., Hammer, U. T., and Kim, W. K. 1964. Isolation and culture of toxic strains of Anabaena flos-aquae (Lyngb.) de Bréb. Verh. Internat. Verein. Limonol., 15:796-804.
- Gorham, P. R., Simpson, B., Bishop, C. T., and Anet, E. F. L. J. 1959. Toxic waterblooms of blue-green algae. Proc. IX. Int. Bot. Congr., 2:137.
- Goryuvova, S. V. 1950. (Chemical composition and kinetics of excretion in the blue-green alga Oscillatoria splendida Grev.) Academy of Sciences, Moscow.
- Gran, H. H. 1930. The spring growth of plankton at More in 1928-29 and at Lofoten in 1929 in relation to its limiting factors. Skrifter utgite av. Det. Norske Vid. Ak. Oslo 1. Mat-Nat. Klasse, 5:1-77.
- Gran, H. H. 1933. Studies on the biology and chemistry of the Gulf of Maine. II. Distribution of phytoplankton in 1932. Biol. Bull., 64:159-182.
- Gran, H. H., and Braarud, T. 1935. A quantitative study of the phytoplankton in the Bay of Fundy and the Gulf of Maine. Jour. Biol. Bd. Can., 1:279-467.
- Grant, G. A., and Hughes, E. O. 1953. Development of toxicity in blue-green algae. Can. Jour. Pub. Health, 44:334-339.
- Gray, R., and Bonner, J. 1948a. Structure determination and synthesis of a plant growth inhibitor, 3-acetyl-6-methoxybenzaldehyde, found in the leaves of Encelia farinosa. Jour. Amer. Chem. Soc., 70:1249-1253.
- Gray, R., and Bonner, J. 1948b. An inhibitor of plant growth from the leaves of Encelia farinosa. Amer. Jour. Bot., 35:52-57.
- Grümmer, G. 1961. The role of toxic substances in the interrelationships between higher plants. IN: Symposia of the Society for Experimental Biology. XV. Mechanisms in Biological Competetion. Academic Press, Inc. New York. Pp. 219-229.

- Guillard, R. P. L., and Ryther, J. H. 1962. Studies of marine planktonic diatoms. I. Cyclotella nana Haestedt and Detonula confervacea (Cleve) Gran. Can. Jour. Microbiol., 8:229-239.
- Guillard, R. P. L., and Wangersky, P. J. 1958. The production of extracellular carbohydrates by some marine flagellates. Limnol. and Oceanogr., 3:449-454.
- Gunter, G., Smith, F. G. W., and Williams, R. H. 1947. Mass mortality of marine animals on the lower West Coast of Florida, November, 1946. Science, 105:257.
- Gunter, G., Williams, R. H., Davis, C. C., and Smith, F. G. W. 1948. Catastrophic mortality of marine animals and coincident phytoplankton bloom on the west coast of Florida. Ecol. Mongr., 18:309-327.
- Guseva, K. A. 1937. The hydrobiology and microbiology of the Ucha Reservoir on the Moscow-Volga Canal. II. Observations of the growth of Anabaena Lemmermannii Richter, Aphanizomenon flos-aquae Bréb. and Asterionella formosa Hassall in the reservoir during the first summer of its existence. Mikrobiologiya, 6: 449-464. (Available as NRC Technical Translation 877, National Research Council, Ottawa, Ontario, 1960.)
- Haagen-Smit, A. J., and Thimann, K. V. 1938. The excystment of Colpoda cucullus. Jour. Cell. Comp. Physiol., 11:389.
- Halim, Y. 1960. Observations on the Nile bloom of phytoplankton in the Mediterranean. Jour. Conseil. Perm. Inter. Explor. Mer., 26:57-67.
- Halldal, P. 1958. Pigment formation and growth in blue-green algae in crossed gradients of light intensity and temperature. Physiol. Plant., 11:401-420.
- Halldal, P., and French, C. S. 1958. Algal growth in crossed gradients of light intensity and temperature. Plant Physiol., 33:249-252.
- Hammer, C. L., Sell, H. M., Klomparens, W., and Vaughn, J. R. 1950. Selective inhibition of green plants and fungi by beta methyl umbelliferone. Bot. Gaz., 112:135-137.
- Hammer, U. T. 1964. The succession of "bloom" species of blue-green algae and some causal factors. Verh. Internat. Verein. Limnol., 15:829-836.

- Harder, R., and Oppermann, A. 1953. Über antibiotische Stoffe bei den Grünalgen Stichococcus bacillaris und Protosiphon botryoides. Arch. Mikrobiol., 19:398-401.
- Hardy, A. C. 1923. Notes on the Atlantic plankton taken off the Coast of England in 1921 and 1922. Publ. Circ. Cons. Int. Explor. Mer., No. 78.
- Hardy, A. C. 1926. The herring in relation to its animate environment. Part II. Reports on trials with the plankton indicator. Minist. Agric. Fish., Fish. Invest. Ser. II, 8, No. 7.
- Hardy, A. C. 1936. The ecological relations between the herring and the plankton investigated with the plankton indicator. Jour. Mar. Biol. Assoc. U. K., 21:147-177.
- Hardy, A. C., and Gunther, E. R. 1935. The plankton of the South Georgia whaling grounds and adjacent waters, 1926-1927. Disc. Reports, 11:1-456.
- Hart, T. J. 1934. Phytoplankton of the southwest Atlantic and the Bellinghausen Sea, 1929-1931. Disc. Reports, 8:1-268.
- Hart, T. J. 1941. Phytoplankton periodicity in Antarctic surface waters. Disc. Reports, 21:261-356.
- Hartman, R. T. 1960. Algae and metabolites of natural waters. IN: The Ecology of Algae. Univ. of Pittsburgh, Spec. Publ., No. 2, pp. 38-56.
- Hartman, R. T., and Graffius, H. J. 1960. Quantitative seasonal changes in the phytoplankton communities of Pymatuning Reservoir. Ecology, 41:333-340.
- Hartwell, J. L., Johnson, J. M., Fitzgerald, D. B., and Belkin, M. 1953. Podophyllotoxin from Juniperus species; Savinin. Jour. Amer. Chem. Soc., 75:235-236.
- Harvey, H. W. 1933. On the rate of diatom growth. Jour. Mar. Biol. Assoc. U. K., 19:253-275.
- Harvey, H. W. 1937. Note on the selective feeding by Calanus. Jour. Mar. Biol. Assoc. U. K., 22:97-100.
- Harvey, H. W. 1938. The supply of iron to diatoms. Jour. Mar. Biol. Assoc. U. K., 22:205-219.

- Harvey, H. W. 1947. Manganese and the growth of phytoplankton. Jour. Mar. Biol. Assoc. U. K., 26:562-578.
- Harvey, H. W. 1949. On manganese in sea and fresh water. Jour. Mar. Biol. Assoc. U. K., 28:155-164.
- Harvey, H. W., Cooper, L. H. N., Lebour, M. V., and Russell, F. S. 1935. Plankton and its control. Jour. Mar. Biol. Assoc. U. K., 20:407-441.
- Hasler, A. D., and Jones, E. 1949. Demonstration of the antagonistic action of large aquatic plants on algae and rotifers. Ecology, 30:359-364.
- Hauschka, T., Toennies, G., and Swain, A. P. 1945. The mechanism of growth inhibition of hexenolactone. Science, 101:383-385.
- Heise, H. A. 1949. Symptoms of hay fever caused by algae. Jour. of Allergy, 20:383-385.
- Heise, H. A. 1951. Symptoms of hay fever caused by algae. II. Microcystis, another form of algae producing allergenic reactions. Ann. of Allergy, 9:100-101.
- Henderson, C. 1949. Manganese for increased production of water-bloom algae in ponds. Progr. Fish. Culturist, 11:157-159.
- Henderson, V. E., and Katznelson, H. 1961. The effect of plant roots on the nematode population of the soil. Can. Jour. Microbiol., 7:163-167.
- Henderson, V. E., and Katznelson, H. 1962. Studies on the relationships between nematodes and other soil microorganisms. I. The influence of actinomycetes and fungi on Rhabditis (Cephaloboides) oxycerca de Man. Can. Jour. Microbiol., 8:875-882.
- Henris, Y., Keller, P., and Keynan, A. 1961. Inhibition of fungal growth by bacteria during cellulose-decomposition. Can. Jour. Microbiol., 7:857-863.
- Hile, R. 1936. Age and growth of the Cisco, Leucichthys artedi (Le Seur), in the lakes of the Northeastern Highlands, Wisconsin. Bull. U. S. Bur. Fish., 48:211.
- Hoffman, L. 1960. Chemotaxix of Oedogonium sperms. The Southwestern Naturalist, 5:111-116.

- Holm-Hansen, O., Gerloff, G. C., and Skoog, F. 1954. Cobalt as an essential element for blue-green algae. *Physiol. Plant.*, 7:665-675.
- Howard, A. 1935. The effects of grass on trees. *Proc. Roy. Soc., London, B.*, 97:284-321.
- Howard, N. J., and Berry, A. E. 1933. Algal nuisances in surface waters. *Can. Jour. Public Health*, 24:377-384.
- Howell, J. F. 1953. Gonyaulax monilata sp. nov., the causative dinoflagellate of a red tide on the east coast of Florida in August-September, 1951. *Trans. Amer. Micr. Soc.*, 72:153-156.
- Hrbáček, J. 1964. Contribution to the ecology of water-bloom-forming blue-green algae - Aphanizomenon flos-aquae and Microcystis aeruginosa. *Verh. Internat. Verein. Limnol.*, 15:837-846.
- Hughes, E. O., Gorham, P. R., and Zehnder, A. 1955. Toxicity of Microcystis aeruginosa in pure culture. *Phycol. News Bull.*, 8:5.
- Hughes, E. O., Gorham, P. R., and Zehnder, A. 1958. Toxicity of a unialgal culture of Microcystis aeruginosa. *Can. Jour. Microbiol.*, 4:225-236.
- Hulburt, E. M. 1956. The phytoplankton of Great Pond, Massachusetts. *Biol. Bull. Woods Hole*, 110:157-168.
- Hutchinson, D., Weaver, R. H., and Scherago, M. 1943. The incidence and significance of microorganisms antagonistic to Escherichia coli in water. *Jour. Bact.*, 45:29.
- Hutchinson, G. E. 1943. Thiamine in lake waters and aquatic organisms. *Arch. Biochem.*, 2:143-150.
- Hutchinson, G. E., and Setlow, J. K. 1946. Limnological studies in Connecticut. VIII. The niacin cycle in a small inland lake. *Ecology*, 27:13-22.
- Hutner, S. H. 1948. Essential constituents of sea water for growth of a marine diatom. *Trans. New York Acad. Sci.*, 10:136-141.
- Hutner, S. H., and McLaughlin, J. J. A. 1958. Poisonous tides. *Sci. Amer.*, 199:92-98.

- Hutner, S. H., Provasoli, L., Schatz, A., and Haskins, C. P. 1950. Some approaches to the study of the role of metals in the metabolism of microorganisms. *Proc. Amer. Phil. Soc.*, 94:152-170.
- Ingram, W. M., and Prescott, G. W. 1954. Toxic fresh-water algae. *Amer. Midland Naturalist*, 52:75-87.
- Jackson, D. F., and McFadden, J. 1954. Phytoplankton photosynthesis in Sanctuary Lake, Pymatuning Reservoir. *Ecology*, 35:1-4.
- Jakob, H. 1954a. Compatibilit  s et antagonismes entre algues du sol. *Compt. Rend. Acad. Sci. Paris*, 238:928.
- Jakob, H. 1954b. Sur les propri  t  s antibiotiques   nergiques d'une algue du sol: Nostoc muscorum. *Compt. Rend. Acad. Sci. Paris*, 238:2018-2020.
- Johnson, W. H. 1933. Effects of population on the rate of reproduction in Oxytrichia. *Physiol. Zool.*, 6:22-54.
- Johnson, W. E., and Hasler, A. D. 1954. Rainbow trout production in dystrophic lakes. *Jour. Wildlife Man.*, 18:113-134.
- Johnston, R. 1964. Sea water, the natural medium of phytoplankton. II. Trace metals and chelation and general discussion. *Jour. Mar. Biol. Assoc. U. K.*, 44:87-109.
- Jones, J. K. N., Hough, L., and Wadman, W. H. 1952. An investigation of the polysaccharide components of certain fresh-water algae. *Jour. Chem. Soc.*, pp. 3393-3399.
- Jones, L. R., and Morse, W. J. 1903. The shrubby cinquefoil as a weed. *Vt. Agr. Exp. Sta.*, 16th Ann. Rep., pp. 173-190.
- J  rgenson, E. G. 1956. Growth inhibiting substances formed by algae. *Physiol. Plant.*, 9:712-726.
- J  rgenson, E. G. 1959. The growth of Chlamydomonas reinhardtii and Scenedesmus quadricauda in unialgal and mixed cultures. *Carnegie Inst. Wash. Year Book*, 58:341.
- J  rgenson, E. G. 1960. Effect of cell extracts on the growth of Bacillus subtilis. *Carnegie Inst. Wash. Year Book*, 59:349.

- Jørgenson, E. G., and Steenmann-Nielson, E. 1959. Effects of filtrates from cultures of unicellular algae on the growth of Staphylococcus aureus. Int. Oceanogr. Congress, 1959. Am. Assoc. Advancement Science, p. 923.
- Jørgenson, E. G., and Steenmann-Nielson, E. 1961. Effects of the filtrates from cultures of unicellular algae on the growth of Staphylococcus aureus. Physiol. Plant., 14:896-908.
- Jubb, K. V. F., and Kennedy, P. C. 1963. Pathology of Domestic Animals. Vol. II. Academic Press. New York.
- Juday, C., and Birge, E. A. 1931. The second report on the phosphorus content of Wisconsin lake waters. Trans. Wis. Acad. Sci., Arts, Lett., 26:353-382.
- Juday, C., Birge, E. A., Kemmerer, G. I., and Robinson, R. J. 1927. Phosphorus content of lake waters of northeastern Wisconsin. Trans. Wis. Acad. Sci., Arts, Lett., 23:233-248.
- Kalmbach, E. R. 1934. Western duck sickness, a form of botulism. U. S. Dept. Agric. Tech. Bull. No. 411, p. 81.
- Katznelson, H., and Strzelczyk, E. 1961. Studies of the interaction of plants and free-living nitrogen-fixing microorganisms. I. Occurrence of Azotobacter in the rhizosphere of crop plants. Can. Jour. Microbiol., 7:437-446.
- Ketchum, B. H. 1939a. The absorption of phosphate and nitrate by illuminated cultures of Nitzschia closterium. Amer. Jour. Bot., 26:399-407.
- Ketchum, B. H. 1939b. The development and restoration of deficiencies in the phosphorus and nitrogen composition of unicellular plants. Jour. Cell. Comp. Physiol., 13: 373-381.
- Ketchum, B. H. 1951. Plankton algae and their biological significance. IN: Manual of Phycology. Smith, G. M., Ed. Chronica Botanica Co. Waltham, Massachusetts. Pp. 335-346.
- Ketchum, B. H. 1954. Mineral nutrition of phytoplankton. Ann. Rev. Plant Physiol., 5:55-74.

- Ketchum, B. H., and Keen, J. 1948. Unusual phosphorus concentrations in the Florida red tide sea water. Jour. Mar. Res., 7:17-21.
- Ketchum, B. H., Lillick, L., and Redfield, A. 1949. The growth and optimum yields of unicellular algae in mass culture. Jour. Cell. Comp. Physiol., 33:267-280.
- Ketchum, B. H., and Redfield, A. C. 1938. A method for maintaining continuous supply of marine diatoms by culture. Biol. Bull., 75:165-169.
- Ketchum, B. H., and Redfield, A. C. 1949. Some physical and chemical characteristics of algae grown in mass culture. Jour. Cell. Comp. Physiol., 33:281-299.
- Ketchum, B. H., Vaccaro, R. F., and Corwin, N. 1958. The annual cycle of phosphorus and nitrogen in New England coastal waters. Jour. Mar. Res., 17:282-301.
- Keynan, A., Henis, Y., and Keller, P. 1961. Factors influencing the composition of cellulose-decomposing microflora on soil crumb plates. Nature, 191:307.
- Kidder, G. W. 1941. Growth studies on inhibition of ciliates. V. The acceleration and inhibition of growth in biologically conditioned medium. Physiol. Zool., 14: 209-226.
- Kimball, R. F. 1942. The nature and inheritance of mating types in Euplotes patella. Genetics, 27:269.
- Kimball, R. F. 1943. Mating types in the ciliate Protozoa. Quart. Rev. Biol., 18:30.
- Knyp1, J. S. 1963. A fungistatic action of coumarin. Nature, 200:800-802.
- Koch, L. W. 1955. The peach replant problem in Ontario. I. Symptomatology and distribution. Can. Jour. Bot., 33:450-460.
- Kratz, W. A., and Myers, J. 1955a. Nutrition and growth of several blue-green algae. Amer. Jour. Bot., 42:282-287.
- Kratz, W. A., and Myers, J. 1955b. Photosynthesis and respiration of three blue-green algae. Plant Physiol., 30:275-280.

- Krauss, R. W. 1958. Physiology of the fresh-water algae. *Ann. Rev. Plant Physiol.*, 9:207-244.
- Krauss, R. W. 1961. Fundamental characteristics of algal physiology. IN: *Algae and Metropolitan Wastes*. Rob't. A. Taft Eng. Cent. Tech. Rept. W61-3:40-47.
- Krauss, R. W., and Thomas, W. H. 1954. The growth and inorganic nutrition of Scenedesmus obliquus in mass culture. *Plant Physiol.*, 29:205-214.
- Kudo, R. R. 1954. Protozoology. Charles C. Thomas Publisher. Springfield, Illinois. Pp. xi + 966.
- Kylin, H. 1946. On the nature of the cell wall constituents of the algae. *Jour. Indian. Bot. Soc.*, 25:97-99.
- Lackey, J. B. 1938. A study of some ecological factors influencing the distribution of protozoa. *Ecol. Monogr.*, 8:501-528.
- Lackey, J. B. 1942. The effects of distillery wastes and waters on the microscopic flora and fauna of a small creek. *Pub. Health Reports*, 57:253-260.
- Lefevre, M., and Farrugia, G. 1958. De l'influence, sur les algues d'eau douce, des produits de décomposition spontanée des substances organiques d'origine animal et végétale. *Hydrobiologia*, 10:49-65.
- Lefevre, M., and Jakob, H. 1949. Sur quelques propriétés des substances activées tirées des cultures d'algues d'eau douce. *Compt. Rend. Acad. Sci. Paris*, 229:234-236.
- Lefevre, M., Jacob, H., and Nisbet, M. 1950. Sur la sécrétion, par certaines Cyanophytes, de substances algostatiques dans les collections d'eau naturelles. *Compt. Rend. Acad. Sci. Paris*, 230:2226.
- Lefevre, M., Jakob, H., and Nisbet, M. 1951. Compatibilités antagonismes entre algues d'eau douce dans les collections d'eau naturelle. *Proc. Intern. Assoc. Theor. Appl. Limnol.*, 11:224-229.
- Lefevre, M., Jakob, H., and Nisbet, M. 1952. Auto- et hétéroantagonisme chez les algues d'eau douce in vitro et dans les collections d'eau naturelles. *Ann. Sta. Centr. Hydrobiol.*, 4:5-198.

- Lefevre, M., and Nisbet, M. 1948. Sur la sécrétion par certaines espèces d'algues de substance inhibitrices d'autres espèces d'algues. Compt. Rend. Acad. Sci. Paris, 226:107-109.
- Lefevre, M., Nisbet, M., and Jakob, J. 1949. Action des substances excrétées en culture par certaines espèces d'algues, sur le métabolisme d'autres espèces d'algues. . Proc. Internat. Assoc. Theor. Appl. Limnol., 10:259-264.
- Lewin, R. A. 1956. Extracellular polysaccharides of green algae. Can. Jour. Microbiol., 2:665-672.
- Lewin, R. A. 1957. Excretion of glycolic acid by Chlamydomonas. Bull. Japan. Soc. Phycol., 5:74-75.
- Lillick, L. C. 1937. Seasonal studies of the phytoplankton off Woods Hole, Massachusetts. Biol. Bull. Woods Hole, 73:488-503.
- Lillick, L. C. 1940. Phytoplankton and planktonic protozoa of the off-shore waters of the Gulf of Maine. II. Qualitative composition of the planktonic flora. Trans. Am. Phil. Soc., 31:193-237.
- Ljunggren, H., and Fahraeus, G. 1959. Effect of Rhizobium polysaccharide in the formation of polygalacturonase in lucerne and in clover. Nature, 184:1578-1579.
- Lockwood, J. L. 1960. Lysis of mycelium of plant pathogenic fungi by natural soil. Phytopath., 50:787-789.
- Loehwing, W. K. 1937. Root interactions in plants. Bot. Rev., 3:195-239.
- Loomis, W. F., and Lenhoff, H. M. 1956. Growth and sexual differentiation of Hydra in mass culture. Jour. Exp. Zool., 132:555-574.
- Loosanoff, V. L., Hanks, J. E., and Ganaros, A. E. 1957. Control of certain forms of zooplanktons in mass algal cultures. Science, 125:1092-1093.
- Louw, P. G. J. 1950. The active constituent of the poisonous algae, Microcystis toxica Stephens, (with a note on experimental cases of algae poisoning in small animals, by Smit, J. D.). So. African Indust. Chem., 4:62-66.

- Lucas, C. E. 1936. On certain interrelationships between phytoplankton and zooplankton under experimental conditions. *Jour. Cons. Int. Explor. Mer.*, 11:343-362.
- Lucas, C. E. 1938. Some aspects of integration in plankton communities. *Jour. Cons. Int. Explor. Mer.*, 13:309-322.
- Lucas, C. E. 1947. The ecological effect of external metabolites. *Biol. Rev.*, 22:270-295.
- Lucas, C. E. 1949. External metabolites and ecological adaptation. *Symp. Soc. Exp. Biol.* III. Selective toxicity and antibiotics. Academic Press, Inc. New York. Pp. 336-356.
- Lucas, C. E. 1961. Significance of external metabolites in ecology. IN: *Symposium of the Society for Experimental Biology*. XV. Mechanisms in Biological Competition. Academic Press, Inc. New York. Pp. 190-206.
- Lund, J. W. G. 1949. Studies on Asterionella. I. The origin and nature of the cells producing seasonal maxima. *Jour. Ecol.*, 37:389-419.
- Lund, J. W. G. 1950. Studies on Asterionella formosa. II. Nutrient depletion and the spring maximum. *Jour. Ecol.*, 38:1-35.
- Lund, J. W. G. 1954. The seasonal cycle of the plankton diatom Melosira italica (Ehr.) Kütz. subsp. subartica O. Müll. *Jour. Ecol.*, 42:151-179.
- Lund, J. W. G. 1955. Further observations on the seasonal cycle of Melosira italica (Ehr.) Kütz. subsp. subartica O. Müll. *Jour. Ecol.*, 43:90-102.
- Lund, J. W. G. 1959. Buoyancy in relation to the ecology of the freshwater phytoplankton. *Brit. Phycol. Bull.*, 7:1-17.
- Lynn, W. G., and Edelman, A. 1936. Crowding and metamorphosis in the tadpole. *Ecology*, 17:104-109.
- Mackenthun, K. M., Herman, E. F., and Bartsch, A. F. 1948. A heavy mortality of fishes resulting from the decomposition of algae in the Yahara River, Wisconsin. *Trans. Amer. Fish. Soc.*, 75:176-180.
- MacKinnon, A. F. 1950. Treatment of algal poisoning. *Can. Jour. Comp. Med. and Vet. Sci.*, 14:208.

- Marshall, K. C., and Alexander, M. 1961. Competition between soil bacteria and Fusarium. Plant and Soil, 12:143-153.
- Mason, M. F., and Wheeler, R. E. 1942. Observations upon the toxicity of blue-green algae. Fed. Proc. Amer. Soc. Exp. Biol., 1:124.
- Massey, A. B. 1925. Antagonism of walnuts (Juglans nigra L. and Juglans cinerea L.) in certain plant associations. Phytopath., 15:773-784.
- Maizel, J. V., Burkhardt, H. J., and Mitchell, H. K. 1964. Avenacin, an antimicrobial substance from Avena sativa. Biochem., 3:424-431.
- Mautner, H. G., Gardner, G. M., and Pratt, R. 1953. Antibiotic activity of seaweed extracts. II. Rhodomela larix. Jour. Amer. Pharm. Assoc. (Scientific ed.), 42: 294.
- McCombie, A. M. 1953. Factors influencing the growth of phytoplankton. Jour. Fish. Res. Bd. Canada, 10:253-282.
- McLachlan, J. 1957. Some aspects of the autecology of Aphanizomenon flos-aquae Born. et Flah, studied under cultural conditions. Dissertation. Oregon State University.
- McLachlan, J., and Gorham, P. R. 1961. Growth of Microcystis aeruginosa Kütz. in a precipitate-free medium buffered with TRIS. Can. Jour. Microbiol., 7:869-882.
- McLachlan, J., and Gorham, P. R. 1962. Effects of pH and nitrogen on growth of Microcystis aeruginosa Kütz. Can. Jour. Microbiol., 8:1-11.
- McLachlan, J., Hammer, U. T., and Gorham, P. R. 1963. Observations on the growth and colony habits of ten strains of Aphanizomenon flos-aquae. Phycologia, 2: 157-168.
- McLeod, J. A., and Bodnar, G. S. 1952. A case of suspected algal poisoning in Manitoba. Can. Jour. Pub. Health, 43: 347-350.
- Medcaf, J. C. 1960. Shellfish poisoning - another North American ghost. Can. Med. Assoc. Jour., 82:87-90.

- Medcaf, J. C., Leim, A. H., Needler, A. B., Needler, A. W. H., Gibbard, J., and Naubert, J. 1947. Paralytic shellfish poisoning on the Canadian Atlantic coast. Bull. Fish. Res. Bd. Canada, No. 75. Pp. 32.
- Mergen, F. 1959. A toxic principle in the leaves of *ailanthus*. Bot. Gaz., 121:32-36.
- Metz, C. B. 1948. The nature and mode of action of the mating type substances. Amer. Nat., 82:85.
- Moore, B. G., and Tischer, R. G. 1964. Extracellular polysaccharides of algae: effects on life-support systems. Science, 145:586-587.
- Mortimer, C. H. 1941. The exchange of dissolved substances between mud and water in lakes. Jour. Ecol., 29:280-329.
- Muir, R. 1941. Textbook of Pathology. Edward Arnold & Co. London. Pp. vi + 991.
- Muller, C. H. 1953. The association of desert annuals with shrubs. Amer. Jour. Bot., 40:53-60.
- Muller, C. H., Muller, W. H., and Haines, B. L. 1964. Volatile growth inhibitors produced by aromatic shrubs. Science, 143:471-473.
- Muller, W. H., and Muller, C. H. 1956. Association patterns involving desert plants that contain toxic products. Amer. Jour. Bot., 43:354-361.
- Myers, H. E., and Anderson, K. L. 1942. Bromegrass toxicity vs. nitrogen starvation. Jour. Amer. Soc. Agron., 34:770-773.
- Myers, J. 1951. Physiology of the algae. Ann. Rev. Microbiol., 5:157-180.
- Nall, R. W. 1962. Toxic algae. IN: Ecology of phytoplankton blooms. University of Louisville. Pp. 31-54.
- Naylor, A. W., and Gerner, G. 1940. Fluorescent lamps as a source of light for growing plants. Bot. Gaz., 101: 715-716.
- Needler, A. B. 1949. Paralytic shellfish poisoning and Gonyaulax tamarensis. Jour. Fish. Res. Bd. Canada, 7:494-504.

- Nelson, N. P. B. 1903-1904. Observations upon some algae which cause "water blooms." Minn. Bot. Stud. 3, Bot. Serv., VI:51-56.
- Newcombe, C. L. 1940. Studies on the phosphorus content of the estuarine waters of Chesapeake Bay. Proc. Amer. Phil. Soc., 83:621-630.
- Newcombe, C. L., and Brust, H. F. 1940. Variations in the phosphorus content of estuarine waters of Chesapeake Bay near Solomons Island, Maryland. Jour. Mar. Res., 3:76-88.
- Newcombe, C. L., and Long, A. G. 1939. The distribution of phosphates in the Chesapeake Bay. Proc. Amer. Phil. Soc., 81:393-420.
- Nickell, L. G., and Burkholder, P. R. 1947. Inhibition of Azotobacter by soil actinomycetes. Jour. Amer. Soc. Agron., 39:771-779.
- Nielsen, K. F., Cuddy, T. F., and Woods, W. B. 1960. The influence of the extract of some crops and soil residues on germination and growth. Can. Jour. Plant Sci., 40: 188-197.
- Nightingale, H. W. 1936. Red water organisms--their occurrence and influence upon marine aquatic animals with special reference to shellfish in waters of the Pacific coast. Argus Press. Seattle.
- Norman, A. G. 1955. The effects of polymyxin on plant roots. Arch. Biochem. Biophys., 58:461.
- Norstadt, F., and McCalla, T. M. 1963. Phytotoxic substance from a species of penicillium. Science, 140:410-411.
- Nutman, P. S. 1949. Nuclear and cytoplasmic inheritance of resistance to infection by nodule bacteria in red clover. Heredity, 3:263-293.
- Nutman, P. S. 1954. Symbiotic effectiveness in nodulated red clover. Heredity, 8:35-60.
- O'Colla, P. S. 1962. Mucilages. IN: Physiology and Biochemistry of Algae. Lewin, R. A., Ed. Academic Press, Inc. New York. Pp. 337-356.

- O'Donoghue, J. G., and Wilton, G. S. 1951. Algal poisoning in Alberta. *Can. Jour. Comp. Med.*, 15:193-198.
- Olson, T. A. 1949. History of toxic plankton and associated phenomena. *Sew. Works. Eng.*, 20:71.
- Olson, T. A. 1951. Toxic Plankton. Paper presented to the Inservice Training Course in Water Works Problems, Ann Arbor, Mich. Pp. 86-95.
- Olson, T. A. 1960. Water poisoning--a study of poisonous algal blooms in Minnesota. *Amer. Jour. Pub. Health*, 50:883-884.
- Orr, Howard. 1954. Quantitative studies of protozoa populations from two areas of Pymatuning Lake, Penn. *Ecology*, 35:332-334.
- Otterstrom, C. V., and Steenmann-Nielson, E. 1940. Two cases of extensive mortality in fishes caused by the flagellate Prymnesium parvum Carter. *Rep. Danish Biol. Sta.*, 44:6-24.
- Papadakis, J. 1963. Plant population stress and antibiotics in the rhizosphere. *Soil Science*, 96:257-260.
- Park, D. 1957. Behavior of soil fungi in the presence of bacterial antagonists. *Trans. Brit. Mycol. Soc.*, 40:283-291.
- Parke, M. 1949. Studies on marine flagellates. *Jour. Mar. Biol. Assoc. U. K.*, 28:255-286.
- Parnos, I., and Spiegelstein, M. 1963. Effect of illumination of Prymnesium parvum Carter, in an axenic culture and in fish ponds. *Bamidgeh*, 15:50-59. *Biol. Abstr.*, 45: No. 54352, 1964.
- Patrick, R. 1948. Factors effecting the distribution of diatoms. *Bot. Rev.*, 14:473-524.
- Patrick, Z. A. 1955. The peach replant problem in Ontario. II. Toxic substances from microbial decomposition products of peach root residues. *Can. Jour. Bot.*, 33:461-486.
- Payen, J. 1938. Recherches biochimiques sur quelques Cyanophycées. *Rev. Algol.*, 11:1-99.

- Pearsall, W. H. 1932. Phytoplankton in the English lakes. II. The composition of the phytoplankton in relation to dissolved substances. Jour. Ecol., 20:241-262.
- Pennak, R. W. 1946. The dynamics of freshwater plankton populations. Ecol. Monogr., 16:339-356.
- Pennak, R. W. 1949. An unusual algal nuisance in a Colorado mountain lake. Ecology, 30:245-247.
- Pennington, W. 1942. Experiments on the utilization of nitrogen in fresh water. Jour. Ecol., 3:326-340.
- Peralta, F. de, and Estioko, R. P. 1924. A tentative study of the effect of root excretion of common paddy weeds upon crop production of lowland rice. Philippine Agr., 11:205-216.
- Perry, G. S. 1932. Some tree antagonisms. Proc. Pa. Acad. Sci., 6:136-141.
- Petersen, W. H., Fred, E. B., and Domogalla, B. P. 1926. The occurrence of amino acids and other organic nitrogen compounds in lake water. Jour. Biol. Chem., 23:287-295.
- Phinney, H. K. 1952. Some relationships of phytoplankton to pollution. Scientific Monthly, 74:17-20.
- Phinney, H. K., and Peek, C. A. 1960. Klamath Lake, an instance of natural enrichment. IN: Algae and Metropolitan Wastes. Rob't. A. Taft San. Eng. Cent. Tech. Rep't. W61-3, 22-27.
- Pickering, S. U. 1903. The effect of one plant on another. Jour. Roy. Agr. Soc. England, 64:365-376.
- Pickering, S. U. 1917. The effect of one plant on another. Ann. Bot., 31:181-187.
- Pickering, S. U. 1919. Action of one crop on another. Jour. Roy. Agr. Soc. England, 43:372-380.
- Platt, E. L. 1915. The population of the "blanket-algae" of fresh-water pools. Am. Naturalist, 49:752-762.
- Pollock, B. M., Goodwin, R. H., and Greene, S. 1954. Studies on roots. II. Effects of coumarin, scopoletin, and other substances on growth. Amer. Jour. Bot., 41: 521-529.

- Pomeroy, L. R., Haskin, H. H., and Ragotskie, R. A. 1956. Observations on dinoflagellate blooms. *Limnol. and Oceanogr.*, 1:54-60.
- Potter, L. F., and Baker, G. E. 1960. The microbiology of Flathead and Rogers Lakes, Montana. I. Preliminary survey of microbial populations. *Ecology*, 37:351-355.
- Potter, L. F., and Baker, G. E. 1961. The microbiology of Flathead and Rogers Lakes, Montana. II. Vertical distribution of the microbial populations and chemical analyses of their environment. *Ecology*, 42:338-348.
- Prakash, A. 1963. Sources of paralytic shellfish toxin in the Bay of Fundy. *Jour. Fish. Res. Bd. Canada*, 20:983-995.
- Prakash, A., and Medcalf, J. C. 1961. Hydrographic and meteorological factors affecting shellfish toxicity at Head Harbour, New Brunswick. *Jour. Fish. Res. Bd. Canada*, 19:101-112.
- Pratt, D. M. 1950. Experimental study of the phosphorus cycle in fertilized sea water. *Jour. Mar. Res.*, 9:29-84.
- Pratt, R. 1942. Studies on Chlorella vulgaris. V. Some properties of the growth-inhibitor formed by Chlorella cells. *Amer. Jour. Bot.*, 29:142-148.
- Pratt, R. 1943. Studies on Chlorella vulgaris. VI. Retardation of photosynthesis by a growth-inhibiting substance from Chlorella vulgaris. *Amer. Jour. Bot.*, 30:32-33.
- Pratt, R., and Fong, J. 1940. Studies on Chlorella vulgaris. II. Further evidence that Chlorella cells form a growth-inhibiting substance. *Amer. Jour. Bot.*, 27:431-436.
- Pratt, R., and Fong, J. 1944. Chlorellin, an antibacterial substance from Chlorella. *Science*, 99:351-352.
- Pratt, R., Mautner, H., and Gardner, G. M. 1951. Report on antibiotic activity of seaweed extracts. *Jour. Amer. Pharm. Assoc. (Scientific Ed.)*, 40:575.
- Pratt, R., Oneto, J. F., and Pratt, J. 1945. Studies on Chlorella vulgaris. X. Influence of the age of the culture on the accumulation of chlorellin. *Amer. Jour. Bot.*, 32:405-408.

- Prescott, G. W. 1931. A report on a study of some biological problems in certain Iowa lakes. Rep't. to Iowa State Fish and Game Comm.
- Prescott, G. W. 1933. Some effects on the blue-green algae, Aphanizomenon flos-aquae on lake fish. The Collecting Net, 8:77-80.
- Prescott, G. W. 1938. Objectionable algae and their control in lakes and reservoirs. Louisiana Municipal Review 1 (unpaged reprint).
- Prescott, G. W. 1939. Some relationships of phytoplankton to limnology and aquatic biology. IN: Problems in Lake Biology. Amer. Assoc. Adv. Sci. Pub. No. 10:65-78.
- Prescott, G. W. 1948. Objectionable algae with reference to the killing of fish and other animals. Hydrobiologia, 1:1-13.
- Prescott, G. W. 1960. Biological disturbances resulting from algal populations in standing water. IN: The Ecology of Algae. Univ. of Pittsburgh Spec. Publ. No. 2:22-37.
- Prescott, G. W. 1962. Freshwater Algae of the Western Great Lakes Area. Wm. C. Brown Company Publishers. Dubuque, Iowa. Pp. xiii + 977.
- Pringsheim, E. G. 1949a. Pure Cultures of Algae. Cambridge University Press. Cambridge. Pp. 119.
- Pringsheim, E. G. 1949b. The relationship between bacteria and myxophyceae. Bacteriol. Rev., 13:47-98.
- Pringsheim, E. G. 1951. Methods for the cultivation of algae. IN: Manual of Phycology. Smith, G. M., Ed. Chronica Botanica. Pp. 347-358.
- Proctor, V. W. 1957a. Some controlling factors in the distribution of Haematococcus pluvialis. Ecology, 28:457-462.
- Proctor, V. W. 1957b. Studies of algal antibiosis using Haematococcus and Chlamydomonas. Limnol. and Oceanog., 2:125-139.
- Proesbsting, E. L. 1950. A case history of a "peach replant" situation. Am. Soc. for Hort. Sci., 56:46-48.

- Proesbsting, E. L., and Gilmore, A. E. 1940. The relation of peach root toxicity to the re-establishing of peach orchards. *Proc. Am. Soc. Hort. Sci.*, 38:21-26.
- Provasoli, L. 1958. Nutrition and ecology of protozoa and algae. *Ann. Rev. Microbiol.*, 12:279-398.
- Provasoli, L. 1961. Micronutrients and heterotrophy as possible factors in bloom produced in natural waters. IN: *Algae and Metropolitan Wastes*. Rob't. A. Taft San. Eng. Cent. Rep't. W61-3:48-50.
- Provasoli, L., McLaughlin, J. J. A., and Droop, M. R. 1957. The development of artificial media for marine algae. *Arch. Mikrobiol.*, 25:392-428.
- Prowse, G. A., and Talling, J. F. 1958. The seasonal growth and succession of plankton in the White Nile. *Limnol. and Oceanog.*, 3:222-238.
- Quinn, A. H. 1943. Sheep poisoned by algae. *Jour. Amer. Vet. Med. Assoc.*, 102:229.
- Ragotzkie, R. A., and Pomeroy, L. R. 1957. Life history of a dinoflagellate bloom. *Limnol. and Oceanog.*, 2: 62-69.
- Rangaswami, G., and Vasantharajan, V. N. 1962. Studies of the rhizosphere microflora of citrus trees. *Can. Jour. Microbiol.*, 8:473-489.
- Rangaswami, G., and Vidyasekeran, P. 1963. Antibiotic production by Streptomyces sp. in corn rhizosphere. *Phytopath.*, 53:995-997.
- Rao, C. B. 1953. Distribution and periodicity of algae in some small ponds. *Jour. Ecol.*, 40:62-71.
- Raper, J. R. 1952. Chemical regulation of sexual processes in the thallophytes. *Bot. Rev.*, 18:447-545.
- Raper, J. R. 1957. Hormones and sexuality in lower plants. IN: *Symposia Soc. Exptl. Bio.*, XI:143-165.
- Ray, S. M., and Wilson, W. B. 1957. Effects of unialgal and bacterial-free cultures of Gymnodinium brevis on fish. *Fishery Bulletin No. 123 of the Fish and Wildlife Service*, 57:469-496.

- Redfield, A. C., Smith, H. P., and Ketchum, B. H. 1937. The cycle of organic phosphorus in the Gulf of Maine. Biol. Bull. Woods Hole, 73:421-443.
- Reich, K., and Aschner, M. 1947. Mass development and control of the phytoflagellate Prymnesium parvum in fish ponds in Palestine. Palestine Jour. Bot., 4:14.
- Reish, D. J. 1963. Mass mortality of marine organisms attributed to the "red tide" in southern California. Calif. Fish. and Game, 49:265-270.
- Reisner, G. S., and Thompson, J. F. 1956. Manganese deficiency in Chlorella under heterotrophic carbon nutrition. Nature, 178:1473-1474.
- Rice, T. R. 1949. The effects of nutrients and metabolites on populations of planktonic algae. Dissertation. Harvard University.
- Rice, T. R. 1953. Phosphorus exchange in marine phytoplankton. Fish. Bull., U. S. Fish. and Wildlife Serv., 80: 77-89.
- Rice, T. R. 1954. Biotic influences affecting population growth of planktonic algae. Fish. Bull., U. S. Fish. and Wildlife Serv., 54:227-245.
- Richards, C. M. 1958. The inhibition of growth in crowded Rana pipiens tadpoles. Physiol. Zool., 31:138-151.
- Riley, G. A. 1943. Physiological aspects of spring diatom flowerings. Bull. Bingham Oceanog. Coll., 8:1-53.
- Riley, G. A. 1946. Factors controlling phytoplankton populations on Georges Bank. Jour. Mar. Res., 5:54-73.
- Riley, G. A. 1956. Oceanography of Long Island Sound, 1952-1954. IX. Production and utilization of organic matter. Bull. Bingham Oceanog. Coll., 15:324-344.
- Riley, G. A., and Bumpus, D. F. 1946. Phytoplankton-zooplankton relationships on Georges Bank. Jour. Mar. Res., 6:33-47.
- Robinson, F. A. 1953. Antibiotics. Pitman Publishing Corporation. New York. Pp. vii + 132.

- Rodhe, W. 1948. Environmental requirements of fresh-water plankton algae. Experimental studies in the ecology of phytoplankton. *Symbolae Botanica Upsalienses*, 10:1-149.
- Rose, E. T. 1934. Notes on the life history of Aphanizomenon flos-aquae. *Univ. Iowa Stud. Nat. Hist.*, 16:129-141.
- Rose, E. T. 1953. Toxic algae in Iowa lakes. *Proc. Iowa Acad. Sci.*, 60:738-745.
- Rose, S. M. 1958. Failure of survival of slowly growing members of a population. *Science*, 129:1026.
- Rose, S. M. 1959. Population control in guppies. *Amer. Mid. Nat.*, 62:474-481.
- Rose, S. M. 1960. A feedback mechanism of growth control in tadpoles. *Ecology*, 41(1):188-199.
- Rose, S. M., and Rose, F. C. 1961. Growth-controlling exudates of tadpoles. IN: *Symposia of the Society for Experimental Biology. XV. Mechanisms of Biological Competition*. Academic Press, Inc. New York. Pp. 207-218.
- Round, F. E. 1961. Studies on bottom-living algae in some lakes of the English Channel district. V. The seasonal cycles of the Cyanophyceae. *Jour. Ecol.*, 49(1):31-38.
- Rovira, A. D. 1956. Plant root excretions in relation to the rhizosphere effect. *Plant and Soil*, 7:178.
- Rugh, R. 1934. The space factor in growth rate of tadpoles. *Ecology*, 15:407-411.
- Ryther, J. H. 1954. The ecology of phytoplankton blooms in Moriches Bay and Great South Bay, Long Island, New York. *Woods Hole Oceanogr. Inst. Biol. Bull.*, 106:198-209.
- Ryther, J. H. 1955. Inhibitory effects of phytoplankton upon the feeding of Daphnia magna with reference to growth, reproduction and survival. *Ecology*, 35:522-533.
- Ryther, J. H. 1959. Potential productivity of the sea. *Science*, 130:602-608.
- Ryther, J. H. 1960. Organic production by plankton algae and its environmental control. IN: *The Ecology of Algae*. Univ. of Pittsburgh Spec. Publ. No. 2:72-83.

- Ryther, J. H., Yentsch, C. H., Hulburt, E. M., and Vaccaro, R. F. 1958. The dynamics of a diatom bloom. Biol. Bull., 115:257-268.
- San Antonia, J. P. 1952. The role of coumarin in the growth of roots of Melilotus alba. Bot. Gaz., 114:79-95.
- Saunders, G. W. 1957. Interrelations of dissolved organic matter and phytoplankton. Bot. Rev., 23:389-409.
- Schneiderman, F. J. 1927. The black walnut (Juglans nigra L.) as a cause of the death of apple trees. Phytopath., 17:529-540.
- Schreiner, O., and Reed, H. S. 1908. The toxic action of certain organic plant constituents. Bot. Gaz., 45:73-102.
- Schreiner, O., and Shorey, E. C. 1908a. The isolation of dihydroxystearic acid from soils. Jour. Amer. Chem. Soc., 30:1599-1607.
- Schreiner, O., and Shorey, E. C. 1908b. The isolation of picoline carboxylic acid from soils and its relation to soil fertility. Jour. Amer. Chem. Soc., 30:1295-1307.
- Schreiner, O., and Sullivan, M. X. 1909. Soil fatigue caused by organic compounds. Jour. Biol. Chem., 6:39-50.
- Schwimmer, M., and Schwimmer, D. 1955. The Role of Algae and Plankton in Medicine. Grune and Stratton, Inc. New York and London.
- Scott, R. M. 1952. Algal toxins. Public Works, 54-55: 65-66.
- Scutt, J. E. 1964. Autoinhibitor production by Chlorella vulgaris. Amer. Jour. Bot., 51:581-584.
- Sehgal, J. M. 1961. Antimicrobial substances from flowering plants. Antibiot. Bull., 4:3-29.
- Senior, V. E. 1960. Algal poisoning in Saskatchewan. Can. Jour. Comp. Med., 24:26-31.
- Shelubsky, M. 1951. Observations on the properties of a toxin produced by Microcystis. Verh. Internat. Verein. Limnol., 11:362-366.

- Shilo, M. 1964. Review of toxigenic algae. Verh. Internat. Verein. Limnol., 15:782-795.
- Sieburth, J. M. 1960. Acrylic acid, an "antibiotic" principle in Phaeocystis blooms in Antarctic waters. Science, 132:676-679.
- Simonds, D. H. 1954. Analogues of diaminopimelic acid as inhibitors of bacterial growth. Biochem. Jour., 58:520-523.
- Simpson, B., and Gorham, P. R. 1958. Source of the fast-death factor produced by unialgal Microcystis aeruginosa NRC-1. Phycol. Soc. Amer. News Bull., 11:59-60.
- Siu, R. G. H., and Reese, E. T. 1953. Decomposition of cellulose by microorganisms. Bot. Rev., 19:377-416.
- Singh, R. N. 1955. Limnological relations of Indian inland water with special reference to waterblooms. Verh. Internat. Verein. Limnol., 12:831-836.
- Smayda, T. J. 1957. Phytoplankton studies in lower Narragansett Bay. Limnol. and Oceanogr., 2:342-358.
- Smith, J. D. 1950. Experimental cases of algae poisoning in small animals. So. African Industrial Chemist, 4:66.
- Sokoloff, Boris. 1949. The Miracle Drugs. Ziff-Davis Publishing Company. Chicago. Pp. 308.
- Somers, I. I., and Shive, J. W. 1942. The iron-manganese relation in plant metabolism. Plant Physiol., 17:582-602.
- Sommer, H. 1939. Marine plankton and paralytic shellfish poisoning. Proc. 6th Pacific Science Congress, 5:415-416.
- Sommer, H., and Meyer, K. F. 1941. Mussel poisoning--summary. Weekly Bull. Calif. State Dept. Publ. Health, 20:53-55.
- Sommer, H., Monier, R. P., Riegel, B., Stanger, D. W., Moid, J. D., Wikholm, D. M., and Keralis, E. S. 1948. Paralytic shellfish poisoning. I. Occurrence and concentration by ion exchange. Jour. Amer. Chem. Soc., 70:1015-1108.

- Sommer, H., Reigel, B., Stanger, D. W., Moid, J. D., Wikholm, D. M., and McCaughey, M. B. 1948. Paralytic shellfish poisoning. II. Purification by chromatography. Jour. Amer. Chem. Soc., 70:1019-1021.
- Sonneborn, T. M. 1939. Paramecium aurelia: mating types and groups; lethal interactions, determination and inheritance. Amer. Natural., 73:390-413.
- Sonneborn, T. M. 1942. Sex hormones in unicellular organisms. Cold Springs Harb. Symp. Quant. Biol., 10:11.
- Sonneborn, T. M. 1943. Gene and cytoplasm. Proc. Nat. Acad. Sci., 29:329-345.
- Spencer, C. P. 1954. Studies on the culture of a marine diatom. Jour. Mar. Biol. Assoc. U. K., 33:265-290.
- Spencer, R. R. 1930. Unusually mild recurring epidemic simulating food infection. Public Health Reports, 45:2867-2877.
- Stalker, M. 1886. Stalker on the Waterville cattle disease. 4th Biennial Rep., Board of Regents, Univ. Minn. Suppl. 1, Dept. of Agric., 105-108.
- Starr, Richard C. 1964. The culture collection of algae at Indiana University. Amer. Jour. Bot., 51:1002-1044.
- Starr, T. J. 1958. Notes on a toxin from Gymnodinium brevis. Texas Rep. Biol. and Med., 16:500-507.
- Steen, E. B. Dictionary of Biology. Barnes and Noble. New York. To be published.
- Steenmann-Nielson, E. 1955a. An effect of antibiotics produced by plankton algae. Nature, Lond., 176:553.
- Steenmann-Nielson, E. 1955b. The production of antibiotics by plankton and its effect upon bacterial activities in the sea. Deep-Sea Research, 3 Suppl.:281-286.
- Stevenson, I. L. 1956a. Antibiotic activity of actinomycetes in soil and their controlling effects on root of wheat. Jour. Gen. Microbiol., 14:440-448.
- Stevenson, I. L. 1956b. Antibiotic activity of actinomycetes in soil as demonstrated by direct observation techniques. Jour. Gen. Microbiol., 15:372-380.

- Stevenson, I. L. 1962. The effect of decomposition of various crop plants on the metabolic activity of soil microflora. *Can. Jour. Microbiol.*, 8:501-509.
- Stewart, A. G., Barnum, D. A., and Henderson, J. A. 1950. Algal poisoning in Ontario, Canada. *Can. Jour. Comp. Med.*, 14:197-202.
- Stewart, W. D. P. 1963. Liberation of extracellular nitrogen (amino acids) by two nitrogen-fixing blue-green algae (Nostoc entophytum, Calothrix scopulorum). *Nature*, 200:1020-1021.
- Steyn, D. G. 1943. Poisoning of animals by algae in dams and pans. *Farming in South Africa*, 18:489-492.
- Steyn, D. G. 1945a. Poisoning of animals and human beings by algae. *South Africa Jour. Sci.*, 41:243-244.
- Steyn, D. G. 1945b. Poisoning of animals by algae (scum or waterbloom) in dams and pans. Union of South Africa, Dept. of Agri. and Forestry, Gov. Ptg. Off., Pretoria, South Africa.
- Strong, M. C. 1944. Walnut wilt of tomato. *Mich. Quart. Bull.*, 26:194-195.
- Strzelczyk, E. 1961. Studies on the interaction of plants and free-living nitrogen-fixing microorganisms. II. Development of antagonists of Azotobacter in the rhizosphere of plants at different stages of growth in two soils. *Can. Jour. Microbiol.*, 7:507-513.
- Sverdrup, H. U. 1952. On conditions of the vernal blooming of phytoplankton. *Jour. Cons. Int. Explor. Mer.*, 18:287-295.
- Sweeney, B. M. 1951. Culture of the dinoflagellate Gymnodinium with soil extract. *Amer. Jour. Bot.*, 38: 669-677.
- Talling, J. F. 1957. The growth of two plankton diatoms in mixed culture. *Physiol. Plant.*, 10:215-223.
- Thatcher, R. W. 1923. The effect of one crop on another. *Jour. Amer. Soc. Agron.*, 15:331-338.
- Thimann, K. V. 1955. *The Life of Bacteria*. Macmillan. New York. Pp. 755.

- Thimann, K. V., and Barker, H. A. 1934. Studies on the excystment of Colpoda cucullus. Jour. Exp. Zool., 69:37.
- Thimann, K. V., and Bonner, W. D., Jr. 1949. Inhibition of plant growth by protoanemonin and coumarin, and its prevention by BAL. Proc. Nat. Acad. Sci., 35:272-276.
- Thimann, K. V., and Haagen-Smit, A. J. 1937. Effects of salts on emergence from the cyst in protozoa. Nature, 140:645.
- Thompson, W. K. 1958. Toxic algae. V. Study of toxic bacterial contaminants. DRKL Rep. No. 63. Defence Research Board of Canada. Pp. 6.
- Thompson, W. K., Laing, A. C., and Grant, G. A. 1957. Toxic algae. IV. Isolation of toxic bacterial contaminants. DRKL Rep. 51. Defence Research Board of Canada. Pp. 7.
- Thornton, H. G. 1929a. The influence of the number of nodule bacteria applied to the seed upon nodule formation in legumes. Jour. Agr. Sci., 19:373-381.
- Thornton, H. G. 1929b. The role of the young lucerne plant in determining the infection of the root by the nodule-forming bacteria. Proc. Roy. Soc. London B., 104:481-492.
- Tiffany, L. H. 1951. Ecology of freshwater algae. IN: Manual of Phycology. Smith, G. M., Ed. Chronica Botanica Co. Waltham, Massachusetts. Pp. 293-311.
- Tisdale, E. S. 1931a. Epidemic of intestinal disorders in Charleston, W. Va., occurring simultaneously with unprecedented water supply conditions. Amer. Jour. Pub. Health, 21:198-200.
- Tisdale, E. S. 1931b. The 1930-1931 drought and its effect upon the public water supply. Amer. Jour. Public Health, 21:1203-1215.
- Tolbert, N. E., and Zill, L. P. 1956. Excretion of glycolic acid by algae during photosynthesis. Jour. Biol. Chem., 222:895-906.
- Tolbert, N. E., and Zill, L. P. 1957. Excretion of glycolic acid by Chlorella during photosynthesis. IN: Research in Photosynthesis. Gaffron, H., Ed. Interscience. New York. Pp. 228-231.

- Tucker, A. 1957a. The relation of phytoplankton periodicity to the nature of the physico-chemical environment with special reference to phosphorus. I. Morphometrical, physical and chemical conditions. *Amer. Mid. Nat.*, 57: 300-333.
- Tucker, A. 1957b. The relation of phytoplankton periodicity to the nature of the physico-chemical environment with special reference to phosphorus. II. Seasonal and vertical distribution of the phytoplankton in relation to environment. *Amer. Mid. Nat.*, 57:334-370.
- Tukey, H. B. 1954. *Plant regulators in Agriculture*. John Wiley and Sons, Inc. New York. Pp. x + 269.
- Vaccaro, R. F., and Ryther, J. H. 1960. Marine phytoplankton and the distribution of nitrite in the sea. *Jour. Cons. Int. Explor. Mer.*, 25:260-271.
- van Niel, C. B. 1955. Classification and taxonomy of the bacteria and blue-green algae. IN: *A Century of Progress in the Natural Sciences, 1853-1953*. California Academy of Sciences. San Francisco.
- Van Overbeek, J., Blondeau, R., and Horne, U. 1951. Trans-cinnamic acid as antiauxin. *Amer. Jour. Bot.*, 38:589-595.
- Veldee, M. V. 1931. Epidemiological study of suspected water-borne gastroenteritis. *Amer. Jour. Public Health*, 21:1227-1235.
- Venkataraman, G. S. 1962. The effect of the extracellular substances produced in culture by Nostoc sp. and Chlorella vulgaris on their growth. *Indian Jour. Microbiol.*, 2:121-126.
- Verduin, J. 1951. Comparison of spring diatom crops of western Lake Erie in 1949 and 1950. *Ecology*, 32:662-668.
- Verduin, J. 1952. Photosynthesis and growth rates of two diatom communities in western Lake Erie. *Ecology*, 33: 163-169.
- Verduin, J. 1956a. Energy fixation and utilization by natural communities in western Lake Erie. *Ecology*, 37: 40-50.

- Verduin, J. 1956b. Primary production in lakes. *Limnol. and Oceanogr.*, 1:85-91.
- Vinberg, G. G. 1955. Toksicheskie fitoplankton. *Uspekhi Sovr. Biologii*, 38:216-226. (Toxic Phytoplankton. N.R.C. Tech. Transl. TT-549.)
- Wade, W. E. 1949. Some notes on the algal ecology of a Michigan Lake. *Hydrobiologia*, 2:109-117.
- Wagtendonk, W. J. v , and Zill, L. P. 1947. Inactivation of paramecin ("killer" substance of Paramecium aurelia 51, variety 4) at different hydrogen-ion concentrations. *Jour. Biol. Chem.*, 171:595.
- Waks, C. 1936. The influence of extracts of Robinia pseudoacacia on the growth of barley. *Pub. Fac. Sci. Univ. Charles (Praha)*, 150:84-85.
- Waksman, S. A. 1947. Microbial Antagonisms and Antibiotic Substances. The Commonwealth Fund. New York. Pp. ix + 415.
- Waksman, S. A. 1961. The role of antibiotics in nature. *Perspectives in Biol. and Med.*, 4:271-287.
- Waksman, S. A., and Horning, E. S. 1943. Distribution of antagonistic fungi in nature and their antibiotic action. *Mycologia*, 35:47-65.
- Waldichuk, M. 1958. Shellfish toxicity and the weather in the Strait of Georges during 1957. *Fish. Res. Bd. Can. Pacific Prog. Rep't.*, No. 112:10-14.
- Watanabe, A. 1951. Production in cultural solution of some amino acids by the atmospheric nitrogen-fixing blue-green algae. *Arch. Biochem. Biophys.*, 34:50-55.
- Watanabe, A., Nishigake, S., and Konishi, C. 1951. Effect of nitrogen-fixing blue-green algae on growth of rice plants. *Nature*, 168:748-749.
- Weberg, G. S., and Stephenson, N. R. 1960. Toxicologic studies on paralytic shellfish poison. *Toxicology and Applied Pharmacology*, 2:607-615.
- Wedbury, Stanley. 1963. Paramedical Microbiology. Reinhold Publishing Corporation. New York. Pp. xv + 462.

- Welch, A. M. 1962. Preliminary survey of fungistatic properties of marine algae. *Jour. Bacteriol.*, 83:97-99.
- Welty, J. C. 1934. Experimental explorations into group behavior of fishes: a study of the influence of the group on individual behavior. *Physiol. Zool.*, 7:85-128.
- Went, F. W. 1942. The dependence of certain annual plants on shrubs in Southern California deserts. *Bull. Torrey Bot. Club.*, 69:100-114.
- Went, F. W. 1955. The ecology of desert plants. *Sci. Amer.*, 192:68-75.
- Whaley, W. G., Breland, O. P., Heimsch, C., Phelps, A., Schrank, A. R., and Wyss, O. 1964. *Principles of Biology*. Harper and Row, Publishers. New York, Evanston and London. Pp. xvii + 776.
- Wheeler, R. E., Lackey, J. D., and Schott, S. 1942. Contribution on the toxicity of algae. *Pub. Health Rept.*, 57:1695-1701.
- Whittaker, J. R., and Vallentyne, J. R. 1957. On the occurrence of free sugars in lake sediment extracts. *Limnol. and Oceanogr.*, 2:98-110.
- Williams, A. E., and Burris, R. H. 1952. Nitrogen fixation by blue-green algae and their nitrogenous composition. *Amer. Jour. Bot.*, 39:340-342.
- Williams, R. E. O., and Spicer, C. C., Editors. 1957. *Microbial Ecology*. Cambridge University Press. Cambridge, England. Pp. ix + 388.
- Wilson, P. W. 1940. *The Biochemistry of Nitrogen-Fixation*. Univ. of Wisconsin Press. Madison, Wis. Pp. 73.
- Wilson, W. B., and Ray, S. M. 1956. The occurrence of Gymnodinium brevis in the western Gulf of Mexico. *Ecology*, 37:388.
- Winter, A. G. 1961. New physiological and biological aspects in the interrelationships between higher plants. IN: *Symposia of the Society for Experimental Biology*. XV. *Mechanisms in Biological Competition*. Academic Press, Inc. New York. Pp. 229-244.

- Wolfe, R. N., and Powelson, D. M. 1951. Differences between Bacillus cereus var. mycoides and nonrhizoid mutants of Bacillus cereus. Jour. Bact. Proceedings of 51st General Meeting of the Society of American Bacteriologists. Pp. 55.
- Woodruff, L. L. 1913. The effect of excretion products of Infusoria on the same and on different species, with special reference to the protozoan sequence in infusions. Jour. Exp. Zool., 14:575-582.
- Woods, F. W. 1960. Biological antagonisms due to phytotoxic root exudates. Bot. Rev., 26:546-569.
- Work, E., and Dewey, D. L. 1953. The distribution of α - ϵ -diaminopimelic acid among various microorganism. Jour. Gen. Microbiol., 9:394.
- Wright, J. M. 1954. The production of antibiotics in the soil. I. Production of gliotoxin by Trichoderma viride. Ann. Appl. Biol., 41:280-299.
- Zehnder, A., and Gorham, P. R. 1960. Factors influencing the growth of Microcystis aeruginosa Kütz. emend Elenkin. Can. Jour. Microbiol., 5:645-660.