

ALGAE AND METROPOLITAN WASTES

Transactions of the 1960 Seminar

U. S. DEPARTMENT

OF HEALTH,

EDUCATION,

AND WELFARE

Public Health Service



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METROPOLITAN WASTES

Transactions of the Seminar on Algae and Metropolitan Wastes, held at Cincinnati, Ohio, April 27-29, 1960, under the sponsorship of the Division of Water Supply and Pollution Control and the Robert A. Taft Sanitary Engineering Center.

U. S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE

Public Health Service
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Division of Water Supply & Pollution Control

Robert A. Taft Sanitary Engineering Center Cincinnati 26, Ohio

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FOREWORD

The Public Health Service, particularly the Robert A. Taft Sanitary Engineering Center, has a responsibility to promote free exchange of information on problems in the field of environmental sanitation. This Seminar on Algae and Metropolitan Wastes was the first national meeting devoted solely to this subject. It was part of a continuing program of seminars in the water field at this Center.

The problem of algae is many faceted. Some species are a bane to men on earth, but others are potentially the staff of life of men in space. It is important, however, to consider first those problems most pressing and near at hand, such as the relationship of algae to this Nation's spreading metropolitanism and to the disposal of urban wastes.

The Seminar on Algae and Metropolitan Wastes was limited to discussion of prevention and control of objectionable blooms of algae resulting from enrichment by urban and other wastes. This is a long standing problem which, as urbanization increases, will become more severe if corrective measures are not developed and applied. We, therefore, regard this report as particularly important and are pleased to present it under the sponsorship of this Center and the Division of Water Supply and Pollution Control of the Public Health Service.

H. G. HANSON, Director Robert A. Taft Sanitary Engineering Center

MEETING NOTE

The 1960 Seminar on Algae and Metropolitan Wastes was held in Cincinnati, Ohio, April 27-29, under the sponsorship of the Division of Water Supply and Pollution Control of the Public Health Service. Outstanding scientists and engineers from government, industry, and universities were invited to participate. The seminar was attended by 139 registrants representing 27 states and the District of Columbia, and 4 foreign countries. The meeting consisted of panel discussions on (a) the problem, (b) growth characteristics of algae, (c) sources of nutrients, (d) methods of prevention and control, and (e) research needs. A tour of the Center's facilities was included, and an evening banquet at which Mr. R. G. Lynch of the Milwaukee Journal was the principal speaker. Mr. Lynch's talk is presented as the Introduction to this volume.



Photo courtesy of Kenneth M. Mackenthun, Wisconsin Committee on Water Pollution.

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INTRODUCTION

This paper by Mr. Lynch was delivered as the dinner address of the Seminar. It is presented here to introduce the Transactions because it deals with the basic theme of achievement of better communication between scientist and layman and, indeed, between scientist and scientist.

DO THEY DIG YOU, DADDY-O, OR ARE YOU WAY OUT?

R. G. LYNCH The Milwaukee Journal, Milwaukee, Wisconsin

Mr. Toastmaster, scientists, technicans and any others who may have wandered in:-

My editors sometimes complain that I am too concerned with facts. They would like a lighter touch, and I agree that it makes for better reading, but I have observed that if I write that way and the editor has to cut my copy to fit his space, it is the facts that go out and the froth that remains.

The title of my talk, Do They Dig You, Daddy-o, or Are You Way Out? is not intended to be funny. Beatniks use that lingo as a barrier to outsiders—to set themselves apart. Scientific and technical people are guilty of the same thing. It is a form of snobbishness and I think that you people cannot afford the luxury.

As a fulltime reporter on natural resources, the only one that I know of in the newspaper field, I attend more or less technical meetings, work from papers or summaries prepared by technicans and scientists, and read extensively. And I have observed a very serious lack of communication between scientific and technical people and the public.

It is particularly serious in the natural resources field, where research and studies must solve problems or develop approaches to them. The men engrossed with these things meet to talk over what they are doing, and they meet in something like a vacuum—people who already know much about the subject telling each other more about it.

When they reach decisions or prepare plans, they spring their ideas on the public and expect them to be received gratefully, and implemented. This seems to me to be a major reason why progress is so slow toward wise management of resources.

Usually some changes are involved, some support must be generated, some money must be provided. People just do not accept changes unless they are convinced of good reasons; they or their representatives just will not dig up money for things they don't understand. Except when they are fright-

ened, I should say. People of foreign countries who want American dollars are shrewd enough to learn the American language.

It is important that the people be taken along on the whole trip, and that is not being done. Dr. Carl O. Sauer, University of California geographer and chairman of the 1955 international symposium on "Man's Role in Changing the Face of the Earth", said in summation:

"We are now come in 1955 to a revised version of Aldous Huxley's 'bright new world' of the 1920's — to a faceless, mindless, countless multitude, managed from the cradle to the grave by a brilliant elite of madmen, obsessed with accelerating technological progress".

Now, I don't want to be misunderstood. There is exactness in scientific and technical terms. Also, my friend, Joe Hickey, professor of wildlife management at the University of Wisconsin, has pointed out to me that scientific papers must be written so that men of different languages may understand them.

But technical and scientific people carry their jargon outside and use it in addressing laymen, sometimes unwittingly, sometimes to be impressive. Even in their own meetings, they are sometimes less than comprehensible.

A year ago I attended a seminar on aquatic biology here in Cincinnati. The first session was devoted to radioactivity and the radiobiologists talked a language that the others obviously had difficulty in understanding.

"TLm" was one of the terms tossed around. A speaker was kind enough to say that it was about the same as LD-50, which I know is the dose lethal to 50 per cent of an experiment population, so I groped along with the speakers.

On the way out I asked a Ph.D. of my acquaintance what TLm stood for. He said, "Oh, about the same as LD-50." I said, "Yeah, I heard the man, too, but what do the letters stand for?" He laughed and said he didn't know.

Another man turned around and said, "Toxicity limit median, I think." A third man corrected him, saying, "No, it's TOLERANCE limit median."

Some very interesting things were said at that seminar and I think it is significant that the only local newspaper coverage I saw was in the form of interviews with Ernie Swift and Seth Gordon, a couple of guest speakers from the wildlife conservation field, and they were really just tails on the kite. But newspaper men could understand what they said.

Either at the seminar, or afterward by mail, I got a notice of another seminar on benthic biology out in California. I picked up my Webster's Collegiate dictionary and found benthos defined as fresh water, bottom dwelling organisms. I wanted more, and also had a hunch this was wrong, so I lugged my big Merriam Webster to the dining room table and found that benthos meant the bottom of the sea and its flora and fauna.

Next I consulted encyclopedias at the office. Americana said benthos was the fauna of ocean depths under 100 fathoms; Britannica said it was the sedentary bottom-living animal and plant life of the sea.

And I said, "Why don't people say what they mean!"

Incidentally, I replaced my 1943 Collegiate dictionary with a new one, for benthos was in the newword section. As long as I can remember, I have read with a dictionary at hand. I do not like to pass over a word that I do not understand. But when I must stop too often it destroys the continuity of my reading and I may give up.

I gave up on Papini's <u>Life of Christ</u> and Spengler's <u>Drive to the East and Toynbee's History</u>. I have seven or eight volumes of Toynbee that one of you can buy cheap. I have developed over the years a deep resentment of men like Toynbee, who display their erudition with fancy language and lapses into untranslated Greek, Latin, German and French.

I think that any man who has something of value to say does not have the right to deny it to any mind capable of understanding the thoughts or the facts, simply because the possessor of the mind lacks his own high level education.

Such men as Toynbee have forgotten that semantics also is a science and, I think, an art as well. There are others who realize this and try to communicate but fail because they are too steeped in the jargon of their disciplines.

Perhaps some of the Wisconsin men here are familiar with Prof. John Curtis's new book on Wisconsin vegetation. It is a wonderful book in its field and I feel like a flea on a dog's back when I mention it here, in this way. But it illustrates my point.

Publication notice and wrapper said that the book was written with a minimum of technical terms, for the use of (among others) farmers, conservationists and men in recreation and weed control work. Well, Curtis really had me wearing out that dictionary! I think I found 20 or 30 words in the first couple of chapters that were not in my Collegiate and some were not even in the big Merriam. Mesic and xeric were terms constantly used; neither was in the big dictionary. I called up two foresters and a curator of botany who could not define the words. Finally I got what I wanted from a university professor.

A man could as easily use "dry" and "drier" as "xeric" and "more xeric" if he thought of it.

But Curtis made a sincere effort to get through. He was on the right track when he used "podzolization" with the explanation that it meant topsoil impoverishment by leaching and translocation. That is something the scientific and technical man can do. It is all right for him to employ words which will be useful to his reader or listener, with an explanation of the meaning. The type of reader who wants information is apt to be receptive to new words.

I must say that I stuck with Curtis and was well rewarded, but I wonder how many farmers, weed control men and conservationists went beyond such expressions as "varietal endemism", "morphological subspeciation", "altitudinal retardation of phenological events" or "Gaussian amplitude curve".

How many readers do you think will use a dictionary, much less read with one at hand?

And when the offering is oral, there is no dictionary and you can lose an audience quickly. Dr. Luna Leopold of the geological survey addressed a Milwaukee gathering of conservationists not long ago and his paper was a polished masterpiece. I admired his flow of language. But as we left the hall an attorney said to me, "I should have brought along a dictionary."

The lack of communication is being recognized by more and more men in scientific and technological fields as a serious thing. My presence here is a manifestation of that.

Dr. Marston Bates, University of Michigan zoologist, in a book published only last month, referred to a new word, biocenosis, created as a substitute

Introduction

for "community" in order to pinpoint a meaning.

"Biocenosis," he said, "leads easily to biomes and biochores, to ecosystems and ecotones. These are all lovely words but they don't really say anything new. The trouble is that the word-coiner, sinking blissfully into his additions, gradually loses all communication with the outside world. He emerges from time to time to complain that the world doesn't really understand or appreciate his important thoughts—meaning his big words."

Prof. Hickey, whom I mentioned earlier, edited the <u>Journal of Wildlife Management</u> for three years. He ran across such gems as this:

"While piloting a Polar Cub 30 miles west of Churchill, two foxes were seen in a highly abnormal condition." Aside from indicating that two foxes seem to have been piloting an airplane, this is not a very informative statement.

And this one:

"A porcupine covered with <u>Sarcoptes scabell</u> was brought to the laboratory in a morbid condition."

That Journal, incidentally, recently had a fuss in its letter column over editorial requests for "polishing up" manuscripts, which led Justin Leonard, a Ph.D. in zoology who heads up conservation research in Michigan, to comment:

"The Ph.D. in science can make journal editors quite happy with plain, unadorned, eighth grade-level composition."

Dr. Watson Davis, director of Science Service, in an address last December at the conference on scientific communication of the American Association for the Advancement of Science, said this:

"The great art in telling is to make the words mean what you want them to, not in your own mind, but in the minds of others. There is little need for 'writing down'. This is demonstrated by the daily newspaper which serves children and old folks alike. Clearness and vividness are more needed than vocabulary limitations based on word lists."

I cannot wholly agree with Dr. Davis. How can there be clearness and vividness if the reader or listener does not know the meaning of the words? As for "writing down" (or talking down), it is dangerous even to think in such terms.

Newspapers, too, have a responsibility in this field and not all of them are making a real effort to meet it. A few papers, like the Milwaukee <u>Journal</u>, try to give their readers a good ration of things they should get, along with things they want, which must be provided if papers are to be sold. After all, newspaper publishing is a business. But The Journal has lost no circulation because of its offer-

ing of substantial reading matter.

I think more and more papers in the next decade or two will assign men to natural resources writing and they will try to inform themselves, as I have tried, in order to be interpreters of things scientific and technical.

But now the burden is heavily on you people and your fellows. You must realize that competition for newspaper space is very keen. The editor's wastebasket is large and his reporters' assignment sheet is crowded.

If your press releases do not get into print, if reporters do not come to your meetings or, having come, walk out and print nothing, examine your efforts. Maybe your mimeographed papers are too full of jargon, and also the summaries, which usually are more appealing to the press. I think authors of papers should not prepare the summaries. That is a job for an interpreter, a public relations man.

In my observation, a better job of communication results if a public relations man is a good newspaper man with some scientific and technical knowledge superimposed, rather than a scientific or technical man drafted for a task which he may not understand. Some ignorance of the jargon is an asset; it guarantees a degree of translation.

I think that better meetings of this sort would result if panel chairmen, in addition to summarizing at the end of a discussion, started it off with an easily understood explanation of its purpose and probable content. Such prefaces might well be out on the press table.

As for speaking and writing for popular consumption, I think it is well to remind yourself that, in the present state of biological evolution, the people you approach probably have minds as good as yours; they simply lack the tools to make use of their minds as you use yours. Your job is to supply the tools.

Words are the tools of the mind. I am sure that you know that man could not think until he invented words, or symbols, to think with. If you want people to think about things that concern you and your job, use words they know or supply new, useful words. Do it casually, not in a way which seems to say, "See how much more I know than you do!"

We had an incident in Wisconsin where a game biologist told a group of farmers, "I won't attempt to explain that; you wouldn't understand it anyhow." The dropping of that gem into the water, you may be sure, spread some ripples.

I think it is well to remind yourself, too, that

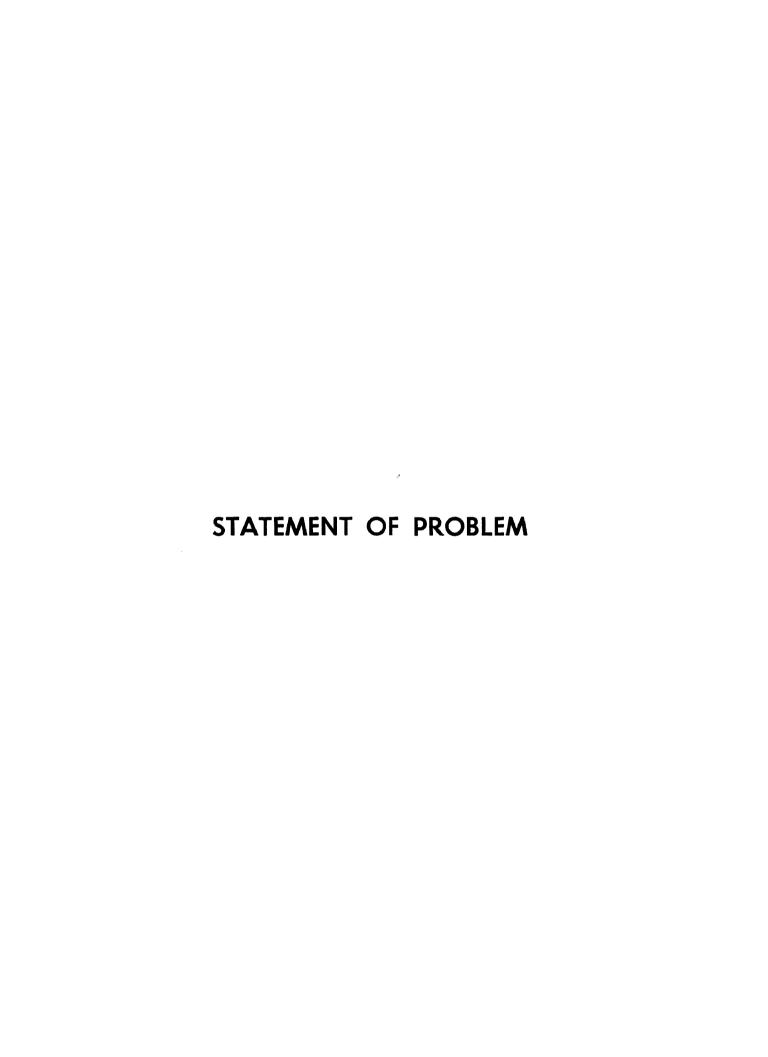
people are not compelled to listen to you or read you. You are in a buyer's market, competing with murder, rape, scandal, politics and comics in the newspaper and sex, whodunits and westerns on television and drugstore book counters.

At least, you must make what you say understandable, and if you have the talent you had better make it interesting. Regard it as a challenge, for that it surely is.

I used to become angry when I heard a speaker giving a very polished dissertation in the jargon of his profession and saw his audience looking at each other with raised eyebrows.

But I have reached the point where I feel sorry for such a man and say to myself:

"There he is, erudite as all get out----and ignorant as hell."



INTRODUCTION

INDUCED EUTROPHICATION - A GROWING WATER RESOURCE PROBLEM

A. F. Bartsch, Assistant Chief Research Branch Division of Water Supply & Pollution Control Public Health Service Cincinnati, Ohio

James Whitcomb Riley wrote sadly on "The Passing of the Backhouse". Many others share his nostalgic feelings and join him in quietly lamenting its passing from the American backyard scene. Although there were sewers in ancient Rome and Baghdad, the water carriage system of the modern world really grew with the industrial revolution that concentrated people in cities during the late 1800's. It is largely due to this passing of the backhouse and the train of events that followed that we are faced now with problems involving algae and metropolitan wastes. Streams and lakes, needed as water supplies or used for other purposes, have been receiving quantities of sewage that serve as nutrients for excessive growths of algae.

Algae of concern are mostly microscopic, freefloating ones. Their spectrum of color includes grass green, gold, brown, red, blue-green, and various shades in between. In moderate quantities, algae are extremely beneficial. Problems arise only when they are too abundant. Although some algae are more notorious than others for causing undesirable blooms, almost all are objectionable if present in sufficient quantity. We are made aware of their presence by the color they impart to the water, by the characteristic odors in the vicinity, and by unsightly scums, drifting windrows and drying drifts along the shore. In some urban centers of the United States, the layman is quite well acquainted with algae and what they do to the utility of water.

Nutrients that support algal growth in lakes and reservoirs originate in the surrounding drainage area and enter with the runoff. If the soil is fertile, nutrients for algae are likely to be abundant. Even the time and pattern of applying fertilizer to agricultural land may influence the contribution of nutrients to the aquatic environment. In their simple existence, algae utilize the mineral nutrients that have come from the land as well as carbon dioxide dissolved from the air or released in decay of organic matter. During warm seasons when growing conditions are otherwise favorable, algal production slows down and finally is stopped by depletion of any one nutrient element. Because nitrogen and phosphorus are not abundant in most surface wa-

ters, more commonly than other elements they seem to act as a brake on further rapid growth as the season progresses. Thus, although some naturally fertile lakes habitually develop algal blooms from year to year, as a rule, such blooms are less frequent and objectionable than in lakes polluted with sewage.

Sewage contains a multitude of different components, of which many have not even been precisely identified. It is a rich source of nutrients capable of growing dense populations of algae. Especially notable are nitrogen, phosphorus, potassium and carbon, as shown in Table 1. There is

Table 1. SELECTED ALGAL NUTRIENTS IN SEWAGE*

Nutrient	Concentration, ppm	
Nitrogen	20 - 50	
NH ₃	7 - 40	
NO ₃	0 - 4	
NO ₂	0 - 0.3	
Org.	3 - 42	
Carbon	66 - 176	
Sol. phosphorus	1 - 13	
Potassium	13 - 44	

^{*}Taken from Fitzgerald and Rohlich, 1958. Based on data for 14 sewages.

reason to suspect that sewage also contains organic substances that may possibly function as algal stimulants at extremely low concentrations. The algae-growing ability of sewage in undiluted form is well known from experiences with laboratory, pilot, and full-scale waste stabilization ponds. Yields from sewage can be as high as 23 to 36 tons per acre per year in ponds only 2 to 12 inches deep (Gotaas, Oswald and Ludwig, 1954). Potential yields obtainable in this way may be 10 to 20 times as great as those from cultivated land. Sewage nutrients are also effective for growing algae, although somewhat restrained, after dilution in surface waters.

The objectives of conventional sewage treatment are removal of suspended solids, microoganisms, and BOD. In most cases, meeting these objectives

makes treated sewage totally acceptable for discharge to surface waters so far as health, esthetic, and most water reuse considerations are concerned. But even with so-called complete treatment, removal of algal nutrients - nitrogen and phosphorus in particular - is negligible. This deficiency of waste treatment has been cited many times. So far, little consideration has had to be given to removal of these elements during or after the conventional treatment process. In the final analysis, conventional treatment will not prepare sewage adequately for discharge to waters physically suitable for algal growth.

It is well known that lakes do not persist forever as permanent marks on the landscape. During the course of history, they change continually mature, grow old, and eventually change to dry land. Examples of this development at all stages can be found in numerous geographic areas. As aging progresses, deep, infertile lakes accumulate sediments and nutrients, become shallower, more fertile and productive and thus qualify to be called eutrophic lakes. Periodically they may have excessive quantities of algae as a sign that the wheels of the aging process are fully in motion. Induced eutrophication through sewage pollution accelerates the aging process regardless of when in the history of the lake such nutrients are applied (Figure 1). At the algal bloom stage, people are

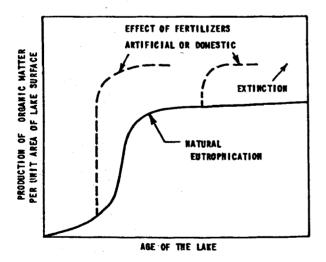


Figure 1. NATURAL AND INDUCED EUTROPHI-CATION. (From Hasler, 1947).

concerned because of the immediate unpleasantness of algae that interfere with water resource enjoyment and not because this is a milestone in the progressive extinction of the lake.

Throughout the world, there are many examples of surface waters made fertile by sewage. Among them are ponds and lakes intentionally enriched to produce more fish or for other purposes. These bodies of water are not the problem. Lakes un-

intentionally enriched with sewage, however, characteristically have changed from sparkling, clear, useful ones to unsightly and malodorous ones. The nature and extent of the problem as it existed worldwide in 1946 was well described by Hasler (1947). A familiar thread of similarity and repetition can be noted in each case. Sometimes it includes all but more commonly only several of the following steps: (1) introduction of raw or treated sewage, (2) replacement of prized deep water trout or whitefish by less desirable kinds, (3) increase in plankton or free-floating plant and animal life, and (4) explosive seasonal appearance of the blue-green alga, Oscillatoria rubescens.

The Zurichsee, a lake in the foothills of the Swiss Alps, is a celebrated example of induced eutrophication. The lake is composed of two basins separated by a narrow passage. The upper basin received no sewage and remained essentially unchanged whereas the lower basin, receiving the sewage from a group of small communities with more than 100,000 people, underwent typical changes as described above. Other Alpine lakes suffered the same fate, as did also lakes in Sweden and England. But, one need not go to Europe to learn about the problems of induced eutrophications; there are excellent examples here in the United States.

Many people know beautiful Madison, Wisconsin, because of its chain of four lakes with the musical names, described poetically by Longfellow. Others know it also as a classical example of the consequences of bringing together lake water, algae, and sewage. The problem has a long history, going back to 1920 or even earlier. Many technical people have been fascinated by it and have studied one aspect or another. The voluminous historical record of the problem – as it emerged and grew, how it aroused the public, the technical, political and legislative actions taken, and finally the remedy—make instructive and fascinating reading.

The algal bloom problem has plagued the property owners on the shores of Connecticut's Lake Zoar also. This body of water is an impoundment on the Housatonic River (Curry and Wilson, 1955; Benoit, 1955) created in 1919. By 1947 production of algae had increased to the nuisance level, stimulating investigative action by the State government. Again sewage and industrial wastes have been implicated as sources of algal nutrients. A number of potential remedies have been investigated.

Across the Nation, at Seattle, Lake Washington is a newer entry to the algal problem field (Sylvester, 1956; Edmondson, 1956; Bogan, 1956; Edmondson, Anderson and Peterson, 1956). The story begins about the turn of the century when Seattle sewage was first discharged to the lake. As the population grew and new lakeside communities

were sewered, the quantity of sewage kept increasing. By 1941 the sewage was finally diverted to a treatment plant with the effluent going to a different drainage. But the problem was not ended by provision of the combined sewer system because storm water and sewage frequently were bypassed to the lake through numerous overflows. Since 1941, the growing communities in the surrounding area provided additional sewage treatment facilities draining their effluents to the lake. These plants and a number of other sources upstream on tributaries supply algal nutrients in quantity. There is a history of parallel biological change also with records going back to 1933. The quantity of algae has increased significantly. Evidence of this is shown both by increasing numbers of algae that have been measured and recorded and by progressively decreasing transparency of the water. By 1955, Oscillatoria rubescens, the rust-colored form that often seems characteristic of induced eutrophication, appeared on the scene and became a prominent part of the algal population. Other limnological changes, including decrease in oxygen resources in the deeper areas, have been stimulated in turn by accumulation and settling out of the increasing amounts of organic matter synthesized in the lake. Intense efforts are being directed to finding and executing a remedial and preventive program for this extremely valuable body of water.

The Potomac River below Washington, D. C., is another susceptible body of water. This tidal reach, where the water rocks back and forth, is as much like a lake as a river. The immediate tributary land area has a concentrated sewered population of 1.7 million people. Records from a survey on the river made by Purdy in 1913 show a moderate amount of algal growth at that time. Many changes have occurred since then in population size and distribution pattern, in land uses upstream, in sewerage and sewage treatment, and in silting of the tidal flats and denuding them of aquatic plants. Limited intermittent studies during the last few years reveal that algal populations are much greater now than in the past. Blooms of blue-green algae are common and become objectionable at least in some of the bays and coves. Undoubtedly, the peak of productivity has not yet been reached.

An interesting deviation from the usual pattern just described occurred on Long Island in the area famous for Long Island ducklings and Blue Point oysters. In raising ducklings, it is customary to have ponds accessible to the fowl for drinking and exercise. Usually it is downslope from the feeding troughs. Because of this arrangement, the pond becomes a depository for manure from ducks puddling in the water. Manure from others waddling near the feed boxes reaches the pond as a result of wet weather drainage down the slope. These fluid wastes, rich in algal nutrients, drained or were pumped to Moriches Bay. As they accumulated,

they stimulated formation of dense blooms of algae of types unacceptable as the normal food of oysters inhabiting the Bay. Oyster production declined until the famous Blue Points were gone from the market.

In spite of considerable study, the duck farm waste problem has not been solved completely, but in 1953 a passage from Moriches Bay to the Atlantic Ocean was reopened by dredging and the action of high seas. Now, the tides scour and flush the wastes from the Bay, and harvesting oysters is again a gainful occupation.

These are but a few examples of the consequences of induced eutrophication. Many others could be cited, but these are sufficient to show that the trouble spots of the past are not isolated and peculiar situations.

During the past year, more than \$650,000,000 were spent for construction of sewerage facilities, including waste treatment. Amounts spent during other recent years are similarly impressive. The citizens who pay this bill, like the people in Madison or in communities on the shores of Lake Washington or Lake Zoar who paid for secondary treatment, have a right to expect safe, clean, attractive, useable water in return. In some cases and fortunately so far they have been few - such great expectations have not materialized. With the mineralizing processes in sewage treatment, nitrogen and phosphorus are made more readily available as algal nutrients, and algal problems have taken the place of the preceding, more typical pollution problems.

It is obvious that the technique of waste treatment has not yet gone far enough, that there are communities that will have to do more than remove suspended solids, microorganisms, and BOD from sewage. It is necessary to think more seriously about additional - or tertiary - treatment to remove algal nutrients where environmental factors require it. Lakes are more susceptible than streams to algal troubles and must be considered with extreme caution as potential basins to receive treated sewage. Where flowing waters are available for dilution, they are preferable. Some time ago, consideration was given to discharging treated sewage to Lake Tahoe. Apprehension of creating an irreversible algal problem in such valuable recreational waters was an important factor in rejecting the idea.

We stand now at a point where the need for more knowledge is unquestioned. It is necessary to examine this growing algal problem, measure its size, determine its exact nature, find how to remedy existing situations, and learn to treat wastes more effectively in order to prevent them. The urgency of this need is emphasized by several facts. Population increases continually. As a re-

sult, there are ever more people in sewered communities – not only to swell the quantity of sewage with which we are concerned, but also to compete for recreational and other uses of available water. As the quantity of sewage increases, its character changes also in many ways. One is the per capita increase in phosphorus content traceable to the recent popularity of phosphorus-bearing detergents. Most of it finds its way to the sewer. As

water resources development goes forward, we find ourselves continually losing many miles of free-flowing rivers and gaining many square miles of quiescent standing water. With all of these changes, we can expect to be faced more frequently with problems of algae and metropolitan wastes. Problems of the past have been costly ones. Problems of the future will be costly also unless we spend the effort now to learn how to cope with them.

REFERENCES

Benoit, R. J. 1955. Relation of phosphorus content to algae blooms. Sewage & Industrial Wastes $\underline{27}$: 1267-1269.

Bogan, R. H. 1956. A new critical phase of the Lake Washington pollution problem: Treatment and control of nutritional elements. The Trend in Engineering, April 1956, pp. 13-14.

Curry, J. J. and S. L. Wilson. 1955. Effect of sewage-borne phosphorus on algae. Sewage & Industrial Wastes 27: 1262-1266.

Edmondson, W. T. 1956. A new critical phase of the Lake Washington pollution problem: Biological aspects of the problem. The Trend in Engineering, April 1956, pp. 11-12. Edmondson, W. T., G. C. Anderson, and Dr. R. Peterson. 1956. Artificial eutrophication of Lake Washington. Limnology & Oceanography 1: 43-53.

Gotaas, H. B., W. J. Oswald, and H. F. Ludwig. 1954. Photosynthetic reclamation of organic wastes. The Scientific Monthly 79: 368-378.

Hasler, A. D. 1947. Eutrophication of lakes by domestic drainage. Ecology 28: 383-395.

Sylvester, R. O. 1956. A new critical phase of the Lake Washington pollution problem: A paradox of water-pollution control. The Trend in Engineering, April 1956, pp. 8-10.

Specific Problems in Lakes

MADISON'S LAKES: MUST URBANIZATION DESTROY THEIR BEAUTY AND PRODUCTIVITY?

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To the geologists, lakes such as those found in and near Madison, Wisconsin, are, in geological time, relatively short-lived widespreads in a river that drains an extensive watershed. The four principal lakes are on the Yahara River which flows from the North to the Southeast where it empties into the Rock River. These lakes are known by Indian names given in 1858 by Dr. Lyman C. Draper, founder of the Wisconsin State Historical Society, as Mendota, Monona, Waubesa, and Kegonsa; a fifth, spring-fed, Lake Wingra, lies South of Lake Mendota, and drains through Murphy's creek into Lake Monona. Dr. Draper chose the names from the language of the Chippewa Indians, who never dwelt in the vicinity of Madison, but preferred to live in northern Wisconsin. His choices were based on the beauty of the sound of the names. However, the Winnebago Indians, who lived in the vicinity of the Madison lakes, called the entire chain "Taychoperah," which means "four lakes." The Winnebago name, "Wingra" means, literally, "dead lake", and it was aptly applied because in the 19th century, that spring-fed, shallow, marl-bottomed body of water was nearly extinct. Extensive dredging operations, carried on from 1912 to 1918, were needed to return Wingra to the status of a lake.

We are concerned mainly with the four lakes of the Yahara drainage basin. They are relatively young, according to the geologists, because it was only 20,000 to 30,000 years ago that the ice of the last glacier melted and left the water level of Lake Mendota at approximately 850 feet above sea level. The water level of Lake Monona stood originally at about 844.5 feet, that of Lake Waubesa at 844.3 feet, and the water of Lake Kegonsa was originally about 842.5 feet above sea level. Government surveyors, who started their work in the southern part of the State, and worked toward the North, designated the lakes by number in the order in which they found them: Thus, Kegonsa was called First Lake, Waubesa, Second Lake, Monona, Third Lake, and Mendota, Fourth Lake. These numerical designations are gradually being dropped, but "old-timers" still use them to show their sophistication, and to confuse newcomers.

The area of land which drains to Lake Mendota is approximately 250 square miles, and the water from it feeds four creeks, one small river (the

Yahara), and an unknown number of springs. According to the best estimates available, the three lower lakes -- Monona, Waubesa, and Kegonsa -- receive drainage from an additional 250 to 300 square miles of land. Hence, the total Yahara drainage basin comprises 500 to 550 square miles of fertile farm lands, wooded slopes, and city or village properties. Lake Mendota is the largest and deepest of the four, Monona the second, Kegonsa the third, and Waubesa the fourth in order of area and depth.

The first white settlers in the Taychoperah area were entranced by the beauty of the lakes and by their productivity. They found that all of the lakes contained an abundance of fish, wildlife and waterfowl. Unfortunately, some of the early settlers introduced carp into Lakes Mendota and Monona sometime between 1850 and 1870.

No one knows exactly when nuisance conditions caused by excessive growths of algae and rooted plants first occurred in the four lakes, but the first written report of such a phenomenon appeared in 1888 in the Transactions of the Wisconsin Academy of Sciences, Arts, and Letters, VII (1883-1887) in a paper written by Professor William Trelease of the University of Wisconsin. This paper, which was an essay, and which contained little factual information, was entitled "The Working of the Madison Lakes": it describes nuisance conditions in Lakes Mendota and Monona as early as June, 1882. Professor Trelease also reported a bloom in Lake Waubesa in 1886. Despite this report's inadequacies as a scientific paper, it establishes the fact that Madison's lakes could become overproductive of algae and thus produce nuisance conditions before urbanization of the area became extensive.

Madison's first water works was completed in 1884. It pumped water from a highly productive aquifer about 850 feet beneath the surface. This water-bearing stratum continues today to supply the needs of the city and its nearby communities with good, but "hard" water. In 1884, the city, built on an isthmus between lakes Mendota and Monona, had a population of 12,000, but no sewerage. Privies, cesspools, and direct drains to the lakes were used to dispose of sewage. Storm water then, as now, flowed directly to the lakes.

In December of 1884, the Mayor and Common Council of the City of Madison stated: "Considering our finances, our sanitary desires, our present and prospective population, we want the cheapest, the best, the safest, and the most flexible system --(of sewage treatment and disposal) -- available." This was a prophetic statement; in it may be found the needs, the limitations, and at least indications of the problems that are with us today. The government of Madison was faced then as now with the problem of collecting, treating, and disposing of the waste water of a rapidly growing community. Also, then, as now, the wheels of government turned slowly, and it was not until June of 1894, ten years later, that Mr. John Nader reported to the City Council that "the lakes are not properly to be used as receptacles for sewage in the crude state" -- and that a sewerage system and treatment plant should be constructed "at the earliest opportunity."

Immediately, arguments arose in the Council over which system for sewage treatment and disposal was the cheapest, the best, the safest, the most flexible, and where the effluent was to be discharged. Councilmen from wards with frontage on Lake Mendota argued with those from wards with frontage on Lake Monona. Finally, it was decided to construct the treatment plant on the Yahara River between Lakes Mendota and Monona with the hope that during flow from the treatment plant to Lake Monona, dilution of the effluent would be sufficient to make the water of the river entering Lake Monona as pure as that of the lake. Furthermore, this location was chosen because it was on low ground at the far northeast edge of the city, and thus fulfilled the "out of sight, out of mind" criterion.

In August, 1894, the seventh of a succession of engineers to study the Madison sewage treatment and disposal problem submitted his report and, in 1895, construction of the sewerage system was started. In October, 1897, construction was commenced of a sewage treatment plant which would use chemical precipitation followed by filtration through sand —a so-called "International Process" — with a predicted capacity of 200,000 gallons per acre of sand filter per day. Unfortunately, the treatment plant could not be made to operate properly, fell far short of the claim that it would produce an effluent "as pure as the water of Lake Mendota", and was abandoned in January, 1901.

Mr. F. E. Turneaure, then City Engineer, later Dean of the College of Engineering, was ordered to construct a new treatment plant which would employ septic tanks followed by cinder filters which would drain into the Yahara River near its outlet into Lake Monona. This was done, and in 1902, boatmen and residents along Lake Monona reported that great improvement had occurred in the "purity of the Lake's water", and that "its odors and excessive weed growths had been decreased."

However, by 1906, the capacity of Turneaure's treatment plant had been reached, by 1911 it was overloaded, and it again became necessary to face up to the problem of getting "the cheapest, the best, the safest, and the most flexible system available." Again, the "out of sight, out of mind" criterion was employed, and on the basis of a study made by engineer John M. Alvord of Chicago, E. E. Parker, then City Engineer, built a new treatment plant in the Town of Burke, two miles North of the Turneaure plant. The Burke plant had a rated capacity of 5,000,000 gallons per day. It consisted of primary settling tanks followed by sprinkling filters which drained into a ditch leading to Starkweather Creek at the North end of Lake Monona, about one mile Northeast of the inlet of the Yahara River. The plant was completed and put into operation in 1914. Thus, from 1906 until 1914, Lake Monona was polluted with poorly treated effluent from the old Turneaure plant.

Nuisance growths of algae and offensive odors occurred with increasing frequency in Lake Monona; these undesirable conditions reached a climax in the summer of 1918. Madison had grown to a population of over 30,000, and the shores of Lakes Monona, Waubesa, and Kegonsa were thickly populated by cottagers. In addition, there were several resorts on the shores of these lakes, and the City of Stoughton, on the Yahara River, to the Southeast of Lake Kegonsa, had grown to a population of near 5,000. The lakes were beginning to show the effects of urbanization, and people were becoming aware of the problems created by use of the lakes as "receptacles" for inadequately treated sewage.

Much time has been spent in presentation of the early history of sewage treatment and disposal in Madison, and the development of public awareness of the Madison lakes problem. This has been necessary to gain an understanding of the studies which have been made, and the steps which have been taken since 1918 in an attempt to achieve desired remedies. Time does not permit detailed description or discussion of investigations made, conclusions reached, governmental agencies formed to cope with the problem, legislation enacted, court tests of new laws, and procedures followed to comply with these laws. All that can be done is to present these events in more or less chronological sequence, and to comment on them briefly.

1919-1920. The Alvord and Burdick study and "Report Upon the Cause of Offensive Odors from Lake Monona, Wisconsin" blamed decomposition of algae for the nuisance conditions encountered in 1918. In this work, the firm of Alvord and Burdick had been aided by studies carried on under supervision of Dean H. L. Russell, a bacteriologist, and Professor Chauncey Juday, a limnologist of the University of Wisconsin. Dean Russell and Pro-

fessor Juday agreed that "although the (treated) sewage (from the Burke plant) may, to some degree, stimulate the growth of the algae, we are of the opinion that these growths would be sufficiently luxuriant from natural causes to constitute a nuisance even if the sewage or effluent were not allowed to reach the lake, or were subject to treatment to exclude whatever nitrogenous matter it may contain that would help support algae in the lake." The report also stated "that there is no present justification for attempting to dispose of the Madison sewage plant effluent elsewhere than in Lake Monona as is now done." The report recommended that the Madison Board of Health, which had been using copper sulphate to kill algae in Lake Monona, should continue to do so only on the basis of continuing laboratory studies directed toward determiation of the need for such treatment.

1920-1921. Studies were made on improvements, recommended in 1920 by Alvord and Burdick, of the Burke treatment plant, and the desirability of building a new treatment plant near Nine Springs Creek, which drains into the Yahara River just above Lake Waubesa. The possibility of diverting Madison's sewage to the Wisconsin River, approximately 18 miles West of the City, was considered, but rejected as being too expensive. The Oscar Mayer and Company packing plant commenced operations in a location to the North and West of the Burke treatment plant, and started to contribute its sewage to that of the City.

1923-24. Offensive odors from Lake Monona were prevalent. The Lake Monona Improvement Association was formed, and the statement was made that "we are through with chemists and engineers; let's get down to business." The Common Council formed a Lakes and Rivers Commission to supervise the sanitary conditions of the lakes and streams. The City of Stoughton expressed opposition to establishment of the proposed Nine Springs treatment plant, but the City of Madison, with approval from the State Board of Health, went ahead with its plans for construction.

1928. The Nine Springs treatment plant, employing Imhoff tanks followed by trickling filters and final clarifiers was put into operation. Effluent from this plant flowed through the Nine Springs Creek into the Yahara River, and thence into Lake Waubesa. The Burke plant was continued in operation, but on a limited basis in line with its rated capacity.

1929-30. The proposed sewage treatment plant of the Village of Middleton, located at the northwest end of Lake Mendota, and designed to discharge effluent into Lake Mendota, was given preliminary approval by the State Board of Health. These plans were opposed by the City of Madison and by owners of property on Lake Mendota. The controversy resulted in creation of the Madison Metropolitan

Sewerage District under provisions of a law passed by the 1927 State Legislature. Establishment of the District made provision for collection and transmission of Middleton's sewage to the Nine Springs treatment plant. After public hearings, the Madison Metropolitan Sewerage District was upheld by order of the Dane County Court on February 3, 1930. The decision of Judge George Kroncke representated thorough study and was a carefully worded statement of the Court's opinions. This decision has stood the tests of time and of higher courts, and may well serve as a model for future use.

1931-33. Interceptor sewers, pumping stations, and sewage treatment plants of the City of Madison were transferred to the Madison Metropolitan Sewerage District. Commissioners of the District stated in their plans for the future that "Inasmuch as the waters of Lakes Mendota and Monona should be maintained as free from contamination as possible, it is desirable to plan for the complete elimination of the flow of effluent from sewage disposal plants into the waters of these two lakes, and to treat the sewage so that no unstable or improper effluent will be permitted to enter the Yahara River." This was a major statement of overall policy that has been followed during the past 29 years.

A new "Clean Lakes Association" was formed in 1931 to protest against and to seek prevention of pollution of Lakes Monona and Waubesa. This association immediately requested that the Burke plant be closed and that all of the District's sewage should be treated in the Nine Springs plant. The summers of 1931 and 1932 were bad ones for Lake Monona, and despite frequent and heavy treatments with copper sulfate, the lake bloomed and gave off bad odors. At the same time, in 1931, Lake Mendota suffered an outbreak of disease in its whitefish and perch. Despite the fact that there had been no chemical treatment of Lake Mendota, the general public blamed the Mendota fish deaths on "sewage and chemicals." This is evidence of the lack of confidence of the public in those responsible for the conditions of the lakes and for the treatment and disposal of sewage.

1933-35. The Dane County Board of Supervisors established a County Parks Commission, one duty of which was to control and improve the condition of Lakes Waubesa and Kegonsa. The Parks Commission was authorized to contract with the Madison Board of Health for copper sulfate treatment of Lakes Waubesa and Kegonsa. The Parks Commission also appropriated a small sum to finance a study of the lower lakes. In 1935, the Lake Kegonsa Protective Association was formed by owners of property on the lake, with the expressed purpose of "preventing the Madison Metropolitan Sewerage District from converting Lakes Waubesa and Kegonsa into their private privies." Members

of this Association then and later expressed themselves in earthy, powerful language; they were action-minded, and were not interested in research or explanations. They carried their problems to the Governor and to the State Board of Health. One member collected water from the Yahara River, and from Lakes Monona, Waubesa and Kegonsa, and mailed the samples to Dr. H. C. Bundeson, City Health Commissioner of Chicago, for analysis. This action exemplified the lack of confidence in local authorities and in agencies of the State government, and the belief that "someone from outside" was needed to "get the answers and do the job." Another very articulate citizen made a prophetic statement: "There is only one way out -pipe the stuff around the lakes." Oscar Mayer and Company built and put into operation a plant for treatment of its wastes that produced an effluent no stronger than domestic sewage.

1936. The Dane County Board appropriated funds to build locks designed to control the levels of Lakes Waubesa and Kegonsa. It was believed that maintenance of the "lower lakes" at the highest possible levels would help remedy the algae nuisances. This same year, the report of an "outside" laboratory that had been retained by the Lake Kegonsa Protective Association proved to be unacceptable because it showed that the Nine Springs sewage treatment plant was functioning efficiently. The Nine Springs treatment plant was enlarged by addition of an activated sludge treatment system which was operated in parallel with the existing facilities.

1937. The Burke sewage treatment plant was closed and all sewage of the District was diverted to the Nine Springs plants. The Dane County Board established a County Sanitary District and a County Conservation Committee. The latter was concerned primarily with protection of fish and other wildlife against the adverse effects of chemicals used to control nuisance conditions in the lower lakes.

1938-40. The Southern Wisconsin Lakelands Association, organized in 1939 to help promote resort and recreational use of lakes in the southern part of the State, added its support to anti-pollution and clean lakes efforts of the County Board. The proposal was made by the County Board that State and Federal aid should be obtained to build a pipeline to carry effluent from the Nine Springs plant to the Yahara River South of Stoughton.

1941-43. Through the efforts of the Southern Wisconsin Lakelands Association, and representatives from the Dane County Board, State Senator H. A. Lewis of Boscoble, Grant County, introduced a bill calling for revision of the Wisconsin antipollution law. The Lewis bill would not permit the effluent from the sewage treatment plant of a city of 10,000 or more population to be introduced into a lake of less than six square miles in area, or

into a stream within 15 miles of the city. In the public hearings on this bill, strong opposition was expressed by representatives of cities throughout the State. Hence, Senator Lewis changed the bill to apply only to cities of 45,000 persons or more; to any lake of more than two square miles or less than six square miles in area located within 10 miles of the treatment plant of the city or sewerage district. Since the bill as changed applied only to the Madison Metropolitan Sewerage District, opposition from other localities in the State was withdrawn. In addition, the effective date of this legislation was changed from "immediately" to January 1, 1943.

The Lewis bill, as amended, passed both houses of the Legislature, but was vetoed by Governor Heil in June of 1941. At that time the Governor said that "I feel this matter should receive very careful study and there should be a definite determination whether this particular disposal plant is the cause of the trouble complained of in the lakes near Madison". The Governor appointed a small committee to study the problem and to report to him immediately. The County Board stopped chemical treatment of Lakes Waubesa and Kegonsa so as to "shatter the public complacency" regarding the lakes problem. Incidentally, copper sulfate was then in short supply because of war needs.

The Governor appointed D. W. Mead, Emeritus Professor of Hydraulic and Sanitary Engineering, as Chairman of his Committee, and included in its membership Clifford Halverson of the County Board, E. J. Tully of the State Board of Health, J. L. Ferebee of the Milwaukee Sewerage Commission, and Dr. W. D. Stovall, Director of the Laboratory of Hygiene of the State Board of Health. This Committee recommended:

- 1. "That facilities be provided for the treatment of the lakes with copper sulphate to abate the nuisance odor caused by algae until a more satisfactory solution can be arrived at. The city and county should pay for this work.
- 2. "That immediate steps be taken to provide the personnel necessary to begin an investigation of the sources from which nitrogenous material, phosphates, and other materials which act as food substance for algae are contributed, and the amount contributed from each source. This investigation should last a year and be financed by the Metropolitan Sewerage District.
- 3. "That the State provide sufficient funds for the investigation of all the factors which are responsible for the growth of algae in the lakes in the state, and that these funds be made available to the State Board of Health."

The County Board's reaction to this program, conditioned by nearly 25 years of debate against

State, Municipal and District agencies, was to recommend:

- 1. "That Dane County Officials ignore the Governor's committee and go ahead with a county program to combat pollution of lakes Waubesa and Kegonsa.
- 2. "That the Dane County Board appropriate \$3,500 to sponsor a chemical study of the pollution with the view of using the information gained as a basis for court action to prevent the Madison Metropolitan Sewerage District from continuing to dump effluent in the lakes.
- 3. "That the County Board provide an appropriation to spray the lakes with copper sulphate until such time as conditions are corrected."

In June of 1942, the problems of the District, the Governor's Committee, and the County Board were further complicated by the decision of the Federal Government to recondition and to use the Burke plant to treat sewage of Truax Field, an Army Air Corps base located to the North of Madison.

At the end of June, 1942, Dr. Clair N. Sawyer was engaged by the Governor's Committee to conduct the sanitary chemical studies needed to implement the Committee's program. The County Board was unimpressed, and decided to conduct its own survey.

The Governor's Committee made a preliminary statement of the problem as follows:

"After considerable study and observation of the local lake problem, the conclusion was reached that the major part of the offensive conditions and stenches produced are a direct result of the accumulations of algal growths in advanced stages of decomposition. It was further concluded that the stimulation of such algal growths was not a direct result of pollution of the lakes in an ordinary sense, but rather was related to the fertilization of the water by enrichment with minerals such as nitrogen, phosphorus, potassium, and possibly other fertilizing elements and compounds. These conclusions were based on many considerations of which the following are a few:

- 1. "Algal problems are mostly prevalent in shallow lakes receiving drainage from rich agricultural areas and/or urban communities.
- 2. "Chemical analysis of algae show nitrogen to be one of the major component elements with phosphorus a minor element.
- 3. "Stream pollution surveys where domestic sewage is involved have shown zones of increased biological productivity to occur in regions of the

streams below the sewer outfall.

- 4. "The science of hydroponics is based on the principles of ordinary agricultural fertilization applied to water.
- 5. "Fertilization of lakes is being practiced in some areas with ordinary commercial fertilizers to stimulate greater plankton growths which in turn support fish populations.
- 6. "Some authorities place considerable importance on agricultural land drainage and thereby infer that there is a correlation between fertilization and algal blooms.
- 7. "The growths causing nuisances in inland lakes are largely members of the plant kingdom. All ordinary plant growth is interdependent upon a substrate containing nitrogen, phosphorus, potassium, and other elements.
- 8. "Sewage of domestic origin although highly purified by modern methods of treatment remains, after treatment, abundantly rich in phosphorus, nitrogen, potassium, and possibly other elements of significance.
- 9. "High concentrations of certain fertilizing elements which accumulate in lake waters during the winter months are rapidly depleted in the surface waters during the growing period of warm weather."

SCOPE OF SURVEY

"Although the Committee was charged with studying the pollution of Lakes Waubesa and Kegonsa, it was deemed advisable to include Monona in the survey as it also is one of the lakes causing considerable trouble. It was anticipated that sewage effluent from Truax Field would be added to Lake Monona during the course of the survey, and this was further reason for studying the lake as diligently as the others mentioned above. Lakes Wingra and Mendota were relegated to minor investigations because of their normally good behavior so far as stench producing ability is concerned."

METHODS OF SURVEY

- 1. "Measurement of fertilizing elements.
- 2. "Biological studies.
- "Observations on other southeastern Wisconsin lakes.
- 4. "Research in laboratories."

SIGNIFICANCE OF CHEMICAL OBSERVATIONS

"The soluble phosphorus, potassium, and inorganic nitrogen content of tributary waters are considered more indicative of its fertilizing potency since these represent readily available forms of the elements. The organic nitrogen content is considered of secondary importance because the nitrogen is in a form which is made available incompletely and slowly.

"The suspended organic matter contained in the tributary waters is given consideration mainly for the reason that certain groups have placed considerable emphasis on the solids in the Madison sewage plant effluent as being the cause of the stenches and unsightly conditions along the shores of Lake Waubesa".

Early in 1943, Dr. James B. Lackey of the United States Public Health Service studied the problems faced by the Governor's Committee, conducted investigations independently and in cooperation with Dr. Sawyer, and got the County Board to suspend its proposed program of investigations that would have duplicated the efforts of the Governor's Committee.

Senator Lewis reintroduced his anti-pollution bill, but changed the date of enforcement to: "one year after the termination of the present war as proclaimed by the President or Congress." The bill passed, and was signed by Governor Goodland in April, 1943.

The first report of the Governor's Committee, prepared by Dr. C. N. Sawyer, with consultation and advice of Drs. J. B. Lackey and A. T. Lenz, and covering investigations made from July 1942 to July 1943, was published in mimeographed form. Excerpts from this report follow:

THE GOVERNOR'S COMMITTEE - SUMMARY AND DISCUSSION

"This study shows that a large amount of nutritive and pollutional material is being added to the lakes from various sources. It further shows that the inorganic nitrogen contributed from these sources and from deposits on the lake bottom is a critical substance with relation to lake blooming. It appears that a significant reduction in the amount of the nutritive material reaching the lake would reduce the frequency and density of lake blooms. This should not be interpreted to mean that algae growths in the lake will cease or that blooms will be completely eliminated. While the Madison sewage effluent contributed by far the largest amount of this substance to Lake Waubesa, 76.9%, it is not the sole contributor. Lake Kegonsa receives 67.2% of its inorganic nitrogen and 88.6% of its organic nitrogen from Lake Waubesa. In the case of Lake Monona the storm and industrial sewers of Madison are large contributors.

"As our studies have progressed, it has been more and more apparent that the Madison sewage disposal plant is functioning efficiently. The popular impression that this plant is not purifying the sewage in a satisfactory manner is not correct. There is a question as to the capacity of this plant to handle the sewage and wastes from the metropolitan area if all of that waste which is now going into Lake Monona by various industrial and storm sewers was passed through the plant.

".. The nuisance, as the results of this investigation show, is due to the excessive growth of algae, and the odor created by it. Even though the Madison sewage plant effluent is not directly the cause of the odor that comes from the lakes in the summer, the data clearly show that it is the largest contributor of nutritive material upon which algae feed."

SOME EXCERPTS REGARDING TECHNICAL ASPECTS OF THE SURVEY

"The question was, is there a critical relation between these substances (nitrogen and phosphorus) and the frequency of lake blooming and the density of the bloom? The question bore directly upon the quantitative relation between these substances, inorganic nitrogen and phosphorus, and the sources from which they are contributed to the lake. In other words, if these nutritive materials or any one of them could be shown to be critical, that is, necessary in certain amounts to support algal growth, then the amounts contributed from various sources would be helpful in determining the significance of the various sources to the blooming of the lakes."

"In June, before a heavy bloom, the inorganic nitrogen in solution in the surface water of Lake Waubesa amounted to almost 1.0 part per million. After the algal growth had become so dense that it was recognized as a scum on the lake surface, the inorganic nitrogen in solution in the surface water was found to have been reduced to 0.3 parts per million. In other words, the algae in order to grow to the density attained between these dates in June used up more than half of the inorganic nitrogen in solution in the surface waters. It is important to note that this reduction of inorganic nitrogen took place in water where the algal growth was most dense.

"The organic nitrogen represents decomposable matter whereas inorganic nitrogen does not. The organic nitrogen is considered pollutional because it undergoes decomposition in the lake. The inorganic nitrogen is the result of completed decomposition. It is stabilized nitrogen and is therefore considered to be non-pollutional, nutritive material."

"Raw sewage is by-passed from the city sewers into Lake Monona during periods of mechanical or prolonged electrical failure and during periods of heavy rainfall. The main exit for the sewage to Lake Monona is via the Burke Plant outfall sewer. The sewage enters this sewer at the No. 1 pumping station located at East Johnson and First streets."

"During the course of the survey no untreated sewage was found in the effluent from the Madison Sewage Plant. A total of 116 separate samples were tested and the results showed the effluent to be of better quality on an average than the effluents produced by plants of similar design and facilities operating elsewhere in the United States."

CONCLUSIONS BY DR. CLAIR N. SAWYER

- 1. "The biological productivity of the local lakes is a function of the loading of inorganic nitrogen on each lake.
- 2. "The soluble phosphorus content of water may be a factor in limiting the rate of biological activity and in determining the nature of the growths when its concentration drops below 0.01 part per million.
- 3. "Drainage from improved marsh land is approximately two to three times as rich in inorganic nitrogen as drainage from ordinary farm lands.
- 4. "On the basis of agricultural drainage, Lake Mendota should be from two to three times as productive of biological life as Lake Monona and Waubesa and Kegonsa should be some one to two times as productive as Mendota. Lake Wingra on the basis of lake volume should be the most productive of all.
- 5. "High biological productivity and nuisance conditions do not always occur simultaneously. For example, Lake Wingra, a very shallow lake, does not develop offensive conditions in spite of its high productivity. The reason for this has not been ascertained but may be related to its lower phosphorus content."

THE FUTURE IN REGARD TO THE MADISON LAKES PROBLEM AS SEEN BY THE GOVERNOR'S COMMITTEE AT THE TIME OF THE FIRST "SAWYER" REPORT

"Any solution of the local problem must take into consideration developments in the near and perhaps distant future. The City of Madison and its suburbs has increased its population by 9,430 persons during the past three years. The day is not far distant when this center of government and education will reach a population of 100,000, then it will be 150,000 etc. It is reasonable to assume that industrial growth will increase similarly.

Thus it would be logical to conclude that in the course of another 50 or 100 years the contribution of pollution and fertilization by the metropolitan area will be doubled. During a similar period, it is doubtful whether the contribution from unnatural sources will increase materially. In the year 2000 A. D., the contribution of inorganic nitrogen from the city may approximate 85% of the total contribution to Lake Waubesa, and the soluble phosphorus 94% unless marked changes are made in current practices."

DISCUSSION OF THE REPORT FINDINGS BY DR. LACKEY, UNITED STATES PUBLIC HEALTH SERVICE

"There can be no dispute as to the fact that there is heavy fertilization of the lakes by the materials poured in. The use of sewage for fertilizing lakes in Germany is too well known; the use of sewage sludge as a fertilizer is widespread in this country. These two facts constitute proof of the fertilizing value of sewage, the first as to raw sewage, and the second as to solids and mineralized portions of treated sewage. Snell gives calculated monetary values of human excreta as fertilizer and points out that 85% of the fertilizer value of excreta is contained in the urine and is therefore lost in the effluent of ordinary sewage treatment plants. In other words, it passes on through the plants and out with the effluent, not being incorporated into the sludge. This fact should at once render untenable any argument that by-passed sewage solids, if found, are a major cause of blooming in the lakes.

"It would be well to remember that any removal of nitrogen and phosphorus from effluent waters to these lakes might not have an immediate remedial effect of great magnitude. The layman might be inclined to take such a view and be sharply critical if obnoxious blooms did not stop at once following removal or further treatment of the effluent. There are, however, at least three reasons why the lakes might continue to bloom for a considerable period:

"First - There may be other chemical factors closely related to blooming in addition to nitrogen and phosphorus contributed by sources enumerated herein. These considerations do not obviate the fact that present contributions of nitrogen and phosphorus are readily available forms and extensively used; probably a 'first choice' relationship exists. But should these sources be removed, other possible sources should be remembered.

"Second - The bottom deposits which have been building up over a long period of time should be considered. One component part of these results from sedimentation of dead plankton bodies; another results from the annual death and decay of rooted lake vegetation and attached algae (which at times form very heavy growths both on the rooted plants and on the bottom); and a third consists of sludge from sewage treatment plants and sewers. The depth of such deposits in the Madison lakes needs more investigation, but in places at least, according to Birge, Juday, and March, depths of five meters have been encountered. This rich organic stratum is continually producing nitrates, ammonia, and carbon dioxide; furthermore, any of the gas bubbles, including methane and nitrogen, rising from it afford excellent absorption of such gases before the surface is reached, as well as carrying up some bottom materials. One has but to observe the black sludge brought up by gas bubbles at the Johnson Street sewer to realize this last factor is an important one. Any mud or sludge deposits, therefore, constitute reservoirs of nutritive material from which the overlying water is being continually enriched.

"Third - Another consideration has to do with the standing crop of weeds in the lakes. In protected places the surface may be completely covered with floating weeds, and they provide vast amounts of food as they die and decay. That they tend to cause heavy plankton production is well shown by some of the other southeastern Wisconsin lakes."

- 1944. The second report of the Governor's Committee, covering work done from July 1943 to July 1944, was prepared and published as before. This report "strengthened the conviction that inorganic forms of nitrogen and phosphorus are the main factors in providing fertilizing elements for algal blooms." The report summarized the lake studies made as follows:
- 1. "Those lakes having large drainage areas and/or receiving domestic sewage or sewage plant effluents are most productive of plankton.
- 2. "Those lakes receiving sewage or sewage plant effluents are most productive of nuisance blooms, mainly due to blue-green algae.
- 3. "Inorganic nitrogen and phosphorus were found to be critical factors in the productivity of lakes, the inorganic nitrogen appearing to be a limiting factor in regard to the amount of growth which could be produced and inorganic phosphorus acting largely as a governor upon the rate at which growths occurred.
- 4. "For Lake Waubesa, the most heavily fertilized lake in the survey, during the year 1942-43 at least 65% of the inorganic nitrogen and 89% of the inorganic phosphorus entering the lake was derived from non-agricultural drainage. The contribution of inorganic nitrogen and phosphorus in the effluent and drainage reaching this lake is equivalent to that derived from a population of 140,000."

The second report also summarized significant laboratory and experimental studies, and made

proposals for future work. However, the program of the Governor's Committee was terminated in July of 1944 because the Legislature failed to provide funds for continuation, and the Madison Metropolitan Sewerage District, having contributed \$20,000 to the two-year program, decided to end its support.

In 1947, the late Thomas E. Brittingham, Jr., contributed \$50,000 to the University of Wisconsin to support a five-year study of lake pollution problems and of possible improvements in methods of sewage treatment. His contribution was supplemented by funds from the Wisconsin Alumni Research Foundation, the Lake Mendota Association, Oscar Mayer and Company, the National Institutes of Health, the Office of Naval Research, and the Wisconsin Conservation Department. The studies made since 1947 are too numerous and extensive to be reported here in detail. They have been aimed at finding explanations for nuisance conditions, improvement of methods of sewage treatment, thorough surveys of normal and of polluted lakes and streams, and the development of management practices which might be used to abate nuisance conditions in lakes and streams. Results of some of these studies will be reported to this Seminar. It is our firm belief that out of such basic studies will come the facts needed for improved practices and for practical management proce-

In 1947, Dr. A. F. Bartsch reported studies that he had made during 1945-47, with the help of G. W. Lawton, on Lake Mendota. These investigations were made to get information needed by the State Committee on Water Pollution to supplement the 1942-1944 studies of the Governor's Committee. Dr. Bartsch's conclusions were that the "relative infrequency of blooms in Lake Mendota, as compared with the remaining three lakes, is directly related to its lower rate of nutrient receipt. At the same time, it is to be noted that Lake Mendota, receiving non-urban drainage primarily, has sufficient supplies of nutrients available to allow the production of objectionable algal blooms when other environmental factors are favorable." The report also said that "algal conditions in Lake Mendota are likely to become more severe unless necessary steps are taken to decrease the rate at which nutrients enter the lake."

Also, in 1947, the Burke treatment plant, which had been shut down when Truax Field was closed in 1946, was returned to operation by the Metropolitan Sewerage District with the permission of the State Board of Health. This action was taken because of the need to build a new pressure sewer to carry sewage around the East end of Lake Monona to the Nine Springs plant; the city's volume of sewage exceeded the capacity of the existing interceptors and pressure sewers.

Return of the Burke plant to operation, and the impatience of the increasing population living on the shores of lakes Waubesa and Kegonsa, led in 1948 to the formation of the Southern Wisconsin Anti-pollution Federation. The chief objectives of this organization were: To combat pollution of lakes and streams in southern Wisconsin; to encourage enforcement of existing laws on pollution; and to secure additional anti-pollution legislation. The thirteen organizations belonging to this Federation claimed a membership of 13,000. The Federation persuaded Governor Rennebohm to request the State Committee on Water Pollution to order the Madison Metropolitan Sewerage Commission to present preliminary plans for compliance with the "Lewis" law when it was to become effective. This order was issued jointly by the State Board of Health and the State Water Pollution Committee in July of 1948. By a 2 to 1 vote, the Commission voted to petition the State agencies for a public hearing on their joint order.

The public hearing, lasting four days, was held by the State Committee on Water Pollution. The joint order was upheld, and the Metropolitan Sewerage District was ordered to submit preliminary plans by December 31, 1948 for diversion of the Nine Springs treatment plant effluent to a river not less than ten miles from the plant.

The District appealed the affirmation of the joint order, and the appeal was heard in Circuit Court, Judge A. W. Kopp presiding, in January of 1949. In March, Judge Kopp filed his decision which declared void the order of the State agencies, based on provisions of the Lewis act, because the President or Congress had not proclaimed that World War II had ended.

The decision of the Dane County Circuit Court, Judge Kopp presiding, was appealed to the State of Wisconsin Supreme Court. In August of 1951 the Supreme Court upheld the order of the State Board of Health and the State Committee on Water Pollution. Consequently, these agencies reaffirmed their previous order, and the Madison Metropolitan Sewerage District was thus forced to prepare plans for diversion of its effluent to a river not less than ten miles from its Nine Springs plant.

Much more could be written concerning events of the past nine years, but additional information is believed to be unnecessary. Effluent from the Nine Springs Sewage treatment plant is now diverted via the Badfish Creek to the Yahara River South of Stoughton.

Will it be possible to extend the limits of the Madison Metropolitan Sewerage District, or to create new sewerage districts to treat the sewage of the growing populations surrounding Madison's lakes? Can methods of sewage treatment be improved to the degree necessary to produce an effluent free from plant nutrients? Can lake and stream management practices be developed that will control nuisance conditions, but will not harm fish or aquatic life?

When we know the answers to these questions we shall be able to answer the question raised in this paper's title.

Note: References to papers or reports cited are not listed because these documents are not available in libraries. Much general information has been taken from the Master's thesis, entitled "The Madison Lakes Problem", by James J. Flannery, University of Wisconsin, Department of Political Science, 1949. Views or opinions expressed are those of the author (W. B. Sarles) and do not carry any official significance.

ALGAE BLOOMS IN LAKE ZOAR, CONNECTICUT

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The authors gratefully acknowledge the contributions of Dr. R. L. Holmes, Dr. S. L. Wilson, M. Hupfer, J. Masselli, N. Masselli and B. Parker.

In 1919, when Lake Zoar was first formed by Stevenson Dam on the Housatonic River, there was no algae problem. As the years passed, algae in the lake increased and by 1947 they were so plentiful that a serious nuisance was created for lakeside property owners. The problem was first referred to an ad hoc committee comprising the agricultural experiment station, the water commission, the fish and game department, and the health department, all of the State of Connecticut.

Although no funds were specifically appropriated, the Connecticut Agricultural Experiment Station, being a research agency, conducted a general study of the problem with the support of the other interested agencies. In July 1954 a research study was authorized.

PHYSICAL ASPECTS

Lake Zoar was formed behind a run-of-theriver hydroelectric plant built by the Connecticut
Light and Power Company in 1919. The drainage
area of the Housatonic River at this point is about
1545 sq. mi. This company also operates a pump
storage facility at Lake Candlewood. Although
Lake Candlewood is not on the Housatonic River
proper, the water in the Lake is Housatonic River
water pumped to it for storage (Figure 2).

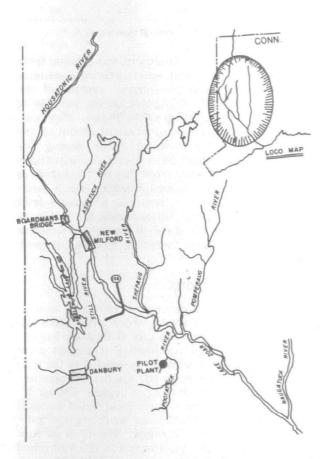


Figure 2. LAKE SYSTEM OF THE HOUSATONIC RIVER AREA IN CONNECTICUT.

Before entering Connecticut, the Housatonic River flows through the western end of Massachusetts where its waters are used extensively by industries in the Pittsfield region. The processes of nature almost completely eliminate any pollution in the water by the time it reaches the Connecticut

state line. As it flows through Connecticut, the river receives little pollution before it reaches New Milford. It is an esthetically satisfactory stream and is used extensively for fishing and recreation. However, there are two aspects of the water quality which are unusual for streams in this area: (1) Flowing through limestone formations, the water is hard (75 ppm.) compared with other streams in the state; (2) total solids average about 170 ppm., whereas other esthetically acceptable waters in the state average 65 ppm.

LIMNOLOGY

Below Boardman Bridge, the quality of the Housatonic River changes materially. The major factors causing this change were: (1) raw sewage pollution from the Town of New Milford, totalling approximately 200,000 gal. per day; (2) a quantity of organic industrial wastes from the hat manufacturing industry in Danbury and Bethel, and the trickling filter effluent from the Danbury sewage treatment plant carried by the Still River. The Shepaug, Pomperaug, and Pootatuck Rivers are relatively large-sized tributaries carrying no serious pollution. The Connecticut State Health Department has eliminated practically all pollution from development adjacent to Lake Zoar.

The lake volume is estimated to be 42,800 acreft. and the average flow of the Housatonic River at Lake Zoar is approximately 2,500 cfs, indicating that the lake waters are completely replaced every 9 days. During the algae season, the low monthly flow (550 cfs) of August and September results in a replacement of the lake volume every 40 days. However, power production causes great fluctuations in the rate of flow.

Preliminary investigation showed that during late July, as the seasonal reduction of river flow becomes apparent, it might be possible for stratification to develop. More detailed studies revealed that the stratification was transient and did not exist to an extent that would affect the problem.

TYPES OF ALGAL BLOOM

Although original complaints had specified rooted aquatic weeds as the cause of the disagreeable condition, algal blooms were an equally objectionable part of the problem. By mid-July Hydrodictyon was found in great quantities in all the shallow, quiet waters where it was intimately associated with aquatic weeds such as Elodea, and Ceratophyllum. Large mats moved from place to place during changes of flow. Early in the season large quantities of Microcystis and Anabaena also appeared (Curry and Wilson, 1955) usually in the upper four feet of lake water.

The most direct approach would have been the addition of algicides such as copper sulfate. It was calculated that a single treatment would cost

\$15,000. Laboratory tests with fungicides, bacteriocides such as quarternary ammonium compounds, and a wide variety of experimental compounds showed some promise. Almost all of the materials tested are included in the list reported by Palmer (1956).

PHOSPHATE ASPECTS

Repeated tests indicated the level of nitrates and phosphates in Lake Zoar was similar to that noted in other lakes at the time of blooming. The well-known ability of some blue-green algae to fix atmospheric nitrogen indicates that the addition of phosphate alone might result in intense blooms in a lake like Zoar where all other aspects of the environment are favorable for bloom development. Phosphorus concentrations varied from 12 to 41 parts per billion (ppb) and averaged about 25 ppb. (Table 2).

Table 2. CONCENTRATIONS OF PHOSPHORUS IN LAKE ZOAR

Sample date, Flow		Phosphorus,	
1950-51	c.f.s.	p.p.b.	lb./day
Sept. 2	372	33	66
Oct. 2	462	15	25
April 27	3,670	30	594
May 7	1,880	41	416
June 4	1,270	26	178
June 26	1,100	22	131
July 3	1,060	25	143
July 13	1,250	31	209
July 18	809	12	52
July 23	2,300	18	224
July 30	987	22	117
Aug. 10	312	28	47
Average	1,289	25	183

The volume of phosphates entering the streams in Connecticut from sewage outlets has reflected the increased use of synthetic detergents. Most of the sewage that is discharged to the Housatonic River in this region is given a high degree of treatment. Such treatment oxidizes the organic phosphate to the mineral form making it available as fertilizer to the aquatic plants.

The bulk of the phosphates fed into Lake Zoar come from the Still River and the Pootatuck River. The estimated amounts of phosphorus coming into Lake Zoar from various sources are shown in Table 3. The values from the analyses and computation check well in view of the fact that many more individual samples were averaged for main stream values than for tributary values. Contributions of

the Still River to the total phosphorus available in Lake Zoar account for about 40 per cent of the total. If this source were eliminated, the phosphorus concentration in Lake Zoar would be reduced to 15 ppb. The critical concentration of phosphorus for blue-green algae growth is believed to be about 10 ppb. A reduction in phosphate should have an important effect on the algae bloom.

Table 3. SOURCES OF PHOSPHORUS CONTRI-BUTORY TO LAKE ZOAR

Location	P, lb./day	Cumulative P, lb./day
Housatonic River at Boardman		
Bridge	98 a/	
Sewage from New Milford	9 ~	
Still River.	77	
Housatonic River at Rt. 133	232 a/	184 ≠
Shepaug River	2 -	
Housatonic River above Pootatuck	167 a/	186 ≁
Pootatuck River	10 -	
Pomperaug River	5	
Housatonic River at Lake Zoar	183 a/	201 ≠

a/ Based on analyses and flow rates at these points.

Lake Candlewood is filled with water taken from the Housatonic River immediately below Boardman Bridge. The soluble phosphorus content of the Housatonic River at this point during periods of high water is probably less than 30 ppb. However, Lake Candlewood produces no algal bloom of any consequence. Determinations made during the algae season indicated phosphorus concentrations of only 8 ppb. It is suggested that the phosphate is utilized early in the season by diatoms which eventually sink to the bottom, removing phosphate from surface waters. Since little additional water is pumped into Lake Candlewood during the summer the supply of nutrient is not renewed as it is in Lake Zoar.

CHANGING CONDITIONS

During the past eight years pollution control agencies have taken action which may affect the problem in the future. The city of Danbury has enlarged and renovated its sewage treatment plant so the quantities of sewage by-passed to the Still River are materially reduced. Six hat manufacturing industries which discharge organic wastes directly to the Still River have constructed screens and settling basins which keep much organic matter from entering the stream. The town of New Milford and two state institutions in the watershed have constructed sewage treatment facilities.

A dam has been constructed on the Housatonic immediately below the confluence of the Shepaug, creating another lake of similar characteristics to Zoar. The new lake is in a position to intercept the phosphorus load originating in the Still River. Already there have been reports of a degree of blooming but no lessening of bloom in Zoar has been noted.

PHOSPHATE REMOVAL

The physiographic features at the sewage treatment plant of the State Hospital at Newtown on a branch of the Pootatuck River offered an ideal opportunity to study the removal of phosphate by the method of Lea, et al (1954) on a pilot plant scale. This method uses alum and lime to precipitate phosphate, with alum being largely recovered and recycled. A pilot plant was constructed on the site (see figure 3) with a special legislative appropria-

The ponds were 400 ft. long by 100 ft. wide with depths from 4.5 to 8.0 ft. The pilot plant and pond design enabled the simulation of conditions in Lake Zoar and provided sufficient flexibility for a variety of controlled experiments. Both ponds were filled with brook water and one of them was inoculated with bloom algae from Lake Zoar. Both developed a good growth of algae, but no field experiments were undertaken before the floods of August and October 1955. The August flood des-

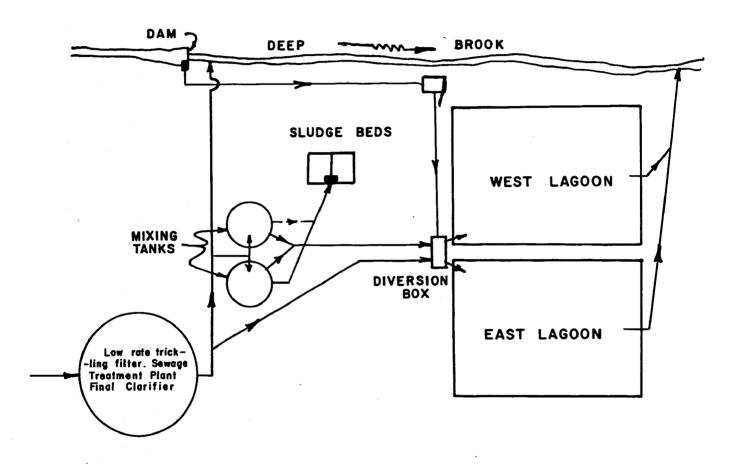


Figure 3. SCHEMATIC DIAGRAM, ALGAE CONTROL PILOT PLANT. Fairfield State Hospital, Newtown, Connecticut

tion. Two ponds were built for use in field experiments to establish the critical levels of phosphate for algae blooms. One pond was to receive sewage or secondary sewage effluent and the other was to serve as a control and receive only creek water or secondary effluent from which phosphate had been stripped.

Preliminary studies were made in 4000 gal. batches in the pilot plant under conditions of 10-minute mixing and 2-hour settling. Using 200 ppm alum, a removal of better than 95 per cent from effluents having a phosphorus content of 3.5 to 4.5 ppm was accomplished.

troyed the pond embankments and the piping from the pilot plant and brook to the ponds.

Negotiations to rebuild the plant were carried out with the United States Engineering Department under their flood rehabilitation program and the project was not back in operation until 1957. During this reconstruction, it was decided to reduce the size of the lagoons to make them more amenable to control. Before any substantial program could be carried out with the restored facilities, an unexplainable increase in the phosphate content of the brook was indicated by analyses from an outside laboratory. The reliability of these analy-

ses had to be accepted in the absence of evidence to the contrary, but no source of the phosphate has ever been established. Nevertheless, the advisability of carrying on the projected field program was questionable since the authorized time of the expenditure was running out. It was therefore deemed prudent to conclude the project while there was still time to make close down expenditures. Such a decision was appropriate because serious doubt had been cast on the feasibility of keeping phosphate in waterways below the critical level for blooms simply by removing phosphate from specific sources with some suitable process.

NEW POSSIBILITIES FOR ALGICIDE TREAT-MENT

Also relevant to the decision was the new opportunity for chemical treatment afforded by the construction of the Shepaug Dam. For the volume of water involved, chemical treatment no doubt will always be very costly, but can be more easily justified for two large lakes with twice the recreation-

al development. Since Lake Zoar is downstream from the new lake behind the Shepaug Dam, it will probably only be necessary to apply algicides to the upper lake to achieve a degree of control in both lakes. Use of the Candlewood Lake discharge as a mixing facility might provide economically attractive chemical treatment for both lake developments.

REFERENCES

Curry, J. J. and S. L. Wilson, 1955. Effect of sew-age-borne phosphorus on algae. Sewage and Industrial Wastes 27(11): 1262-1266.

Palmer, C. M., 1956. Evaluation of new algicides for water supply purposes. Jour. Amer. Water Wks. Assoc. 48:1133-1137.

Lea, W. L., G. A. Rohlich and W. J. Katz, 1954. Removal of phosphates from treated sewage. Sewage and Industrial Wastes <u>26(3):261-275</u>.

KLAMATH LAKE, AN INSTANCE OF NATURAL ENRICHMENT

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The City of Klamath Falls is located in south central Oregon just north of the California State line. The City is named for a turbulent, mile-long stretch of water, now known as the Link River, that lies completely within the city limits. This short river flows between Upper Klamath Lake at the north of the City and Lake Ewauna at the south. Lake Ewauna in turn drains into the Klamath River. In addition to the annual mean flow of 1.1 million acre-feet passing through the Link River, there is a flow of more than 200 thousand acre-feet that leaves Upper Klamath Lake by way of the "A" canal of the Klamath Reclamation Project's irrigation system (Stanley, 1954).

A flow of water of this magnitude would appear to be adequate to supply dilution water for the disposal of wastes from a population of 35 thousand. Despite the fact that there is neither urban development nor heavy industry on the Upper Klamath Lake drainage system, and that there are far more acres of wild than of agricultural land within the basin, the Link and Klamath Rivers carry an organic load so heavy as to present serious problems

even before the discharge of the City's wastes.

A brief summary of geographical information and a map (Fig. 4) are presented as an aid in understanding the scope of this problem. The total area of the Upper Klamath Lake Basin that drains into the Klamath River in Oregon is approximately 7450 square miles. The major source of water flowing into the Klamath River is Upper Klamath lake, which lies in the southwestern portion of the Upper Klamath Basin. Upper Klamath Lake is approximately 23 miles long and 5 miles wide and at the northern end joins Agency Lake, which is approximately 5 miles long by 3 miles wide. The mean depth of Upper Klamath Lake is eight feet and the maximum depth approaches sixty feet. The Lake is supplied by a major tributary, the Williamson River, as well as a number of smaller streams. The Williamson is fed by the Sprague and the Sycan Rivers. Agency Lake receives flow from Wood River and the Seven Mile Canal besides numerous small streams. The Klamath Basin was formed by block faulting followed by local volcanic activity. To the north of Agency Lake, toward the

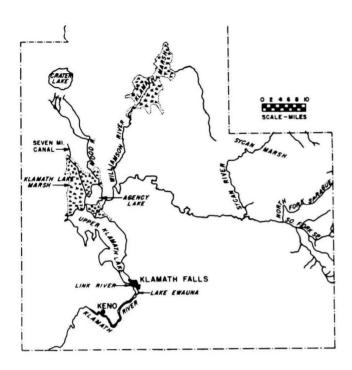


Figure 4. MAP OF THE UPPER KLAMATH LAKE BASIN IN OREGON.

Crater Lake-Mount Mazama site, are vast pumice fields with much subsurface drainage into, or at least in the direction of, the lakes. In the bottom of both lakes and along both margins of Upper Klamath Lake are innumerable springs, some warm, some mineral, some of artesian nature, that feed into the lakes along multitudinous fault joints.

Outflow from Upper Klamath Lake is regulated by a low dam on the Link River, constructed by the Bureau of Reclamation in order to utilize the Lake as a reservoir. Since 1917 the dam has been operated by the California-Oregon Power Company to maintain the surface of the Lake between elevations of 4143.3 and 4137.0 feet. This allows an active storage capacity of 483 thousand acre-feet, which is a little more than half the present total storage capacity of the Lake basin.

The discharge of the Link River flows through shallow Lake Ewauna and into the Klamath River. The Klamath River flows through approximately 16 miles of flat land to Keno, Oregon, where it enters a 236 mile long canyon that it has cut through both the Cascade and the Coast Ranges, debouching into the Pacific Ocean at Requa, California.

THE BIOLOGICAL PROBLEM

For at least sixty years the algal populations of Upper Klamath Lake have been sufficiently large to cause comment and speculation as to the cause and effects of the growth. During the summer months the Lake has been unsightly and has had an

offensive odor. The discharge from the Lake has contained a heavy organic load, high in nitrogen. This has caused portions of the upper reaches of the Klamath River to become periodically anaerobic despite the supersaturation with oxygen that occurs at the exit from the Lake during daylight hours. Because of this situation a serious problem has arisen in the disposal of metropolitan wastes.

From the correspondence files of the Bureau of Reclamation comes evidence of early concern for this situation. As early as January 1906, J. B. Lippencot, Supervising Engineer for the then Reclamation Service (now Bureau of Reclamation) wrote from his Los Angeles office in part: "...I wish to call your attention to the fact that these waters are filled with some sort of organic matter, either animal or vegetable, so that they have a decided green appearance. They are cutting up ice now that has been formed from these waters, and we will probably be asked to use this ice next summer. Last summer we were troubled a great deal up there with stomach complaints. For that reason I am somewhat interested in the sanitary analysis of the water. This same material in the water appears to have some fertilizing properties...."

Analyses were made at this time by the U.S. Geological Survey office in Berkeley and interpreted by them as follows in June 1906: "...the amounts of nitrogen found as free and albuminoid ammonia seem rather large, but as you say there is no possibility of sewage contamination, this would not necessarily condemn the water. The manner in which these ammonias made their appearance and the comparatively small amount of oxygen required point toward this organic matter being of vegetable origin."

On the 25th of August 1928 the Oregon State Board of Health reported to the Bureau on samples from the Link River as follows: "...You will note that our results showed no colon bacillus and therefore no sewage contamination in the water. The dissolved oxygen in the water was between 72% and 80% of saturation...

"... The murky condition of the water is principally due to an algae (sic) or watergrass growth. This algae was found to be mostly spirogyra with a few ulothrix and chroococcus present..."

The year 1933 saw a study of a problem arising from enormous midge populations in the Lake and an effort was made to associate the midge with the plant problem. At this time the plankton algae were identified by Dr. E. I. Sanborn of Oregon State College as: Anabaena spiroides var. crassa, Pediastrum sp., Melosira sp., Navicula sp., Microcystis sp. and Aphanizomenon sp.

The study that has provided most of the infor-

mation reported here was initiated in 1955. It represented an effort to define the problem and to establish cause and effect relationships wherever possible with the hope that ultimately information might be forthcoming that would make possible realistic control measures.

Although this survey has named 29 species of algae as occurring in the plankton with some seasonal regularity, four species of the Cyanophyta (Aphanizomenon n. sp., Gloeotrichia echinulata, Anabaena circinalis, Anacystis (Microcystis or Polycystis) aeruginosa), and two species of diatoms (Asterionella formosa and Melosira sp.) have been most abundant at different seasons.

The prime offender in the summer bloom has been the <u>Aphanizomenon</u>. Germinating spores and single filaments were found in the plankton by the end of March or by the first of April (Table 4). By

Table 4. PHYTOPLANKTON DURING THE GROWING SEASONS OF 1956 and 1957 IN COUNTS PER MILLILITER 1

	Link River		Densest pop	
Date	Aphanizo- menon 2/	Total plankton	Aphanizo- menon 2/	Total plankton
1956				
Apr.	2,5	27.0	9.5	1,921
May	1,481	1,816	3,420	4,150
June	17,637	18,549	18, 349	18, 586
July	11,701	12,837	33, 163	34, 459
Aug.	20,744	20, 759	194, 756	196, 360
Sept.	11,607	11,903	56, 438	57,665
Oct.	3,1173/	3, 196	43,756	43,846
Nov.	21.6	37.5	78.2	85.8
1957			:	
Mar.	0.0	2, 145 ⁴ /	0.0	. 5/
Apr.	0.0	42.74/	0.0	64.5
May	3.0	28.0	841	877
June	6,929	6,935	12,591	12,597
July	12,499	12,570	5/	5/
Aug.	10,748	10,805	5/	5/
Sept.	11,353	12,249	5/	5/
Oct.	1.093	1,124	4, 132	4, 222
Dec.	0.0	41.54	0.0	1,1574/

The study reported herein was supported by the Oregon Klamath River Commission, the California Klamath River Commission, the Klamath County Court, The City of Klamath Falls, The Klamath County Chamber of Commerce, and the Oregon Agricultural Experiment Station.

the end of April the population had developed to the point that several thousand filaments were contained in each liter of water. By the end of May a

million and a half filaments per liter were present and in June, July, August and September the counts were in the tens of millions. Toward the end of September the counts started to drop and through the months of October and November the plants died in great masses. In December, Aphanizomenon was represented only by an occasional spore. The overwintering spores appeared to be produced in September and October. Spores formed earlier than this could not be germinated in culture and their viability in nature is suspect. The density of the population in various areas of the lake varied from day to day as wind drift and currents caused characteristic aggregations. In August 1956 (Table 5) the count at the head of Link River was 20 thousand filaments per milliliter but the densest population in the Lake, measured along Eagle Ridge. was 195 thousand filaments per milliliter.

Table 5. STANDING CROP OF PHYTOPLANKTON AT VARIOUS STATIONS IN UPPER KLAMATH LAKE, AUGUST 22-24, 1956

Station	Counts per ml
1 Link River	20,760
3 Wocus Bay	10, 176
5 Mid Lake	28, 387
6 Algoma Inlet	2,475
7 Algoma Point	21,986
8 Eagle Ridge	196,361
10 North End	5,864
11 Mouth of Williamson River	76
12 Shoalwater Bay	36, 179
14 Pelican Bay	2,077
17 Agency Lake-Westside	191
19 Agency Lake -Eastside	10,643

Throughout the growing season a rather minor depletion of the population occurred through the escape of water into the Link River and finally into the Klamath River. The generally held belief that the limnoplankton is rapidly destroyed or removed in flowing water was not supported by the evidence from an exploratory trip down the Klamath River. Aphanizomenon, considerably reduced in number it is true, survived the trip down the turbulent river very well and was present in relatively large amounts at the mouth of the river. Informants at Requa stated that the characteristic color of the water was distinguishable several hundred yards out at sea.

Wherever along its length the river had been impounded, whether behind a dam or in a backwater or slough, the water had produced blooms comparable with that in Upper Klamath Lake. It can be predicted that the construction of additional impoundments on the Klamath River will greatly increase the organic load of this already impossibly burdened stream and will probably bring an

^{2.} Counts of Aphanizomenon are in filaments per mil-

^{3.} Midlake Station, no sample taken at the Link River.

^{4.} Primarily Asterionella formosa.

^{5.} Also Link River.

end to fish production in this stream.

The weight of organic material exported from Upper Klamath via the "A" canal and the Link River has been calculated from data taken for a very different purpose. While known to be inaccurate, it is also known to err on the conservative side by a factor of between ten and one hundred. In the six month growing period from May through October of 1956, over seven thousand tons of naturally produced organic material were exported. In August (the month of highest production) alone, 23 hundred tons were exported.

Analysis of the freshly dried algal material demonstrated the reason for the surprise of the earlier investigators concerning the high nitrogen content of the water in the absence of contamination by sewage. Over sixty percent of the dry weight of the algae was protein (Table 6). Every-

Table 6. PARTIAL ANALYSIS OF THE COMPOSITION OF FRESHLY DRIED ALGAE FROM UPPER KLAMATH LAKE

Composition	 %
Crude Protein	61.10
Ash	5.73
Phosphorus	0.60
Calcium	0.54
Potassium	1.08

one experienced in pollution problems can visualize the septic conditions that occur from time to time in this basin.

Two additional problems need to be mentioned. The first was the occurrence of limberneck disease affecting thousands of the water fowl inhabiting the lake. This paralytic condition results from the presence of a population of the type C strain of Clostridium botulinum on the bottom. The development of this population is favored by the anaerobic conditions caused by the decomposition of highly proteinaceous organic material. At times the number of birds affected has been so great that, in order to prevent decimation of the water fowl population, it was necessary for the public to join forces with employees of state and national agencies to rescue the diseased birds. The second problem concerns a very potent toxin produced by the bloom organisms. No concrete evidence was obtained as to the effect of this toxin on the biota of the Lake or River but experiments with mice proved that ingestion of the algal material was quickly lethal and intraperitoneal injections of the aqueous extract almost instantaneous in causing death. Since the toxin is water soluble and thermostable it could well be the explanation for the stomach complaints mentioned earlier. At the present time all the water for domestic and industrial use in the area of Klamath Falls is obtained from deep wells. This situation minimizes the danger of the toxin from the public health standpoint but also indicates the probable difficulty of using the Lake water for any purposes other than irrigation or power generation.

CHEMICAL CONSIDERATIONS

The concentration of dissolved minerals in the lake water declined somewhat during the summer as these materials were incorporated into the plankton organisms. After the decline and death of the summer bloom population in the late fall, the concentration of minerals increased in the winter.

There was a similar characteristic seasonal shift in the pH of the Lake water. During the periods of great plankton development the alkalinity generally increased, while during winter it decreased. This shift was attributed to three factors: 1) precipitation came mostly in late fall, winter and early spring and the run-off had an alkalinity approaching neutrality, 2) heavy drainage of humic waters from the marsh areas at the same season increased the H-ion level, 3) in summer the hydroxyl-ion exchange in photosynthesis of the bloom population quickly depleted the H-ion supply and raised the alkalinity (Table 7).

Table 7. SEASONAL VARIATION IN THE CHEMISTRY OF UPPER KLAMATH LAKE WATER

Measurement	Summer (June-July-Aug.)	Winter (Jan.–Feb.–March)		
Temperature in °C.	18.0° - 28.0° 8.2 - 9.9	2.0° - 8.5° 7.6 - 9.2		
,	Values in ppm			
Nitrate	0.06 - 0.2	0,12 - 1,0		
Ammonia	0.155 - 1.290	0.061 - 1.674		
Phosphate	0.0	0.0		
Iron	0.1 - 0.7	0.2 - 2.0		
Calcium	1.6 - 3.2	2.5 - 5.5		
Potassium	2.6 - 37.0	3.0 - 43.5		
Magnesium	5.0 - 29.5	5.0 - 15.0		

Repeated chemical analyses of the water from many sample stations failed to establish the presence of chemical factors such as exceptional concentration or combination of nutrient mineral elements that might explain the exceedingly high productivity. A general statement seems warranted that there are no inorganic chemical constituents that could be singled out as being responsible for this condition. The Lake water was low in calcium, extremely low in dissolved phosphorus, and quite high in chloride. Other nutrients were present in merely nutritionally adequate amounts but could not be described as abundant. A review of the idiosyncrasies of the various influent waters soon concentrated attention upon the great influx of humic waters into the Lake during late winter and early spring.

SOURCES OF HUMIC WATER ENTERING UPPER KLAMATH LAKE

About the northern end of Upper Klamath and Agency Lakes are a number of drainage systems (some mentioned previously) whose basins include approximately 136 thousand acres of marsh land (Table 8). Some agricultural land has been included in this estimate of natural marsh but this is land irrigated by natural flooding and has retained most of the characteristics of natural marsh. Water from these lands drains directly into the local drainage system. In addition to this unmodified marsh there are an additional 92 thousand acres of muck soil agricultural land (Table 8) that has

Table 8 - ACRES OF NATURAL MARSH AND OF IRRIGATED AGRICULTURAL LANDS DRAINING INTO UPPER KLAMATH AND AGENCY LAKES

Natural marsh			
Wood River	24,300 acres		
Upper Klamath Marsh	·		
(Seven Mile Canal)	2,100		
Sycan Marsh	,		
(Sycan River)	25,000		
Klamath Marsh			
(Williamson River)	85,000		
Irrigated agricult	tural land		
Wood River	50,000		
Upper Klamath Lake	11,000		
Sycan Marsh	18,000		
Sprague River	13,000		

been drained and diked. This land lies flooded in fall and winter and is pumped dry in late winter or early spring. The drainage water, like the natural marsh drainage contains humic leachate from the marsh soil. This marsh soil, or muck, consists of a fiberous, Typha peat, several feet in thickness lying on top of variously assorted layers of tuff, pumice and diatomite.

Determination of the composition of the organic leachate has proven quite difficult and only incomplete information is available. A comparison of the analyses of samples showed the content of total dissolved solids per unit volume in samples taken in summer to be twice that in those taken in winter (or early spring). The nitrogen content in summer was more than double that of the winter, but the phenolic content decreased somewhat in the summer (Table 9).

EFFECT OF HUMATES ON THE GROWTH OF ALGAE IN CULTURE

Algal culture media were modified to include a proportion of humic water and the final pH was ad-

Table 9. PARTIAL ANALYSIS OF THE SOLIDS IN SEASONAL SAMPLES OF HUMIC WATERS FROM THE WILLIAMSON RIVER

	January	August
Total solids Kjeldahl nitrogen	103 mg/1 0.7 mg/1	247 mg/1 1.7 mg/1
	Spring	Summer
Aromatic hydroxl groups	3.15 mg/1	2.5 mg/1

justed to a range in which our test organisms grew well. It was found that the production, measured as dry weight of algae, was 70% greater in the humic-enriched cultures than in the unmodified controls. The advantage gained by adding the humates could be overcome by quadrupling the mineral content of the control medium but the added growth appeared in the controls only after three weeks of growth. The vigorous growth stimulated by the presence of the humates rapidly exhausted the relatively lower mineral resources of the experimental medium while the four-fold fortification of the controls allowed ample minerals for a longer period of growth.

The mode of action of the humates remains to be elucidated. Some workers (Burk, et al, 1932a, 1932b) have suggested that the high iron content of humic waters provides considerable stimulation of plant growth. We know, however, that there was sufficient iron in the formulation of the control medium to provide for very vigorous growth of the test organisms for a month. The control medium likewise contained a shotgun mixture of minor elements and it seems relatively unlikely that the presence of one of these was the basis of the stimulation by the humates.

It is our belief, as yet unsupported by concrete evidence, that the action of the humates as chelating and buffering agents was primarily responsible for the observed stimulation. It is also possible that colloidal absorption by the humates inactivated metabolic end products that otherwise would have exerted an anti-metabolic influence in older cultures. Of these three possible explanations, chelation appears the most reasonable. The benefit derived from buffer action as well as action of a protective colloid should appear as a prolongation of the logarithmic phase of the growth curve of the cultures. Contrarily, stimulation in our cultures appeared in the earliest stages of growth and the cultures tended to become exhausted or to stale more quickly than the controls.

CONTROL OF THE ALGAL NUISANCE

Briefly stated, the problems involved in the control of an algal population on such a large scale are manifest. The application of chemical control agents would be expensive in material, manpower and equipment. The dangers to the associated biota would be great. Since such measures would give only temporary relief, effects of the long term use of chemical materials would require intensive study before application. It seems necessary to consider other approaches that lack at least some of these objectionable features. The first suggestion concerns the diversion of all influent humic waters to reservoirs from which they would be

eventually distributed for the irrigation of agricultural land. Besides the very probable major reduction in the algal population that would be effected there would result a conservation of large amounts of humates that are presently being lost.

A second possible approach would be the discharge into the lake or its tributaries of a slurry of some colloidal inorganic material, such as Bentonite clay, in sufficient amount to reduce the light intensity at the bottom over large areas of the lake to below the compensation point. The economic feasibility of these two suggestions must be determined by a study of the engineering considerations involved.

REFERENCES

Burk, D., H. Lineweaver, and C. K. Horner. 1932a. Iron in relation to the stimulation of growth by humic acid. Soil Science 33: 413-453.

Burk, D., H. Lineweaver, and C. K. Horner. 1932b. The physiological nature of humic acid stimulation of Azotobacter growth. Soil Science 33: 455-487.

Stanley, Lewis A. 1954. Report of the Oregon Klamath River Commission on Water Resources and Requirements of the Upper Klamath Basin.

RECENT CHANGES IN THE TROPHIC NATURE OF LAKE WASHINGTON - A REVIEW*

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ABSTRACT

Until a few years ago Lake Washington was a relatively clear, oligotrophic lake. Since the turn of the century, Seattle and other communities bordering the lake have been discharging sewage in increasing amounts, and as of 1957 the lake was receiving the discharge from 10 sewage treatment plants plus septic tank drainage representing 76,300 people. Lake Washington has responded with increased algal productivity and is now eutrophic.

Detailed limnological studies in 1933, 1950 and 1955-1958 illustrate the nature of the lake response. Since 1950, prominent changes have occurred, particularly in 1955 when, for the first time, there appeared an increased growth of phytoplankton made up mainly by the blue-green alga Oscillatoria rubescens, a notorious indicator of pollution in many lakes. A regular annual increase in crop of algae has occurred since this time. Due to the increase in productivity, the transparency of the water has decreased, and oxygen consumption and nutrient release in the hypolimnion during summer stratification have progressively in-

creased. The hypolimnetic oxygen deficit has shown an almost threefold increase since 1933: 1.18 mg/cm²/month in 1933, 3.13 in 1955. In 1957, for the first time, the deepest waters became anaerobic for a short period.

If fertilization of the lake continues, it may be expected that within a very few years nuisance blooms of algae will be produced, unsightly scums and decaying foul-smelling masses of algae will accumulate in places along the shoreline, and well developed stagnant conditions in the hypolimnion will occur. The formation in 1958 of the Municipality of Metropolitan Seattle, a corporation designed to handle sewerage services on a metropolitan basis, was, in part, recognition of the urgency of the Lake Washington situation. Long-range plans include the ultimate diversion of all sewage entering the lake.

INTRODUCTION

The purpose of this paper is to review some of the limnological changes that have been observed in a lake which is undergoing nutrient enrichment by progressively increasing additions of sewage

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discharge resulting from the growth of a large metropolitan area. The situation of undesirable lake response to the waste products of a growing human population has been observed in many places in this country and elsewhere; Hasler (1947) described several such cases. Typically, the addition of nutrient rich material from domestic sewage will hasten the natural process of eutrophication of a lake. In a matter of a few years, depending largely upon the amount of sewage effluent, the size of the lake and the amount and nature of inflow water from natural sources, the lake may respond with increased growths of algae particularly of the blue-green variety, larger populations of zooplankton, depletion of dissolved oxygen in the hypolimnion, and the replacement of game fish with the scrap fish variety. The recreational uses of the water are destroyed, nuisance blooms of algae occur to the extent that undesirable odors are produced from the accumulation of decaying masses, and property surrounding the lake declines in value. The community is faced with the choice of losing the lake permanently as a recreational and esthetic benefit or to correct the situation by the diversion of sewage inflow from the lake. At the present time, Lake Washington is in the process of deterioration from sewage enrichment, but as yet algal blooms have not reached the nuisance level.

The population of Seattle is estimated to rise from 572,000 in 1957 to 1,250,000 in 1980. At present, approximately 20 percent of the population of the metropolitan area reside in the Lake Washington drainage basin; ultimately 50 percent will reside there. In 1957, the flow from ten secondary sewage treatment plants, serving 64,300 people, was discharging into the lake. In addition, the effluent from 4,000 septic tanks representing 12,000 persons reaches the lake. At the beginning of the century, raw sewage entered the lake, but in 1926 steps were taken to remove this discharge and the project reached completion in 1941. However, during periods of appreciable rainfall, combined sanitary and storm-water sewage systems overflow into the lake and contribute about 3.5 percent of the total annual sewage generated in the area.

Lake Washington has received limnological attention from time to time since 1913. Recently, several studies have been conducted, particularly since it was recognized that the lake was responding to ever increasing amounts of treated sewage. The first published observation of the lake consisted of a single series of chemical and plankton samplings taken August 9, 1913 (Kemmerer, Bovard and Boorman, 1923). A detailed investigation was first made in 1933 when an annual study was conducted of the seasonal variation in temperature, oxygen, nutrients and the net plankton (Scheffer, 1936; Robinson, 1938; Scheffer and Robinson, 1939). Unfortunately, phytoplankton estimates were recorded as relative frequency and are

not quantitative. In 1950 a further study was made of the seasonal fluctuations of the physical, chemical, and biological conditions which included quantitative estimates of the phytoplankton and zooplankton populations (Comita, 1953; Anderson, 1954; Comita and Anderson, 1959). In 1952, studies by the Pollution Control Commission involved estimates of bacterial pollution in selected areas of the lake and in tributary rivers (Peterson, Jones and Orlob. 1952). The same group investigated surface chemical and chlorophyll conditions, in 1953, at 26 stations spread over the entire lake (Peterson, 1955). The Department of Oceanography at the University of Washington has obtained data since 1950 on oxygen, temperature and salinity in connection with a study on the intrusion of salt water into the lake through the Lake Washington Ship Canal (Rattray, Seckel and Barnes, 1954; Collias and Seckel, 1954). During the summers of 1955 and 1956, members of the Department of Zoology in cooperation with the Pollution Control Commission sampled the lake for temperature, oxygen, phosphate and phytoplankton (Edmondson, Anderson and Peterson, 1956; Sylvester, Edmondson and Bogan, 1956).

Because of the marked increase in algal productivity noted in the summers of 1955 and 1956, a long-term study was initiated in September 1956 by Drs. W. T. Edmondson and Joseph Shapiro. The immediate purpose of this study has been to obtain a detailed description of the productivity and nutrient condition of the lake and to compare it with past and future conditions (Edmondson, 1960; Shapiro, 1960). Also in 1956, it was realized that a potentially serious civic situation existed, and an engineering firm was appointed by city, county and state officials to prepare a report on a long-range program of sewerage and drainage improvements for the metropolitan Seattle area (Brown and Caldwell, 1958). A particularly useful feature of this study to further limnological investigation of the lake was the preparation of a nutrient budget giving the increment of nitrogen and phosphorus from analysis of measurements made in inlets, sewage effluent and in the lake itself.

During the preparation of this paper, the writer has drawn extensively from those reports cited in the references. Especial thanks are due to Dr. W. T. Edmondson, University of Washington, and Dr. Joseph Shapiro, The Johns Hopkins University, for allowing the writer to examine papers in press and unpublished data from recent years and to utilize some of these data in this review.

PHYSICAL CONDITIONS

Lake Washington lies in an elongate glacial trough sculptured by the Vashon ice sheet. The surface area is 8762 ha, maximum depth 65.2m, mean depth 32.9 m, and volume $2,884.2 \times 10^6 \text{m}^3$ (Comita and Anderson, 1959). In cross section,

the lake is W-shaped rather than the more conventional U-shaped. After the withdrawal of the Vashon glacier, the lake underwent a transitory marine phase, evidence for which was found in deep sediment cores (Gould and Budinger, 1960).

Typically, the lake has an epilimnion of 10 m, thermocline 10 m, and hypolimnion 40 m. Stratification usually takes place in late March or early April and remains so until November or December. After stratification breaks down in late autumn, full circulation takes place during the winter at a temperature of 5 to 8°C. Transparency of the water fluctuates with the season and is highest in winter and lowest during periods of summer algal blooms. The arithmetic mean of Secchi disc measurements made in 1950 was 3.82 m (Comita and Anderson, 1959).

LIMNOLOGICAL CONDITIONS TO 1950

Generally speaking, there were few variations noted in most conditions between the studies made in 1933 and 1950 apart from those which might be expected from normal annual variations due to differences in climatic conditions from year to year. For this reason and because the 1933 study did not include quantitative estimates of the phytoplankton, 1950 has been chosen as representing typical lake conditions prior to its response to added nutrient income. Some of the deteriorative changes between 1933 and 1950 will be mentioned in a later section.

During 1950, the pH was near neutrality except in midsummer when it rose to above 8 due to removal of CO_2 by photosynthesis. Bicarbonate fluctuated between 16-32 mg/L HCO₂. Dissolved oxygen was abundant at all depths, although a minimum of 5.6 mg/L was measured at 55 m during summer stratification. The phosphate concentration remained near 15 ppb PO₄.P during winter mixing but decreased to 0-1 ppb in the epilimnion during summer. An accumulation of phosphate occurred in the hypolimnion as summer stratification progressed, and a maximum of 23 ppb was reached. Data on nitrogen concentrations during this year are scanty, but the maximum observed during the winter was 560 ppb NO3.N which decreased to less than measurable quantity in the epilimnion at times in the summer. The fluctuations of chemical conditions described above compare favorably with those described in 1933 with the exception that the nutrient concentration was lower in the earlier year, i.e., approximately 8 ppb PO₄.P and 150 ppb NO3.N during winter mixing.

The annual fluctuation in quantity of phytoplankton from measurements of cell volume and chlorophyll followed a typical bicyclic pattern. The maximum bloom occurred in spring, a midsummer minimum followed, and a second bloom of lesser extent was observed in late August. Diatoms made up the greatest part of the spring bloom. Common among these were Stephanodiscus niagarae, Melosira varians, M. italica, M. italica var. tenuissima Fragilaria crotonensis, Rhizosolenia gracilis, Asterionella formosa, Synedra acus and Cyclotella bodanica. The late August bloom was composed mainly of dinoflagellates, especially Peridinium divergens and to a lesser extent Ceratium hirundinella. Among the blue-green algae, Oscillatoria agardhi and Phormidium sp. were common but did not contribute significantly to the size of the major blooms. The Chlorophyta, or green algae, were common at times but quantitatively unimportant. In contrast, Anabaena lemmermanni and a number of diatoms were of greatest relative importance in the summer phytoplankton during 1933.

LIMNOLOGICAL CONDITIONS SINCE 1950

Some of the significant changes observed in the lake since 1933 are summarized in Figure 5. It is immediately obvious that the most drastic changes have occurred since 1950.

The standing crop of phytoplankton has increased significantly in recent years as indicated by measurements of phytoplankton volume and chlorophyll concentration (Fig. 5, Panels A and B). During the summer months of July, August, and September, the phytoplankton volume in the epilimnion increased from 0.6 mm³/L in 1950 to 1.6 mm³/L in 1955 and further to 4.2 mm³/L in 1956, the last year for which data on phytoplankton volume are available at the present time. Judging from the measurements of chlorophyll, a further large increase in phytoplankton crop occurred in 1958.

As mentioned previously, the major components of the phytoplankton in 1933 were diatoms and Anabaena lemmermanni. In 1950, diatoms made up the spring bloom and dinoflagellates the late summer bloom. Phormidium sp. and Oscillatoria agardhi were conspicuous components in 1950, but the largest crops attained by the blue-green algae were $0.33 \, \text{mm}^3 / L$ in February and again in September. The maximum standing crop of all phytoplankton observed during the period of sampling in 1955 was $2.89 \text{ mm}^3/\text{L}$ in July of which 2.78mm³/L or 96 percent was composed of the bluegreen alga Oscillatoria rubescens. O. rubescens remained abundant throughout the samplings and Aphanizomenon flosaquae became dominant in September.

O. rubescens is a well-known algal species, especially in the Swiss lakes, as an indicator of pollution. Late in the nineteenth century, Zurichsee, Switzerland, changed in a relatively short period of time from an oligotrophic to an eutrophic lake. The phytoplankton increased rapidly and was composed largely of O. rubescens. About 10 years later, the cladoceran Bosmina coregoni was re-

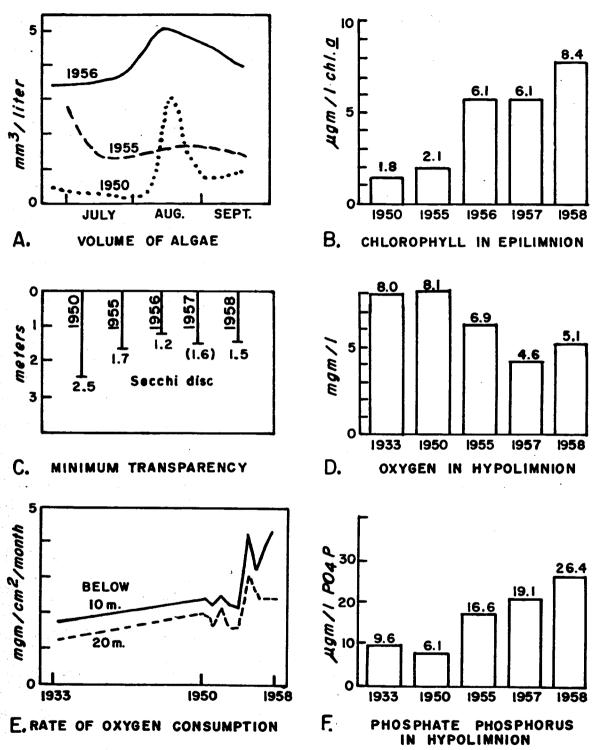


Figure 5. CHANGES THAT HAVE OCCURRED IN SOME PROPERTIES IN LAKE WASHINGTON IN RECENT YEARS.

Panel A - Phytoplankton volumes in the epilimnion.

- B Average chlorophyll concentration in the epilimnion during July, August and September of each year.
- C Minimum observed transparency during each year.
- D Mean dissolved oxygen concentration below 10 m at the end of summer stratification.
- E -Oxygen deficit between June 20 and August 20 below 10 m and below 20 m.
- F Mean concentration of phosphate below 10 m at the end of summer stratification. (Modified from Edmondson, 1960).

placed by B. longirostris. A somewhat similar situation has been observed in Lake Washington although the change of cladocera species occurred prior to the appearance of O. rubescens. As early as 1940, B. longirostris replaced B. longispina Leydig (= B. coregoni longispina). By 1955, phytoplankton volume had increased significantly and O. rubescens became dominant. Deevey (1942) has shown from fossil evidence that the same change in Bosmina species occurred during the transition of Linsley Pond from oligotrophic to eutrophic.

The transparency of a lake is related in part to the quantity of plankton material present. Due to increased algal growth in Lake Washington, Secchi disc measurements are becoming less (Fig. 5, Panel C). The minimum transparency in 1950 was 2.5 m whereas in 1956 it was 1.2 m. One measurement of 4.1 m was made in 1913. Data are not available for 1933.

Seasonal changes in the hypolimnetic waters of lakes are in part related to epilimnetic processes. Organic material, originally produced or derived from the epilimnion, settles into the deeper waters. This material consists of living and dead phytoplankton and zooplankton, fecal material from zooplankton, and other debris entering the lake. As the dead organic matter decomposes by bacterial action and living material respires, oxygen is consumed and nutrients other than those absorbed by bacteria and living algal cells are released to the waters. Some fraction of the material is sedimented to the bottom. It is therefore not surprising that some workers have found the consumption of oxygen in the hypolimnion in a series of lakes to be related roughly to the mean quantity of seston (Hutchinson, 1938) or standing crop of net plankton (Rawson, 1942) in the epilimnion although the true relationship is probably with the total production in the epilimnion rather than with the standing crop.

In Lake Washington after 1950, it can readily be seen that during summer the average concentration of dissolved oxygen in hypolimnetic waters has become much less, and in 1957, the deepest waters became anaerobic (Fig. 5, Panel D). The rate of removal of dissolved oxygen from the hypolimnion, termed the oxygen deficit, has been calculated below 10 m and below 20 m for all years since 1933 that sufficient data are available and expressed on an areal basis (Edmondson, Anderson and Peterson, 1956; Edmondson, 1960). In earlier years, when the lake was relatively transparent, it is likely that some photosynthesis took place in the upper part of the hypolimnion so that values calculated below 10 m in those years are probably minimal and the 20 m estimate is more reliable. However, as the lake has become less transparent, especially since 1955, and because an interesting oxygen minimum between 10-15 m has increased in magnitude particularly since 1956, the values below 10 m may be better estimates in more recent years. In any case it is apparent that a significant increase in the deficit has taken place since 1933 (Fig. 5, Panel E). Although the largest rate (3.1 $\text{mg/cm}^2/\text{month}$ 0₂ consumed below 20 m) was recorded in 1955, the general trend is that of progressive increase.

One of the features of the distribution of dissolved oxygen in the lake is the previously referred to appearance of a metalimnetic oxygen minimum beginning usually early in June and growing progressively stronger until its disappearance at the end of summer stratification. Shapiro (1960) has reported an increase in the magnitude of this minimum correlated with conditions of increasing eutrophication, the minimum recorded level reaching 2.2 mg/L at 15 m in 1957. Shapiro has concluded that the agent responsible for this minimum is the respiration of a metalimnetic population of non-migrating copepods and that the recent increase in magnitude of the minimum is due to an increase in the numbers of copepods.

The concentrations of all measured nutrients have been increasing greatly in recent years, and phosphate, in particular, has shown a marked increase. This is adequately illustrated by noting that phosphate accumulation in the hypolimnion during summer stratification has steadily increased since 1950 (Fig. 5, Panel F). The cause for this has, of course, been attributed to greater amounts of organic material produced in the epilimnion due to an increase in the nutrient income to the lake with a resultant rise in the amount of material settling into the hypolimnion, subsequently decomposing and releasing nutrients to these waters. Epilimnetic waters, in all years studied, contained low, sometimes undetectable, quantities of phosphate, indicating near maximum utilization of nutrients in the trophogenic zone during the summers. As a result, the major differences between the years are reflected in changes in the hypolimnion. For example, the maximum concentration of phosphate reached in the deepest waters was 23 ppb in 1950, 89 ppb in 1957, and 74 ppb in 1958. Although the maximum recorded measurement in 1958 was somewhat less than that in 1957, greater concentrations were found at intermediate depths in 1958 so that the total phosphate present was greater than before.

Although the principal source of nitrogen and phosphorus to Lake Washington at the present time is from the major tributary streams, the amount contributed by sewage discharge would in the near future greatly exceed that from natural sources. Figure 6 shows the enrichment of the lake by phosphorus from natural sources and from sewage sources, and the projected trend from sewage in future years if effluent continues to enter the lake (Brown and Caldwell, 1958). Table 10 gives the increment of N and P to the lake from natural and sewage sources (Brown and Caldwell, 1958). The

estimated amount of nitrogen entering the lake from sewage sources in the past 40 years has more than doubled, and the phosphorus income has almost tripled. The contribution of nitrogen from sewage is expected to increase from the 1957 level of 6.5 percent of the total input from all sources to an ultimate value of 35.2 percent; phosphorus from 43 percent to 92 percent.

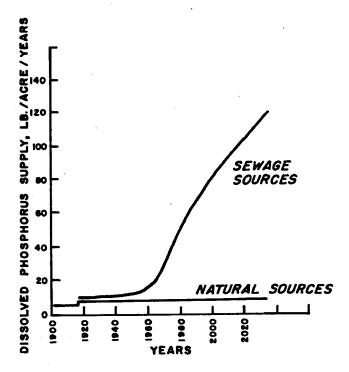


Figure 6. THE TREND OF PHOSPHORUS EN-RICHMENT IN LAKE WASHINGTON FROM NAT-URAL AND SEWAGE SOURCES. In 1916, the Cedar River was diverted to the lake which accounts for the rise in phosphorus from natural sources since that time (from Brown and Caldwell, 1958).

Table 10 - CONTRIBUTION OF TOTAL DIS-SOLVED NITROGEN AND PHOSPHORUS TO LAKE WASHINGTON.

Category	Date	Nitrogen*	Phosphorus*
Natural sources	1957	5,850	151
Sewage sources	1916-1930	181	42
1	1931-1940	240	54
	1941-1950	226	74
	1957	409	114
Total	1957	6, 259	265
Mean content in lake	1933	1,970	114
	1957	4, 180	184

Nutrient values expressed as 1000 lbs/year. Values taken from Brown and Caldwell (1958).

In summary, Lake Washington is exhibiting an increase in productivity, resulting from fertilization with treated sewage. The mean quantity of phytoplankton and nutrient concentration within the

lake has progressively increased, especially within the past decade. Also, significant annual increases have occurred in oxygen consumption and phosphorus accumulation in hypolimnetic waters during summer stratification. If fertilization continues, the series of changes already observed can be expected to increase in magnitude until serious conditions of algal nuisance blooms, odors, scums, bottom stagnation, etc. develop.

The only practicable manner at present of controlling fertilization in a lake of this size is to divert the sewage elsewhere. The Pollution Control Commission, in 1956, established a policy requiring that all sewage treatment plants eventually divert their effluent from the lake into Puget Sound. However, a project on this scale requires the cooperation of several responsible communities bordering the lake and for this reason it has long been felt by many interested parties in Seattle that a central sewerage authority is necessary to deal adequately with the problem. In 1957, an act providing for the formation of Metropolitan Municipal Corporations was passed by the Washington State Legislature. By a vote of the residents of the greater Seattle area on September 9, 1958, the Municipality of Metropolitan Seattle was established and empowered to plan, finance, and administer sewage services on a metropolitan basis. If the present schedule of "Metro" is maintained, more than 80 percent of the sewage discharges presently entering Lake Washington will be diverted by 1962. Limnologists will be keenly interested in the biological response of the lake as the nutrient income declines during and after sewage diversion.

REFERENCES

Anderson, G. C. 1954. A limnological study of the seasonal variations of phytoplankton populations. Ph.D. Thesis. Univ. Washington. 268 pp.

Brown and Caldwell. 1958. (Civil and Chemical Engineers). Sewage disposal in Lake Washington. Chapter 10 in Metropolitan Seattle sewerage and drainage survey. A report for the city of Seattle, King County and the State of Washington on the collection, treatment and disposal of sewage and the collection and disposal of storm water in the metropolitan Seattle area. Seattle, pp. 219-247.

Collias, E. E. and G. R. Seckel. 1954. Lake Washington Ship Canal data. Univ. Washington, Dept. Oceanogr. Spec. Rept. No. 2. pp. 1-27.

Comita, G. W. 1953. A limnological study of planktonic copepod population. Ph.D. Thesis. Univ. Washington. 195 pp.

Comita, G. W. and G. C. Anderson. 1959. The seasonal development of a population of <u>Diaptomus ashlandi</u> Marsh, and related phytoplankton cycles in Lake Washington. Limnol. Oceanogr., 4(1): 37-52.

REFERENCES (Cont'd.)

Deevey, E. S. 1942. Studies on Connecticut lake sediments. III. The biostratonomy of Linsley Pond. Amer. J. Sci., 240: 233-264, 313-338.

Edmondson, W. T. 1960. Changes in Lake Washington following an increase in the nutrient income. Proc. Int. Assoc. Theor. Appl. Limnol. (In press).

Edmondson, W. T., G. C. Anderson and D. R. Peterson. 1956. Artificial eutrophication of Lake Washington. Limnol. Oceanogr., 1 (1): 47-53.

Gould, H. R. and T. F. Budinger. 1960. Control of sedimentation and bottom configuration by convection currents, Lake Washington, Washington. J. Mar. Res. (In press).

Hasler, A. D. 1947. Eutrophication of lakes by domestic drainage. Ecology. 28: 383-395.

Hutchinson, G. E. 1938. On the relation between the oxygen deficit and the productivity and typology of lakes. Int. Rev. Hydrobiol., 36: 336-355.

Kemmerer, G., J. F. Bovard and W. R. Boorman. 1923. Northwestern lakes of the United States: Biological and chemical studies with reference to possibilities in production of fish. Bull. U. S. Bur. Fish., 39: 51-140.

Peterson, D. R. 1955. An investigation of pollutional effects in Lake Washington. Washington Pollution Control Commission. Tech. Bull. No. 18. 18 pp.

Peterson, D. R., K. R. Jones and G. T. Orlob. 1952. An investigation of pollution in Lake Washington. Washington Pollution Control Commission. Tech. Bull. No. 14. 29 pp.

Rattray, M., Jr., G. R. Seckel and C. A. Barnes. 1954. Salt budget in the Lake Washington Ship Canal system. J. Mar. Res., 13: 263-275.

Rawson, D. S. 1942. A comparison of some large alpine lakes in western Canada. Ecology 23:143-161.

Robinson, R. J. 1938. Chemical data for Lake Washington. Typewritten report. Univ. Washington Library.

Scheffer, V. B. 1936. The plankton of Lake Washington. Ph.D. Thesis. Univ. Washington.

Scheffer, V. B. and R. J. Robinson, 1939. A limnological study of Lake Washington. Ecol. Monogr., 9: 95-143.

Shapiro, J. 1960. The cause of a metalimnetic minimum of dissolved oxygen. Limnol. Oceanogr. (In press).

Sylvester, R. O., W. T. Edmondson and R. H. Bogan. 1956. A new critical phase of the Lake Washington pollution problem. The Trend in Engineering, 8 (2): 8-14.

Specific Problems in Rivers

ALGAE IN RIVERS OF THE UNITED STATES

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Concern for the quality of river waters in the United States increases as the many uses for these waters are intensified. For example, the algae, which are microscopic organisms common in rivers, have a significance that is being emphasized in relation to self purification, radioactivity, water treatment, fish and other aquatic animal life, industrial and sewage pollution, taste and odor production, and recreational uses of the stream water.

Unlike studies of lakes, there have been few detailed or long continued investigations of algae in American rivers. The general impression regarding planktonic algae in rivers seems to be that they are relatively few in number, that many of them are transients multiplying in lakes and merely existing passively and temporarily in streams, or that they are primarily benthic organisms that have broken away from their moorings at the bottom and sides of the stream.

The unattached algae in rivers have been considered to be so few that often they have been recorded in numbers per liter or cubic meter, rather than per ml. In such data converted to numbers per ml, the algae are generally fewer than 100. A real difficulty in evaluation, however, has been the dearth of recorded information on the abundance of algae in rivers.

It is necessary to know the algal population of rivers quantitatively and qualitatively, if we are to be concerned with assessing their value or their significance as stream purifiers, pollution indicators, or as excessive growths; their role in water treatment problems; and their function as the primary food for fish. It can be important to know the algal population of a river before any major change is made in the use of the stream. Also, we need to know the algal population of rivers throughout the year and not merely for the warmer months.

This type of basic information has been almost completely lacking in the United States. It should be obtained over a long period of time, for all months of the year, and for several representative locations on all important rivers of the country. The same procedure for enumeration must be used at all stations if the various records are to be easily compared. While records of all species would be helpful, it can hardly be expected that a comprehensive plan could be carried beyond gen-

eric level since much more time would be required for identification of species.

Fortunately some standardized information on stream algae is now becoming available. In 1957 the National Water Quality Network program was inaugurated by the Public Health Service. Sampling stations on 16 rivers and the Great Lakes were chosen where water could be obtained at regular intervals for various examinations, one of these being for plankton organisms (Palange and Megregian, 1958).

During 1958, the Network's first year, plankton analyses were made once per month of samples from 47 stations in the United States (Figure 7). Rivers included in the survey during the first year were the Arkansas, Colorado, Columbia, Delaware, Detroit, Hudson, Merrimack, Mississippi, Missouri, Ohio, Potomac, Red, Rio Grande, Savannah, Snake, and Tennessee. The numbers of stations and rivers sampled are being increased gradually so that the accumulated data may be representative of a larger part of the country. Moreover, samples are now being collected semimonthly rather than monthly.

The clump count procedure is used in recording the plankton organisms as numbers per ml. In this method of enumeration, isolated cells and colonies are recognized as the unit. Analysis is commonly made on unconcentrated samples at a magnification of 200X. Two longitudinal strips (representing about 200 fields) of the Sedgwick-Rafter slide are examined, rather than only 10 fields as often recommended.

The first annual compilation of data has been published (Anon., 1958) together with a statistical summary of selected data (Anon., 1959). The results of the second year's work are completed and ready for publication. From these records some information as to the numbers and kinds of algae in the country as a whole has been obtained for presentation at this time.

Over a period of two years the average count per monthly sample was 3625 algae per ml, the first year averaging 3460 and the second year 3850. The monthly average ranged from 1376 to 6745. November, December, and March had the lowest average counts for the two year period while April, September, and October had the highest. The

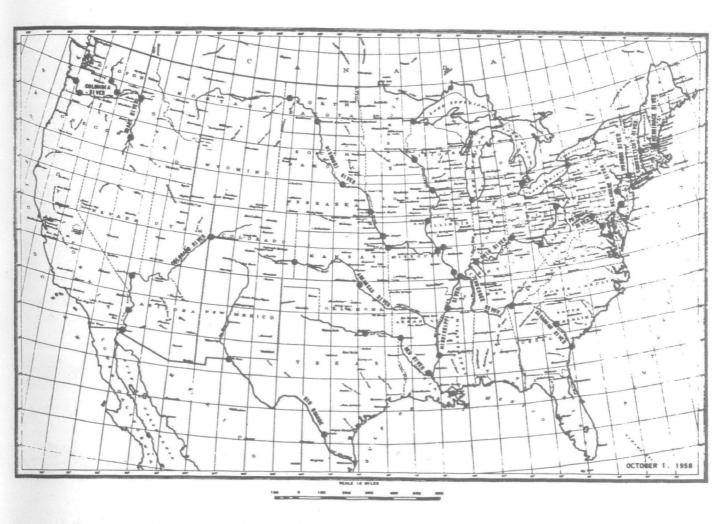


Figure 7. PHS WATER QUALITY BASIC DATA PROGRAM NETWORK OF SAMPLING STATIONS

indications are that the plankton algae in rivers average much larger numbers than has generally been assumed. During the early winter the count may be lower but seasonal fluctuation is less than had been suspected.

Occasional very high counts may raise the averages significantly. For example, the median for all rivers during the first year was only 1460 per ml, in contrast to a mean of 3460 per ml. Some individual counts exceeded 20,000, but only one was above 50,000.

The five rivers with the highest average counts for the first year were the Mississippi, Arkansas, Merrimack, Missouri, and Columbia, while the five with the lowest were the Red, Detroit, Colorado, Savannah, and Tennessee rivers. High plankton counts are to be expected in rivers enriched by land drainage from productive soils, or by treated or untreated sewage from cities and towns, particularly where toxic industrial wastes are not abundant. This relationship of plankton count to enrichment will undoubtedly become more evident when data are available from more fre-

quent sampling and more sampling stations over a period of several years. Plankton fluctuations due to sporadic variations in weather or other environmental factors will then have less influence on data representing average plankton populations.

The same genera of algae tend to be dominant in all of the rivers of the country, and only a limited number of genera comprise the list of the dominant forms that are generally encountered. Leading the list are several diatoms, the first six in order of decreasing frequency being Cyclotella, Synedra, Melosira, Navicula, Asterionella, and Stephanodiscus. Other algae high in the list are Anacystis, Chlorella, Chlamydomonas, and Ankistrodesmus.

Various comparative studies of the plankton records are being made at the Taft Center. One of these deals with the planktonic green algae of the Mississippi and the Ohio rivers. Another is concerned with the number of genera of diatoms in several river systems.

An indication of what the plankton counts may

reveal is found when four-month averages for the year 1959 of the planktonic green algae at 8 stations on the Mississippi River are compared. The average count decreases from the uppermost station at Red Wing, Minnesota, to the lowermost station located at New Orleans, La. (Figure 8). The average count per ml for the whole year was 2087 at Red Wing, and 151 at New Orleans, the count at each intermediate station being less than the one

above. In the upper portion of the river, represented by the first 3 stations, the number of genera and the count were considerably lower during the Nov.-Feb. period than for the remainder of the year. However, from East St. Louis to New Orleans for each third of the year, both the number of genera and the count were fairly constant at all stations.

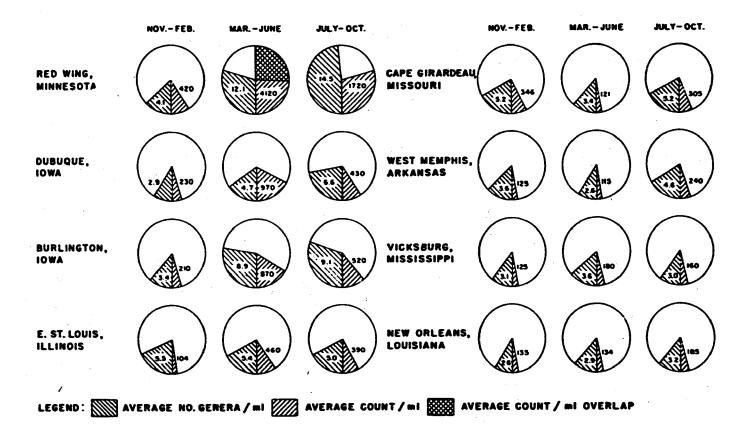


Figure 8. PLANKTONIC GREEN ALGAE -- MISSISSIPPI RIVER -- 1959

For the Ohio River the planktonic green algae, averaged by four-month periods for a total of 2 years, indicate a very distinct difference of population in 1958 and 1959 (Figure 9). During the March-June and the July-Oct. periods of 1959, the average numbers of genera and the average counts were much higher than in the previous year. It is believed that either an increase in algal nutrients or a decrease in toxic materials, such as certain industrial wastes in the rivers, was responsible for the large growth of green algae during 1959.

A report from Pittsburgh, Pa., (Anon., 1960) for September 1959 states that bass, bluegills, and minnows were returning to the Ohio, Monongahela, and Allegheny rivers. This change was ascribed to the temporary absence of acid wastes in the

streams while the steel mills were idled by a prolonged strike in the steel industry.

Determination of the effect of particular factors on the biota of rivers will require detailed studies that are planned for that purpose. The basic data will provide background information giving, for example, an idea of the range in numbers and kinds of algae in different seasons, in different years, and at different locations for all of the larger rivers of the United States.

Appreciation is expressed to Dr. Louis G. Williams, Aquatic Biologist, Sanitary Engineering Center, who is in direct charge of the plankton analyses, for the planning and preparation of Figures 8 and 9.

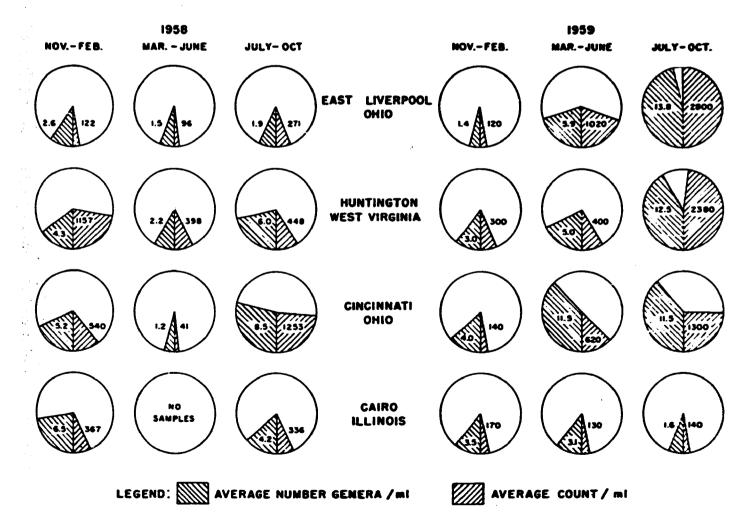


Figure 9. PLANKTONIC GREEN ALGAE -- OHIO RIVER

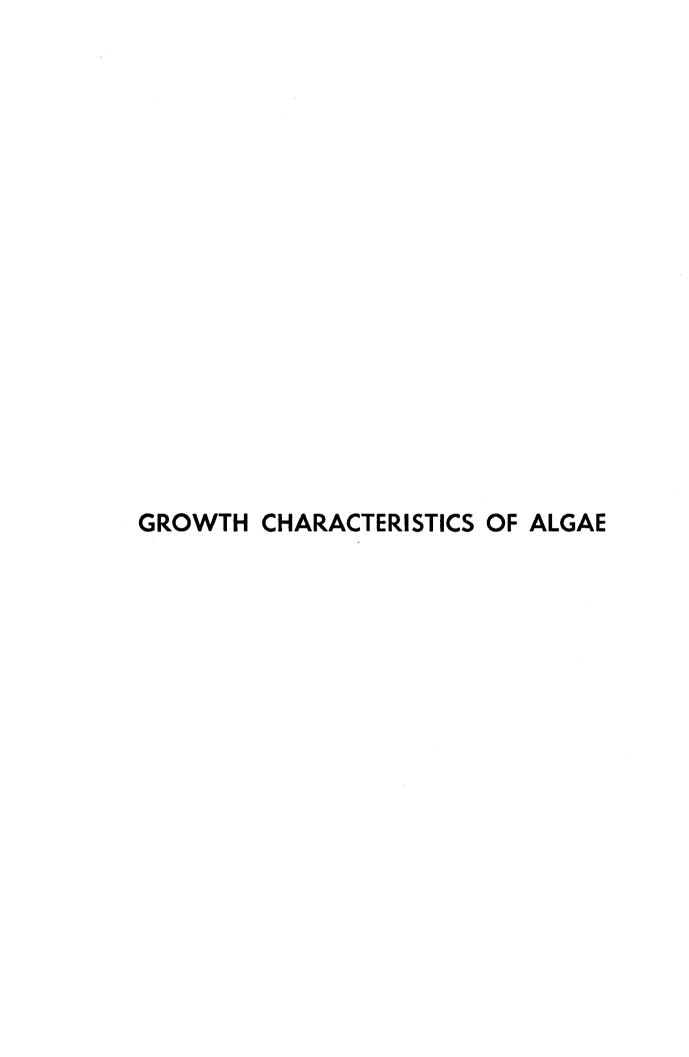
REFERENCES

Anon., 1958. National water quality network, annual compilation of data, October 1, 1957-September 30, 1958. U. S. Dept. Health, Educ., and Welfare, Public Health Service Publication 663.

Anon., 1959. National water quality network, Supplement 1, Statistical summary of selected data, October 1, 1957-September 30, 1958. U. S. Dept. Health, Educ., and Welfare, Public Health Service Pub. 663, Suppl. 1.

Anon., 1960. Percolation and runoff. Jour. Amer. Water Wks. Assn. 52(1):38,40 P&R.

Palange, R. C. and S. Megregian. 1958. Monitoring of stream water quality -- USPHS program. Jour. Amer. Water Wks. Assn. 50:1214-1219.



Nutrition

FUNDAMENTAL CHARACTERISTICS OF ALGAL PHYSIOLOGY

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Control of biological communities as complex as those encountered in natural waters is at best a difficult and at worst an impossible occupation. Yet increasing requirements for water have placed a growing burden of performance on biologists responsible for maintaining water sources in the best possible condition for human consumption and enjoyment. The dual role of predicting and controlling the biological balance in natural waters serving as sources to metropolitan areas requires a thorough appreciation of the subtleties of ecological interaction. The consequences of changing a rapidly moving stream into a relatively stagnant lake, by what may appear to be the praiseworthy conservation practice of damming, are often as difficult to predict as the results of the obviously deleterious practice of using a river as a drain for organic wastes. In spite of what can be determined about the physical and chemical characteristics of a given water source, the degree to which it can initiate the rapid growth of a bacterium or the even more spectacular "bloom" of an alga depends on the specific environmental requirements of these microorganisms. Such requirements, contrary to common belief, are not only nutritional. Accumulating evidence suggests other avenues through which the environment can affect the growth of microorganisms. Sound judgements of cause and effect will be reliable in direct proportion to the completeness of knowledge concerning the nature of the individuals comprising the community. For this reason it is appropriate to focus attention on the physiology of the organisms, especially the algae, which play a major role in determining the aquatic community, and reciprocally affect the quality of the water that supports them.

It would be most desirable if an approach to the fundamental principles of algal physiology could be made with the ambition and hope for intellectual satisfaction that can be enjoyed by physicists in developing the unified field theory. No equivalent advance in biological theory is available for developing such insight into the physiology and biochemistry of any group of organisms, much less the algae. Except for the evolutionary concept of Darwin, and the modern consequences of Mendel's experimentation, biology in general, and physiology more especially, has not been graced with the intermeshing laws and certainties with which the physical sciences have made such striking advances in the control of nature. However, from the masses of data that are recorded in the thousands of biological publications now issued yearly, certain principles seem to be reliable enough for science to accept with some measure of security.

In making certain principles worth stating the student of algal physiology is faced with a bewildering array of differences in the organisms he studies. Chlorella pyrenoidosa was one alga a few years ago, now it is many. Chlorophyll a, the sine qua non of photosynthesis until recently, is now at least 3 chlorophylls a and probably more. One pathway for hexose metabolism is now ancient history, and a given organism may have three or more alternatives in the initial steps, not to mention new modifications in the reactions of the Krebs cycle. Much of this information is recorded and discussed in detailed reviews and books dealing with algal physiology (Fogg, 1953; Krauss, 1958; Myers, 1951; and Smith, 1951). It is not the purpose of this paper to dwell on these details, but rather to discuss some of the generalities, which might be dignified by the term principles, that can be useful in interpreting the performance of algae, and to mention those areas where our understanding is weak, but where the rewards of knowledge should be great in enhancing our present abilities to predict and control the algae in natural populations.

TAXONOMY

Although it may at first seem out of place in a discussion of algal physiology, a few comments should be made about the classification of the organisms which we call algae. There are 7 divisions of algae, each distinct enough in its own characteristics to be worthy of independence as major segments of the plant kingdom ranking with the ferns, gymnosperms, and seed plants. Morphology plays a role in this system of classification, but unlike the criteria for the separation of the groups of higher plants the more critical differences between the divisions of algae have been the peculiarities of their physiology. The bluegreen algae (Cyanophyta) were very early recognized as different from the green (Chlorophyta), red (Rhodophyta), or brown (Phaeophyta) algae because of their pigmentation. Less obvious, but clearly separable because of color, were the yellow-green (Chrysophyta) and the golden-brown (Pyrophyta) groups. Only the Euglenas (Euglenophyta) are not primarily thought of as being independent because of a peculiarity of pigmentation. More careful study over the years has revealed more and more discomforting exceptions to the initially obvious pigmentation. The blue-green algae possess phycobilins essentially the same as those in red algae, and the red algae contain the blue bilins of the blue-greens. Even certain green algae have been shown to contain the blue-green pigments and the cryptomonads are occasionally blue or red (Allen, 1959). Such modifications in the pigmentation of individuals have not resulted in changes in classifications but rather in an awareness of the breadth of biochemical capacity of the members of widely different groups. The nature of the reserve substances of the algae believed to be taxonomically characteristic has not received the attention given the pigments, but it seems certain that further investigations in this area also will show that the organisms have as much disrespect for classification systems in this area as in the case of the pigments. X-ray powder diagrams of the reserve material paramylon, long considered the characteristic of the Euglenophyta, have demonstrated its identity with the hydroglucan of yeast (Kreger and Meeuse, 1952) and leucosin the Chrysophycean reserve is essentially the same as the laminarin of the brown algae (von Stosch, 1951). Other taxonomic criteria based on physiological characters too are showing weakness.

This does not mean that all individuality is necessarily lost by the breaking of man-made taxonomic fences. Evidence of remarkable individuality is also available. The complete absence of brown algae from fresh waters is a characteristic that some might devoutly wish could be shared by other groups. The unique coupling of CO₂ fixation in either light or dark with the normal illuminated photosynthetic intermediates by Euglena is another case in point (Lynch and Calvin, 1953).

The situation concerning the overlapping of physiological characteristics between the major algal divisions is, however, of less interest here than the problem of the identity of individual species. It is becoming increasingly obvious that the former classification of species particularly among the unicellular algae, is only a general guide to the requirements and capabilities of the organisms which fall within the available morphological descriptions. The list of algae at the culture collection at the University of Indiana (Starr, 1960) gives some indication of the situation. Numerous strains, many of them different in their growth characteristics, are listed for Anabaena, Nostoc, Lyngbya, Chlorella, Euglena, Chlamydomonas, Pandorina, and others. Few of the details of the strain differences have been thoroughly investigated, but the degree to which strains of the same species can differ is seen in the tabulation of data for two strains of Chlorella in Table 11 (Sorokin, 1960).

At present we are attempting a revision of the classification of the entire genus Chlorella based on physiological characters alone. It may well be

that the only satisfactory measure of individuality in any genus of algae will be the growth performance under rigidly controlled environmental conditions in specific media.

Table 11 - CHARACTERISTICS OF GROWTH, PHOTOSYNTHESIS, AND RESPIRATION FOR HIGH AND LOW TEMPERATURE STRAINS OF CHLORELLA PYRENOIDOSA

	Strain of Chlorella		
Characteristic	Emerson	7-11-05	
Temperature optimum for:	Degrees C		
Growth	25-26	38-39	
Photosynthesis	32-35	40-42	
Endogenous respiration	30	40-42	
Glucose respiration	30	40-42	
Growth rate at light	Number of doubling		
saturation:	per day		
at 25° C	3.1	3.0	
at 39° C	-	9.2	
Rate of apparent photosyn- thesis at light saturation:	mm ³ 0 _{2/} mm ³ cells/ho		
at 25° C	43	47	
at 39° C	rapidly declining	170	
Rate of glucose			
respiration:	$\mathrm{mm}^3\mathrm{O}_{2}/\mathrm{mm}^3$ cells/h		
at 25° C	4.5	8	
at 39° C	1.6	18	
Saturating light inten-			
sity for growth:	Foot candles		
at 25° C	500	500	
at 39° C	-	1400	

What fundamental principle of algal physiology does this reveal to the student of algal growth in natural waters? It is simply that the performance of a given organism cannot be predicted on the basis of morphological description alone. Neither can it be predicted by references to published physiological characteristics of that species or even of the larger groups. Strain differences preclude certain identification without thorough physiological study of the organisms. A "bloom" of Anabaena flos-aquae in two bodies of water may, or may not, be the result of matching environmental conditions. This will depend on the physiological identity of the strains growing in the separate locations.

Studies of artificially induced mutants have shown that large numbers survive in enriched media and may be peculiarly adapted to survival there (Wetherell and Krauss, 1957). The high number of mutations which can be expected in a large microorganismal population should in time permit a selection of strains peculiarly adapted to the conditions in which they were generated. Environments can be expected to select and perpe-

tuate strains as well as to determine which species will survive.

GROWTH RATES

Regardless of the identity of the organisms the phenomenon most familiar to those concerned with water supplies is the periodic rapid growth of algae. The capacity for generating the population explosion known as the "bloom" apparently is a quiescent characteristic of the organism which manifests itself only when conditions are ideal. In the laboratory, in suitable media and in appropriate apparatus, the maximum potential growth rate of a species can be achieved and maintained indefinitely (Myers and Clark, 1944; and Myers and Graham, 1959). While it might be unwise to give the term normal to a perpetual exponential growth rate, at least the cells from such cultures give uniquely standard populations which have been useful for physiological study.

In nature or, in a self contained laboratory culture, the period of maximum growth rate endures for only a restricted period during the growth of the alga. Figure 10 gives a typical plot of the growth curve of an alga growing in a finite medium in which, in time, one or more of the requirements for growth have become limiting. This sigmoid curve may be distorted somewhat in nature by

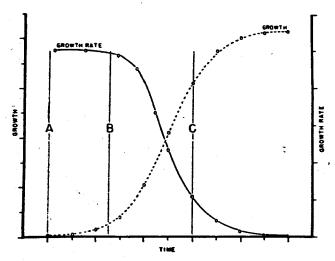


Figure 10. THE RELATION BETWEEN THE PLOTS OF A TYPICAL SIGMOID GROWTH CURVE OF AN ALGAL CULTURE AND THE GROWTH RATE, K, CALCULATED FOR THAT SAME CULTURE USING THE FORMULA, $\log_2 \frac{C_2}{C_1} = K(t_2 - t_1)$.

special conditions, but it is essentially a model for what one might expect during a bloom. Several features of the curve are worth mentioning. First, it should be recognized that the period of maximum growth rate is given by the portion of the curve between A and B. After this time, although the culture continues to grow, the rate is actually falling.

The second curve plotted on the ordinates gives a measure of the rate of change in the growth rate from the optimum, during the exponential phase, to the falling rate during the arithmetic phase. However during this period of falling growth rate the yield of the culture, in terms of increase in weight or volume per unit time, is still increasing because of the larger population base. This means that an algal bloom may be increasing most rapidly long after the conditions that initiated it have ceased to be optimum. It is easy to see how an analysis of waters at the time of maximum bloom may overlook the most important factors causing it.

The fact that a plot of the growth curve of a population of algae is smooth tends to obscure another facet of the multiplication process which may affect the course of growth. Single-celled algae cultured in the laboratory in so called "steady state" cultures are in all stages of maturity. Newly released autospores and mature cells as well as all possible intermediates are mixed and growing together. The rates of respiration, photosynthesis and growth of the cells of different ages are not the same. There is reason to believe that the requirements of growth of cells at different ages may also be different (Sorokin and Krauss, 1959). The coincidence of conditions suitable for the maximum rates of progression through all stages may be rare in natural waters. Conversely the lack of a suitable environment for the passage of the cells through one stage of development may halt a bloom when otherwise conditions are ideal.

The laboratory technique for studying the cells at various stages is known as synchronization. By alternating periods of light and darkness the culture can be made to adjust itself to the point where all of the cell divisions take place at the same time, and, consequently, all of the cells at any given time are the same age. The success of this technique depends on the fact that cell division is inhibited in the light and all cells capable of division divide rapidly in the dark (Sorokin and Krauss, 1959). The useful cycles of light and dark are of the order of 12 hours of light and 12 hours of dark, but the ideal time varies somewhat with the species and with the conditions of culture. This time interval is of the order of natural day/night cycles, and it is quite possible that algae growing at their optimum rate in nature may become synchronized to a considerable degree. Although harder to detect, synchronization can exist in the filamentous blue-greens such as Anabaena or Rivularia, as well as in the essentially unicellular forms such as Microcystis.

The degree of natural synchronization and the opportunities this facet of algal physiology offers to the biologist interested in control are unknown at this time. How much the suddenness of a bloom may be due to the precipitate division of cells that

have been accumulating the necessary metabolites for explosive growth and division can be determined only by investigating this aspect of algal physiology in natural populations.

AUTOTROPHIC AND HETEROTROPHIC GROWTH

A feature of the algae that is basic to an analysis of their physiology is the general capacity for both autotrophic and heterotrophic growth. The capacity for photosynthesis sets the algae apart from most other microorganisms, especially the fungi. Though colorless species are found in all of the major divisions, except the brown algae, the use of light as an energy source and water as the hydrogen donor (photolithotrophy) is the dominant mode of life. Most species, if the medium is satisfactory, act as facultative chemoorganotrophs using sugars or organic acids as both the energy source and as a source of reduced carbon. These two basic forms of existence have variations in numerous species in which the ability to synthesize certain organic moieties such as amino acids and vitamins is limited or blocked completely.

The overall control of metabolic reaction rates in the algae seems linked to the photosynthetic mechanism even when the organism does not employ the light reaction in growth. Beginning with the studies of Bristol-Roach (Bristol-Roach and Muriel, 1928) to the present (Krauss, 1958; and Myers, 1957) the evidence indicates a rate regulating function of the light reaction. The growth rate of algae, with the exception of such photosynthetically crippled forms as Ochromonas (Myers and Graham, 1956), can not be accelerated above that during photosynthesis at saturating light intensities by the addition of a source of reduced carbon. The regulatory mechanism takes the extreme form in those cases of obligate phototrophy where growth of the algae is not possible regardless of the richness of the medium in the absence of light (Wetherell, 1958). Whether this effect is strictly a matter of production of some photosynthetic intermediate or is due to some other regulatory effect of light cannot yet be determined.

Of similar nature is the effect of the photosynthetic process on the fate of the carbon which is fixed during the reaction. Calvin (Bassham and Calvin, 1957) has shown that during illumination the normal path of reduced carbon is into storage products presumably starch and related compounds. In the dark the labeled C14 from C14O2 quickly appears in the compounds of the Krebs Cycle. Whether there is a gate to dark respiration which is closed by light is not yet certain. The gate is open wide enough to permit the incorporation of both exogenous reduced carbon and photosynthetically reduced carbon simultaneously. Myers has demonstrated that when photosynthesis is exogenously subsidized by glucose 50% of the carbon incorporated by the organism comes from glucose and 50% from CO₂ (Myers, 1957).

The dependence of algae on light, and the dominance and even control of the photosynthetic mechanism on the other aspects of the metabolism of the algae, has often led to the assumption that the more light the better. In fact, for even the more hardy autotrophic species like Chlorella and Anacystis the saturation level for the photosynthetic process and for growth is well below 1000 foot candles, or less than 1/10 of full sunlight. The point at which damage to growth takes place is at, or just above, 1000 foot candles for the more susceptible species and only 2000 foot candles for those which are celebrated as resistant to high light intensities. Figure 11 gives the light intensity curves for four algae. Although the light intensities given

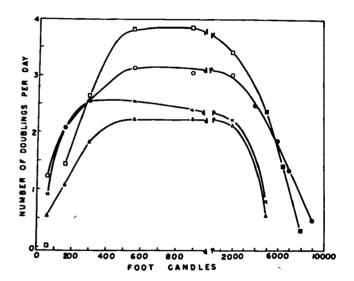


Figure 11. THE GROWTH RATES OF FOUR SPECIES OF ALGAL AT 25°C MEASURED AT LIMITING, SATURATING, AND INHIBITING LIGHT INTENSITIES. The symbols are: Chlorella pyrenoidosa (van Niel), circles; Chlorella vulgaris, crosses; Scenedesmus obliquus, triangles; and Chlamydomonas reinhardti, squares. Open symbols show growth under fluorescent light. Closed symbols show growth under incandescent light.

will vary somewhat with the geometry of the apparatus in which the measurements are taken, it is clear that most of the radiant energy of full sunlight is either not used or is actually damaging to cell growth.

Such data, which should be supplemented by that for other species, coupled with measurements of light intensities at various depths could be useful in computing the theoretical total production capacity of a given body of water during a period of bloom. It would be instructive to know how great a contribution to the total weight of the algae in a given situation was produced by the heterotrophic vs. the autotrophic form of metabolism. Such information could serve to identify the fundamental cause of an algal bloom.

Not only is the source of the carbon of the algae a fact of particular importance, but the reaction sequence by which the algae convert the photosynthate or absorbed organic carbon to energy yielding processes is perhaps of even greater concern. Biochemical studies of algal metabolism have demonstrated alternative pathways for the utilization of organic substrates. The classical Meyerhoff-Embden system for phosphorylation and degradation of hexoses now has two alternative routes established for this process—the glucuronic or gluconic acid shunt (Table 12) and the galactose—6-phosphate route (Figure 12) (Galloway and Krauss, 1959b). Each of these routes for the metabolism of sugar has an entirely different bat-

Table 12. REACTIONS OF THE GLUCONIC ACID SHUNT

6C6H120 hexose	+ 60 ₂ — 6C ₅ H ₁₀ O ₅ + 6CO ₂ + 6H ₂ O	=
4C5H1005 pentose	2C7H1407 + 2C3H603 2C6H1206 + 2C4H804 heptose triose bexose tetrose	
2C5H1005 pentose	+ 2C4Hg04 — 3C6H12O6 tetrose hexose	

tery of enzymes. It seems clear that certain algae utilize one or another of these pathways preferentially, and may, indeed, be obliged to use only one. Similar alternatives exist in the systems involved in the synthesis and degradation of nitrogen compounds within the algae.

It is likely that the variability of these enzyme systems is largely responsible for the differential selectivity of the chemical agents that have been utilized to control algae. Studies have shown that the susceptibility of an alga to a given toxic chemical may be due to the destruction of certain enzymes. Susceptible algae are unable to bypass such blocks, but algae which do have the enzymes for different or alternative pathways can survive at high concentrations of the toxic agent. Evidence for just such an explanation for differential toxicity has been obtained in our laboratory in studies of the susceptibility of phosphoglucose isomerase (Galloway and Krauss, 1959a; Galloway and Krauss, 1959b). The enzymatic reactions of the photosynthetic cycle proposed by Calvin (Bassham and Calvin, 1957) might also be examined in a similar fashion. The characteristics of the unique enzyme, carboxydismutase, in the initial step of carbon dioxide incorporation into the pentose cycle of photosynthesis should be better known. This one key biochemical reaction is characteristic of photosynthesizing algae and is a pregnant subject for study by those with control as an ultimate aim.

It is not possible to cover all the aspects of the photosynthetic process. Reviews, some especially related to the algae, may be found elsewhere (Bassham and Calvin, 1957; Krauss, 1956; and

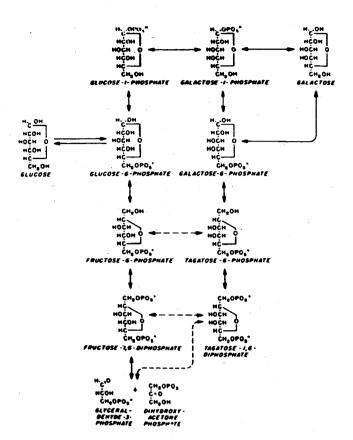


Figure 12. THE SEQUENCE OF HEXOSE INTER-MEDIATES OF AN ALTERNATE PATHWAY IN RELATION TO THE CLASSICAL GLYCOLYTIC SCHEME.

Anon., 1958). In fact most of the current investigation in this area is moving into the realms of solid state physics. However, one aspect of the process which has been uncovered in recent years and is especially pertinent here is the identification of the process of cyclic photosynthetic phosphorylation. The intricacies of this process are involved, but can be studied in detail in the papers of Arnon (Arnon, 1959), Jagendorf (Jagendorf, 1959) and others. The outstanding feature of this system is that ATP can be generated in the chloroplast through a cyclic process by which water is first split into H+ and OH-, and then, after passage through certain enzymes, is recombined to yield water again. This process, quite different from the Hill reaction which provides the reducing power for CO2 reduction and liberates oxygen, results in no oxygen liberation at all. Nevertheless, although no gas exchange can be measured, electromagnetic energy is converted to chemical energy for use by the plant. How extensive this system may be and what portions of the ATP requirement are satisfied in this fashion are not established. Nevertheless it is certain to be of major significance in the energy economy of growing populations, and must be taken into account in the calculations of efficiency. Gas measure of the energy exchange can no longer be considered the exclusive trapping activity of the cell.

NUTRITION

Studies of algae concerned with their growth in nature have been strongly oriented toward inorganic nutrition. The availability of good data has not, however, always made the picture clearer. Attempting to decipher the producing capacity of a body of water based on its nutrient composition is as difficult as determining what the algae require in terms of their internal composition to insure optimum growth. Apparent contradictions in the data stem from three problems related to algae growth. The first deals with the selection of the most useful criterion for measuring growth; the second relates to the availability of nutrients in waters; and the third involves the estimation of requirements when more than one of the factors affecting growth are in rate-limiting supply.

With regard to the first and third of these it is important to recall that there are various ways of evaluating growth in microorganisms. An alga, blocked from further protein synthesis by lack of nitrogen, may continue to synthesize carbohydrate reserves. The capabilities of the cells in this regard have been well tabulated (Spoehr and Milner, 1949; and Thomas and Krauss, 1955). In a real sense this cell is still growing. In fact, as it shifts from protein synthesis to carbohydrate or even fat synthesis, the increase in cell weight may be reduced very little. The amount of light reaching the cell may actually be more influential in determining the amount of cell growth than the nitrogen limitation. This becomes increasingly involved when the factor limiting growth is not limiting growth absolutely, but only reducing the rate of growth. Then as the cell grows in an environment shifting from darkness to damaging light intensities, as it shifts to heterotrophic or autotrophic growth, and as its synthetic directions change, the degree to which each of the factors can be said to be the "limiting" one becomes almost impossible to determine. A sophisticated system of integration, perhaps employing computers, would be necessary for an effective solution. Nor is it even safe to say what the minimum levels of a given essential metabolite may be for the different forms of nutrition. An excellent example is the low requirement for manganese shown by algae when growing heterotrophically in contrast to a requirement, at least two times higher, for the same organism obtaining its reduced carbon by photosynthesis (Reisner and Thompson, 1956). Such considerations are borne out by the difficulty encountered by Gerloff and Skoog (Gerloff and Skoog, 1954; and Gerloff and Skoog, 1957) in deciding the degree to which nitrogen limits growth in the notorious Wisconsin lakes.

The second problem has been of interest for a number of years. It concerns the way in which the algae absorb their inorganic components from the medium. The elemental requirements for the algae are fairly well worked out and can be shown in comparison to those of other microorganisms in Table 13. These requirements have been described in detail in several reviews (Ketchum, 1954; and Pirson, 1955). However the presence of a given

Table 13. CHEMICAL REQUIREMENTS OF MICRORGANISMS

Element	Algae	Fungi	Bacteria	Protozoa
Well established				
and general:				
C,H,O	*	. *	*	*
N, P, S	*	*	*	*
Mg	*	*	*?	*
Ca	*	*?	*?	* .
Co	*	*?	*?	*?
Cu	* ?	*	*?	*?
Fe	*	*	•	*
Me		*	*	
к	*	*?	*?	*
Zn	*?	*	*?	*?
Poorly established or less general:				
В	*?	?	*	0
Ga	0	*?	0	Õ
Mo	*	*		Ô
Si	*?	0	0	Ŏ
Na	*?	Ō		• ?
Va	*?	Ö	0	0
Sr	*?	Ô	Ō	Ö
Rb	*?	•	-	•

- * Signifies demonstrated essentiality
- *? Signifies essentiality in doubt although a requirement has been demonstrated in some species.

element in the medium does not in itself assure that the algae can utilize this element. Though exogenous proteolysis and amino acid sources for nitrogen are common in the algae, even nitrogen in some organic combinations is unavailable. This can be often overlooked when waters show a high nitrogen analysis. In a similar fashion numerous phosphate compounds, even the polyphosphates, are not suitable for algal growth.

The micronutrients which are just as important to algae as nitrogen in spite of their quantitatively inconspicuous characters are probably most subject to conditions which make them unavailable. The shift from soluble molecules to colloidal aggregates may be too subtle to be detected by ordinary analytical means, but is widely accepted as the cause of failure of discrete media to support optimum growth in laboratory cultures. Routine dictates the use of some type of chelating agent in the preparation of all media now used in studies of algal physiology. Nevertheless relatively little is known about the levels and effectiveness of chelating agents found in nature, and these may determine the availability or lack of availability of an element much more than elementary analyses indicated.

Of recent concern has been the mechanism of exchange by which chelated trace metals are absorbed by algae. It appears that chelates may release the metals to the cell by weak though progressive dissociation; that the chelates may be destroyed by such agents as light thereby liberat-

ing their metal complement; or the entire chelate complex may be absorbed and metabolized by the algae (Krauss and Specht, 1958). Further investigations of the mechanism of chelate action should prove useful in understanding their role in supporting algal growth in nature as well as in the laboratory.

ALGAL DECOMPOSITION AND ALGAPHAGE

So far we have directed attention to the factors in the environment responsible for algal growth and to the ways in which the algae utilize these factors in their metabolism. The final question is what happens to the algae after they have grown and exhausted the supply of whatever has generated them? In a pure laboratory culture a mature population may live for months intact in a condition characterized by extremely low endogenous respiration. Even if the culture is exposed to outside bacteria it may continue to exist indefinitely. This is not the case in nature. Algal blooms do disappear and this very fact is some cause for hope in their control. Of course in moving waters the wash-out rate will account for the rapid dissolution of a bloom once growth ceases, but they disappear almost as rapidly in relatively stagnant bodies. One should expect that if the algae decomposed that they would liberate into the water those same products that gave them life and the cycle would regenerate. In general this is not the case and it seems likely that we must look to the activity of other organisms for the answer.

Grazing by larger animals undoubtedly accounts for the consumption of some of the bloom, but most of the algae are disposed of by other microorganisms. Little is known of algal pathology. With the exception of the Chytrids no parasite has been identified on them. A great deal of laboratory experience does show that pure cultures of algae contaminated with bacteria or fungi grow poorly, but in fact many grow very well on inorganic media

with bacteria and some even grow better when contaminated. Anaerobically the algae decompose rapidly (Golueke, Oswald and Gotaas, 1957) and this is the fate of some of the bloom which has settled into the bottom mud. However in the light of what we know about microorganismal infection of other higher plants, animals, and microorganisms it seems that a very large vacuum exists with regard to our knowledge of this phase of algal growth. Especially pertinent is the question of whether phages play a role in the degeneration of algae in nature.

It is not the purpose of this paper to make more than a general comment in this regard. However it seems almost impossible to avoid the conclusion that phages or related viruses must infect algae. About this subject which could be of the greatest practical value we know absolutely nothing. Some experiments are being performed in our laboratory on a limited scale exploring the techniques of phage identification and culture, but results are too new to warrent further statements. From the point of view of the physiologist few things could be more exciting than being able to study the nucleic acidprotein relationships of algae and infecting phages. Much of what we know of inheritance and DNA-RNA mechanisms in other organisms have come from experiments with viruses. It will be a distinct service to algal physiology and to practical aquatic biology when algaphages are finally identified and examined.

CONCLUSION

The general impression that these remarks were intended to convey is that there are sound beginnings to the understanding of the ways of algal life. Already the exploitation of what is known should be a help to the practical biologist. Even greater rewards must come from a study of the algae for the sake of our own intellectual curiousity.

REFERENCES

Allen, M. B. 1959. Studies with Cyanidium caldarium, an anomalously pigmented chlorophyte. Archiv fur Mikrobiologie 32: 270-277.

Anon., 1958. The photochemical apparatus its structure and function. Report of Symposium held June 16-18, 1958. Biology Department, Brookhaven National Laboratory, Upton, N. Y.

Arnon, D. I. 1959. Chloroplasts and photosynthesis. In: The Photochemical Apparatus Its Structure and Function. p. 181-235. Brookhaven National Laboratory, Upton, N. Y.

Bassham, J. A. and Melvin Calvin. 1957. The path of carbon in photosynthesis. Prentice Hall Inc., Englewood Cliffs, N. J. 104 pp.

Bristol-Roach and B. Muriel. 1928. On the influence of light and of glucose on the growth of a soil alga. Ann. Bot. 42: 317-345.

Fogg, G. E. 1953. The metabolism of algae. Methuen and Co. Ltd., London. 149 pp.

Galloway, R. A. and Robert W. Krauss. 1959a. The differential action of chemical agents, especially Polymyxin B, on certain algae, bacteria, and fungi. Am. Jour. Bot. 46: 40-49.

Galloway, R. A. and R. W. Krauss. 1959b. Mechanism of action of Polymyxin B on <u>Chlorella</u> and <u>Scenedesmus</u>. Plant Physiol. 34: 380-389.

REFERENCES (Cont'd.)

Gerloff, Gerald C. and Folke Skoog. 1954. Cell contents of nitrogen and phosphorous as a measure of their availability for growth of Microcystis aeruginosa. Ecology 35: 348-353.

Gerloff, Gerald C. and Folke Skoog. 1957. Nitrogen as a limiting factor for the growth of Microcystis aeruginosa in southern Wisconsin Lakes. Ecology 38: 556-562.

Golueke, C. G., W. J. Oswald, and A. B. Gotaas. 1957. Anaerobic digestion of algae. Applied Microbiol. 5: 47-55.

Jagendorf, A. T. 1959. The Relationship between electron transport and phosphorylation in spinach chloroplasts. In: The Photochemical Apparatus Its Structure and Function. p. 236-258. Brookhaven National Laboratory, Upton, N. Y.

Ketchum, B. H. 1954. Mineral nutrition of phytoplankton. Ann. Rev. Plant Physiol. 5: 55-74.

Krauss, R. W. 1956. Photosynthesis in the algae. Ind. and Eng. Chem. 48: 1449-1458.

Krauss, R. W. 1958. Physiology of the fresh water algae. Ann. Rev. Plant Physiol. 9: 207-244.

Krauss, R. W. and Alston Specht. 1958. Nutritional requirements and yields of algae in mass culture. In: Transactions of the Conference on the Use of Solar Energy -- The Scientific Basis. p. 12-26. Edwin F. Carpenter, Editor. Vol. IV. Photochemical Processes. University of Arizona Press, Tucson, Arizona.

Kreger, D. R. and B. J. D. Meeuse. 1952. X-ray diagrams of <u>Euglena paramylon</u>, of the acid-insoluble glucan of yeast cell walls, and of Laminarin. Biochim. et Biophys. Acta 9: 699-700.

Lynch, V. H. and M. Calvin. 1953. CO₂ fixation by Euglena. Ann. N. Y. Acad. Sci. <u>56</u>: 890-900.

Myers, J. 1951. Physiology of the algae. Ann. Rev. Microbiol. $\underline{5}$: 151-180.

Myers, J. 1957. Suppression of carbon dioxide assimilation by glucose in <u>Chlorella</u>. Plant Physiol. 32: Suppl. xvi-xvii.

Myers, J. and L. B. Clark. 1944. Culture conditions and the development of the photosynthetic mechanism. II. An apparatus for the continuous culture of Chlorella. J. Gen. Physiol. 28: 103-112.

Myers, J. and J. R. Graham. 1956. The role of photosynthesis in the physiology of Ochromonas. J. Cell and Comp. Physiol. 47: 397-414.

Myers, J. and J. R. Graham. 1959. On the mass culture of algae. II. Yield as a function of cell concentration under continuous sunlight irradiance. Plant Physiol. 34: 345-352.

Pirson, A. 1955. Functional aspects in mineral nutrition of green plants. Ann. Rev. Plant Physiol. 6: 71-114.

Reisner, G. A. and J. F. Thompson. 1956. Manganese deficiency in <u>Chlorella</u> under heterotrophic carbon nutrition. Nature 178: 1473 -1474.

Smith, G. M. (Editor) 1951. Manual of phycology. 375 pp. Chronica Botanica Co., Waltham, Mass.

Sorokin, C. 1960. Tabular comparative data for the low and high temperature strains of Chlorella. Nature 184: 613-614.

Sorokin, C. and R. W. Krauss. 1959. Maximum growth rates of Chlorella in steady state and in synchronized cultures. Proc. Natl. Acad. of Sci. (U. S.) 45: 1740-1744.

Spoehr, H. A. and H. W. Milner. 1949. The chemical composition of Chlorella; effect of environmental conditions. Plant Physiol. 24: 120-149.

Starr, Richard C. 1960. The culture collection of algae at Indiana University. Am. Jour. Bot. $\underline{47}$: 67-86.

Thomas, W. A. and R. W. Krauss. 1955. Nitrogen metabolism in <u>Scenedesmus</u> as affected by environmental changes. <u>Plant Physiol.</u> 30: 113-122.

von Stosch, H. A. 1951. Uber das Leukosin, den Reservestoff der Chrysophyten. Die Naturwissenshft. 38: 192-193.

Wetherell, D. F. 1958. Obligate phototrophy in Chlamydomonas eugametos. Physiologia Plantar-um 11: 260-274.

Wetherell, D. F. and R. W. Krauss. 1957. X-ray induced mutations in Chlamydomonas eugametos. Am. Jour. Bot. 44: 609-619.

MICRONUTRIENTS AND HETEROTROPHY AS POSSIBLE FACTORS IN BLOOM PRODUCTION IN NATURAL WATERS

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INTRODUCTION

The heterotrophic abilities and needs of the algae are receiving increased attention. Since the algae are at the origin of the vegeto-animal cleavage they offer a variety of nutritional potencies, from obligate photoautotrophy to facultative heterotrophy and to phagotrophy (consult Lwoff, 1943; and Provasoli and Pintner, 1953b, for evolutionary trends). In our discussion we will consider only the transition phototrophy-osmotrophic heterotrophy without discussing phagotrophy. The word heterotrophy, of nebulous etymology (hetero = other), is now generally accepted to signify a need for preformed organic molecules. The early distinction between need of organic carbon sources and "building blocks" has become untenable because of the complexity of the web of pathways of synthesis. Though the need for growth factors is clearly heterotrophy, a new name, auxotrophy, has gained recognition. It seems opportune to accept it, especially in the algae, because this need is often the only heterotrophic need, is independent of the source of energy utilized, and is quantitatively extremely small in contrast to the large heterotrophic needs for carbon sources and building blocks. For clarity we will consider auxotrophy and heterotrophy separately.

ORGANIC MICRONUTRIENTS

For many years the algae were considered to require only inorganic salts for growth. We know now that many of them require vitamins. Though photoautotrophic algae requiring growth factors depend quantitatively almost solely on inorganic nutrients, the micro-requirement for growth factors is as important because of the absolute nature of this requirement, hence the necessity of considering them as ecological factors.

Most of the ecologically important species, both

marine and freshwater, have not been cultured and we do not know if they are auxotrophic or not. Some guiding principles can, however, be postulated because the phototrophic algae are quite stereotyped in their vitamin requirements. It is remarkable that the trends perceived several years ago on about two-dozen auxotrophs (Provasoli and Pintner. 1953b) have remained the same for a much larger number of species. The sampling is still very small, but the apparent constancy of trends lends weight to them. The available data were tabulated in a recent review (Provasoli, 1958). Since then J. and R. Lewin (1959) have studied the requirements of 25 species of marine diatoms, Droop (1959) of Oxyrrhis marina, McLaughlin and Zahl (1959) of two symbiotic dinoflagellates, Fries (1959) of a red alga, and E. G. and O. Pringsheim (1959) of 23 species of Volvocales. Table 14 summarizes the old and new data. All the phototrophic species and their related counterparts (Chilomonas, Polytoma, Astasia, etc.) require only vitamin B₁₂, thiamine and biotin, alone or in various combinations. This restriction to only three vitamins is unexpected because the other groups of microorganisms (bacteria and molds) have widely diverse vitamin requirements. The specificity of the algae can hardly be fortuitous because they colonize environments rich in all vitamins. In contrast, phagotrophic colorless species, such as Peranema and Oxyrrhis, need additional vitamins and building blocks. This narrow selection appears, then, as a peculiarity of the numerous algae and flagellates of the transition "chlorophyte-leucophyte" (sensu Lwoff, 1943). Whatever the reason, the task of the ecologist and the water engineer is then limited to the charting of these three vitamins in the wa-

The general order of incidence (Table 14, total of single vitamins) appears to be cyanocobalamin (vit. B₁₂), thiamine, and biotin. Even though most

Table 14. VITAMIN REQUIREMENTS OF ALGAE

Algal group	Number of species	No vitamin requirement	Require vitamins	B ₁₂	Thiamine	Biotin	B ₁₂ + thiamine	Biotin + thiamine	B12 + biotin + thiamine
Chlorophyceae	58	25	33	3	6		24		
Eugleninae	9	0	9	2	1		6		
Cryptophyceae	9	0	. 9	2	2		5		
Dionophyceae	17	1	16	11			1	1	3
Chrysophyceae	13	ī	12	3	1		5	1	2
Bacillariophyceae	37	20	17	10	3		4		
Cyanophyceae	10	9	1	1					
Rhodophyceae	1	Ô	1	1					
Totals	154	56	98	33	13		45	- 2	5
Totals for single	- / -		,						
vitamias			•	83	63	7			

algae require B_{12} , it is dangerous to attribute more ecological importance to this vitamin as a possible bloom factor, than to thiamine and biotin. The role and relative importance of the vitamins can only be assessed when we know in detail their seasonal and spacial fluctuations, (i.e. the balance between producers and consumers) and we correlate them with the growth of the different algae in the waters.

The general trends are:

- 1. No correlation seems to exist between need of vitamins and the ability of algae to employ various sources of energy. Strictly photoautotrophic species, such as several marine dinoflagellates, the fresh-water Woloszynskia limnetica, Synura petersenii, S. caroliniana, and several marine Cryptomonads need vitamins just as do photosynthetic species endowed with developed heterotrophic abilities, such as several freshwater Euglena, Phacus, Trachelomonas, and Ochromonas. The permanent loss of chlorophyll (colorless species) is not necessarily accompanied by loss of the power of synthesizing vitamins. Polytoma uvella and P. obtusum do not need vitamins, while other colorless Polytoma, Polytomella coeca and Chilomonas paramoecium are auxotrophic. Thus auxotrophy represents a loss of function which occurs independently.
- 2. No correlation can be found between environment and need for vitamins. Synura needs vitamins as well as Euglena, Volvox, Trachelomonas, etc., which colonize polluted waters. Asterionella and Tabellaria do not need vitamins, but neither do the mesosaprobe Fragillaria and Chlorella which can live in the oxidation ponds. Furthermore, the marine, brackish, and freshwater species belonging to the same algal group have apparently the same order of incidence of auxotrophic species.
- 3. On the contrary, there is a definite homogeneous trend in each algal group. The Cyanophyceae, the Bacillariophyceae, and the Chlorophyceae are the algal groups in which photoautotrophy probably predominates and the need for vitamins is restricted to fewer species. In our table we have included only the species of Chlorophyceae and Cyanophyceae for which the vitamin requirements have been specifically studied. This requires a series of precautions for chemical asepsis which is not normally employed in the maintenance of cultures. We have excluded a few hundred Chlorophyceae and 20 or more species of blue-green algae which are maintained in various culture collections on mineral media in cotton plugged tubes. Cotton during sterilization releases vitamins and Robbins et al. (1951) have shown that microorganisms grow and produce appreciable vitamin B₁₂ in distilled water allowed to stand for a few days in the laboratory. Nonetheless, judging from the general trend of the well-studied species, it is most

probable that the majority of the Chlorophyceae and Cynanophyceae not included in our table does not require vitamins. The blue-green algae, Anabaena cylindrica, A. variabilis, Nostoc muscorum (1013J Wisconsin), Anacystis nidulans, Phormidium autumnale do not require vitamins; only Phormidium persicinum requires cyanocobalamin. Robbins et al. (1951) found that Plectonema nostocorum, Aphanizonemon flos-aquae, Diplocystis aeruginosa, and Calothrix parientina produce B12 (the uninoculated mineral medium was assayed and had no B12).

Conversely, in all the other algal groups, nearly the totality of the species so far studied needs vitamins. It seems safe to expect that most Eugleninae, Dinophyceae, Chrysophyceae, and Cryptophyceae are auxotrophic. We believe that the present sampling, though small, is sufficiently representative because the newly studied species were not preselected by the choice of media. They were obtained bacteria-free either directly from nature or from bacterized cultures, and grown initially in media enriched by a mixture of the known B vitamins.

The data indicate that auxotrophic species predominate in algal groups with strong animal tendencies (high incidence of colorless, holozoic forms, predominance of the flagellated or ameboid species) like the euglenids, dinoflagellates, chrysomonads, and cryptomonads and that few species require vitamins in the algal groups which have well developed vegetal tendencies. This might explain why the need for vitamins is correlated with algal groups and not with environments.

4. Minor trends that might not be at all significant with a larger sampling are that: a) the thiamine requirement is more evident in the Chlorophyceae, while the need for vitamin B_{12} predominates in the other algal groups; b) both B_{12} and thiamine are required by the majority of the Eugleninae, Cryptophyceae and Chrysophyceae; c) most Dinophyceae require only B_{12} .

ECOLOGY OF VITAMINS

The above data clearly indicate that B₁₂, thiamine, biotin, and perhaps other unknown growth factors, are ecological factors which we cannot afford to neglect. The main problem, then, is how to measure them. No difficulty stands in our way for the measurement of vitamins in freshwaters. Vitamin B₁₂ is routinely measured in blood, urine, and other organic fluids and organs, and many hospitals now have an algological section with temperature-controlled rooms and fluorescent lightbanks, because cyanocobalamin is titrated with the freshwater algae Euglena gracilis and Ochromonas malhamensis. Freshwaters are easier to analyze than blood and other organic fluids yet no ecological laboratory is equipped with a bioassay section.

Curiously, the little advance achieved has been for the marine environment where salinity makes things difficult. The assay of vitamin B₁₂ in seawater has been done by using the above-mentioned freshwater Euglena and reducing interference from inorganic salts either by dialysis (Provasoli and Pintner 1953, Kashiwada et al. 1957), by extracting the B₁₂ (Cowey 1956), by diluting the sea water (Daisley, 1958), or by employing marine organisms for the assay (Adair and Vishniac 1958). This problem does not exist for freshwaters; Euglena gracilis and Ochromonas malhamensis can be employed reliably for measuring the various cobalamins present in the waters and many suitable bacteria for bioassay are available for thiamine and biotin. In fact, long ago Hutchinson (1943) and Hutchinson and Setlow (1946) measured thiamine, biotin, and nicotinic acid in Lindsley Pond and Bantam Lake. A recent paper by Hutner et al. (1958) covers adequately the methods, selection of bioassay organism, and known pitfalls.

We will discuss now what is known of the cycle of vitamins in waters. The situation being on the whole similar, data will be taken from work done both on freshwaters and the sea. Bacteria and other microorganisms are the main producers of vitamins in nature.

A great part of the vitamins in freshwaters and in the littoral zone of the sea can be assumed to come from any soil run-off especially during the spring floods. The Lochhead school (Burton and Lochhead, 1951; and Lochhead and Thexton, 1951) found that 65% of the actinomycetes and 70 to 84% of the bacteria in the soil produce vitamin B₁₂ and that 14% required soil extract for growth (for 50% of these bacteria the soil extract requirement could be met by B_{12}). The predominance of B_{12} producers over B12 consumers in the soil should result in an accumulation of B₁₂. This is actually so: Robbins et al. (1950) found 1-8 µg. of B₁₂ per liter of cold water soil extract; soil extract has been a requirement for all sorts of algae and its effect can often be duplicated by vitamins plus trace metals.

Muds are another source of vitamins. Starr (1956) and Burkholder and Burkholder (1956) followed the fate of vitamin B_{12} from a Spartina marsh into a tidal river, a sound, and then the open sea on the coast of Georgia. The vitamin is concentrated by adsorption on the suspended particles which flow toward the sea; it originates in the marsh where the content of B₁₂ in the mud is 0.7 µg/g dry matter. Burkholder and Burkholder (1956) found that suspended solids in brown waters from several Georgia rivers had an extremely high content of B₁₂ in October (1-6 µg B₁₂/g dry weight). Depending upon the amount of particles suspended in the river waters, the B₁₂ content per liter varies from 2-40 mpg. The same authors (1958) found 0.4-3 µg. of vitamin B12 per gram of suspended particle in the phosphorescent bay of La Parguera, Puerto Rico. The sediments of the same bay had an average content of 280 myg. of B_{12} , 7 mug. of biotin and 73 mug. of thiamine per gram of dried mud. Starr et al. (1957) found that 70% of 34 isolates of marine bacteria produced various cobalamins. Burkholder (1959) studied the production of B vitamins by 344 bacteria isolated from waters and muds of Long Island Sound. They found that when cultured with appropriate enrichments, 27% of these bacteria give off vitamin B₁₂, 50% biotin, 60% thiamine, and 11% nicotinic acid. The same picture holds for freshwaters: Robbins et al. (1950) report that fungi and many bacteria, isolated from the water and mud of a pond in which Euglena blooms, produce B₁₂; they also demonstrated that these bacteria, grown with Euglena on agar plates of a medium deprived of B₁₂, diffused enough vitamin to support growth of Euglena.

The exchange of nutrients between microorganisms and algae can be quite direct as in the case of filamentous algae and seaweeds which produce a mucilaginous slime inhabited by bacteria and yeast. Most bacteria epiphytic on seaweeds produce B₁₂ (Ericson and Lewis 1953).

A third source is the vitamins present as solutes in waters. For B_{12} , the ratio between the quantity absorbed on suspended particles and the quantity dissolved in seawater was about 2.5 (Burkholder and Burkholder, 1958). The quantity of dissolved vitamin B_{12} in sea water varies greatly. Coastal waters are the richest; Lewin (1954) and Droop (1955) found 5-10 mpg B_{12}/liter ; Kashiwada et al. (1957b) found up to 55 mpg/liter in the fertile Kagoshima Bay. In the open waters the content is far less: Cowey (1956) found in the North Sea and Norwegian deeps values from 0.1-2 mpg/liter and Kashiwada et al. (1957) in the North Pacific, values fluctuating from 0 to 1 mpg/liter, for surface waters.

The data on vitamin content in freshwater are even scantier. Thiamine varied seasonally in Lindsley Pond from 0.1-0.2 μ g/liter; lower values (0.03-0.04 μ g/liter) were found in Waramung and Bantam Lakes, (Hutchinson, 1943); biotin in Lindsley Pond varied from 0.3-4 μ g/liter, (Hutchinson and Setlow, (1946); Benoit (1957) found 80 μ g/liter of B₁₂ in the same pond, and Kashiwade et al. (1957b) found variations between 0-10 μ g B₁₂/liter in the first ten meter depth of Lake Ikedo.

The seasonal variations in vitamins found by Hutchinson were also found by Cowey in sea water: the dip from 2 mµg/liter in March to 0.3 mµg/liter in May-June coincides with the diatom blooms. Similarly Vishniac and Riley (1959) record for Long Island Sound in the early spring a fall in B_{12} which parallels the consumption of NO_3 . However, vitamin B_{12} even at the low value (4.5 mµg/liter) appears not to be limiting. On the contrary, thiamine is barely detectable in Long Island Sound

(0-20 mug/liter) and they suggest that thiamine is derived from land drainage (63 mug/liter at the breakwater of Indian River) and is diluted as the river mixes with sound waters, or that thiamine is destroyed in alkaline seawater more rapidly than it is produced. Even before the data of Vishniac and Riley, which tend to support Droop's opinion, Droop (1957) concluded that vitamin B12 is not limiting in the sea because it is always present in quantities far exceeding those needed by Skeletonema costatum. In fact many species of algae requiring B₁₂ are very sensitive and require in vitro quantities between 0.1-5 mug/liter. Very sensitive organisms could then still grow in the depleted summer waters of the North Sea which contain 0.1 mug/liter (Cowey, 1956) and bloom in the lowest values (4.5 myg/liter) found in Long Island

However, Daisley (1957) pointed out that in nature the rate of cell division controlled by the various levels of B_{12} was more important than the possible total yield. Another important consideration is that data in vitro of cell yield per given concentration of B_{12} are determined in a system lacking the competition of other organisms requiring vitamin B_{12} ; in nature, on the contrary, we have such a competition. The competition for vitamin B_{12} is further complicated by two other peculiarities.

- 1. Holm-Hansen et al. (1954) have shown that blue-green algae not requiring B_{12} employ B_{12} readily as a cobalt source; as cobalt is generally scarce in water, even organisms not requiring B_{12} may compete for it.
- 2. Several forms of cobalamins are produced by bacteria; Burkholder and Burkholder (1958) have shown that both in Puerto Rico and Long Island muds B_{12} analogs predominate over cyanocobalamin (="true" B_{12} = antipernicious anemia factor). Droop et al. (1959) show that most auxotrophic al-

gae utilize only cyanocobalamin, while several diatoms utilize all cobalamins; the ratio of cyanocobalamin total cobalamins therefore becomes ecologically important.

These intricacies call for far more detailed studies. Since the assay organisms which permit differential assay for cyanocobalamin and the other cobalamins are freshwater organisms, a freshwater location would be preferred. A further advantage is that we can select oligotrophic and eutrophic locations where the runoff, river contribution, flow, and circulation can be followed more easily. Introduction of radioactive Co may help to trace the production of cobalamins by microorganisms and their consumption by phytoplankton, zooplankton, and other microorganisms. The situation for biotin and thiamine is even more vague because of the scarcity of data: thiamine seems, however, to be a limiting fac tor.

The water environment favors the exchange of "external metabolites" postulated by Lucas (1955), creating a very complex situation. The cycle of vitamins may be far more intricate than that of phosphorus and the other scarce and needed metabolites. Not only do the algae compete for vitamins between themselves but against other microorganisms which, like the bacteria, reporduce and metabolize more rapidly. Burkholder (1959) (Table 15) shows the amazing interrelations of vitamin exchange between some marine bacteria. Similar interrelations were found by the Lochhead school between B12-producers and B12-users in soil. Here the nutritional interdependency is dramatically illustrated by vitamin producers themselves being dependent on other vitamins. This interdependency is a chemical symbiosis mediated and enhanced by the continuum which is the water environment.

HETEROTROPHIC ABILITIES OF THE ALGAE

The subject has been recently reviewed by

Table 15. PRODUCTION OF B VITAMINS BY VITAMIN-REQUIRING MARINE BACTERIA (BURKHOLDER 1959)

No. of	Pattern of requirements				No. of vitamin producers			
cultures	Biotin	Thiamine	Niacin	B ₁₂	Biotin	Thiamine	Niacin	B ₁₂
29	+					3	29	1
7	1	+			6		7	
$\overset{\cdot}{2}$			+		1			
3	1			+	3	1	3	
3	+			+	ļ		3	
5	1	+		+	ļ		5	
ĭ		+	+		1			
î	+	+	+					
î	1	+	•	+			1	

Krauss (1958) and Pringsheim (1959). Since algae are at the origin of plants and animals, and the evolution still proceeds, the energy requirements vary greatly. The commonest carbon source for the photosynthetic algae is probably CO2. Many such algae are obligate autotrophs; they cannot be grown in darkness on exogenous carbon sources and do not utilize carbon sources even in light. Among them are several Chlamydomonas; many of the marine flagellates (Dunaliella, Rhodomonas, Amphidinium, Gonyaulax, Gymnodinium, Peridinium, Isochrysis, Syracosphaera); the freshwater Synura caroliniana, S. petersenii, Asterionella formosa, Fragilaria capucina, Tabellaria flocculosa, and Woloszynskia limnetica (Provasoli and Pintner, unpublished); Anabaena cylindrica (Fogg 1953); Anabaena variabilis, Anacystis nidulans and Nostoc muscorum (Kratz and Myers, 1955). Conversely, all the colorless species (Polytoma, Polytomella, Chilomonas, Astasia, etc.) are dependent completely on heterotrophy, and, according to E. and O. Pringsheim (1959) so are several colonial Volvocales. For them a carbon source is needed even in light: glucose is indispensable for Gonium sacculiferum, and acetate for Chlamydobotrys, Astrophoneme gubernaculifera, Gonium octonarium, G. quadratum, Stephanosphaera pluvialis, and Volvulina steinii. These species grow even better in peptone and yeast extract, and reproduce in darkness.

These two extremes, obligate phototrophy and obligate heterotrophy, are bridged by species which are bipotent; however they have different degrees of phototrophic and heterotrophic abilities. Some are predominantly photoautotrophic and the addition of organics is only stimulatory: Eudorina elegans, Gonium sociale, (E. & O. Pringsheim, 1959) Trachelomonas pertyi, T. abrubta, Phacus pyrum, Cryptomonas ovata, var. palustris (Provasoli and Pintner, unpublished), and several species of Chlorella.

Euglena gracilis and Ochromonas danica grow exceedingly well phototrophically and also heterotrophically.

Several marine pennate diatoms are able to grow in darkness on glucose, lactate, or acetate (J. C. & R. A. Lewin, 1959). Pleodorina californica, P. illinoiensis and Volvox globator (Pringsheim 1959) grow poorly phototrophically; good growth is obtained with dilute peptone, yeast extract, and acetate.

Other species are predominantly heterotrophic like Ochromonas malhamensis which grows poorly phototrophically (Myers and Graham 1956). Species with very high heterotrophic abilities like <u>Euglena gracilis</u> and the two <u>Ochromonas</u> in opportune conditions are able to utilize a variety of organic compounds such as fatty acids, alcohols, sugars, amino acids, etc. Other species are apparently more

exacting: Chlorella pyrenoidosa grows with high efficiency in darkness but only on glucose, galactose and acetate (Samejima & Myers, 1958); the "azetatflagellaten" utilizes acetate and not glucose and the "zuckerflagellaten" utilizes glucose and not acetate (Pringsheim 1935, 1954, 1958). However, this apparently specialized utilization may depend in part on preferential penetrabilities of the other metabolites which were not met by the experimental or natural pH tried (Hutner and Provasoli, 1951, 1955). In some cases special triggers may be needed: E. gracilis (an "azetatflagellaten") can utilize glucose only in high CO2 or in the presence of small amounts of organic acids (Cramer and Myers 1952) or amino acids (Hutner et al. 1956). In general the species that utilize very well the exogenous carbon sources grow well in darkness (Euglena, Ochromonas, some Gonium, Chlorella, Chlororogonium, Astrophoneme, Volvulina, Stephanosphaera). Less efficient heterotrophs do not grow in darkness (Pleodorina), or grow poorly . (two Oscillatoria, Lyngbya sp., Phormidium foveo-<u>larum, Plectonema notatum and Nostoc muscorum)</u> (M. G. Allen 1952).

CONSIDERATIONS ON HETEROTROPHY AS A POSSIBLE ECOLOGICAL FACTOR

The effect of organics in natural waters varies greatly with concentration. Therefore we will consider separately three-types of environment differing essentially in organic content.

Richest are the polysaprobic (i.e. septic, saprobic) waters. The flora of these waters consists predominantly of heterotrophic and phagotrophic algae. These environments can be colonized only by algae which can both withstand and utilize high concentrations of organic matter, products of bacterial decomposition (including NH₄), high CO₂ concentrations, reducing condition and, often, gradients of H2S. Colorless flagellates (Distigma, <u>Astasia, Menoidium, Peranema, Monas, etc.),</u> euglenids, and chrysomonads are the usual algal members of these communities. Oxidation ponds are an artificial environment of this type, especially the lagoon receiving the organic influent. The succeeding lagoons have intermediate conditions leading, in the best cases, to a B-mesosaprobic (i.e. subcontaminated) water.

The flora of the first two lagoons differs greatly depending upon the type of waste being processed. The richest in organics are those receiving meat and milk products. The very high concentrations of organics at high load favor active microbial fermentations and anaerobiosis -- conditions favorable only to a few tough obligate heterotrophic algal species which do not contribute oxygen. It is questionable whether the pigmented algae which can live there (especially at low organic load) contribute net oxygen. Depending on their heterotrophic powers, they may prefer heterotrophy to

photosynthesis. It will be interesting to determine the CO₂/O balance of the facultative and obligate heterotrophic pigmented algae in the presence of graded concentrations of highly utilizable carbon sources and building blocks. An extreme case is Ochromonas malhamensis (Hutner et al. 1953). When the medium is extremely rich, the organisms grow at first almost chalk-white in light (they prefer to live heterotrophically and the chloroplast is reduced to 1/10 - 1/20 or more of the normal area); when the medium has become depleted the organisms require a normal chloroplast but even then, as shown by Myers and Graham (1956), they photosynthesis. It will be interesting to determine the CO2/O balance of the facultative and obligate heterotrophic pigmented algae in the presence of graded concentrations of highly utilizable carbon sources and building blocks. An extreme case is Ochromonas malhamensis (Hutner et al. 1953). When the medium is extremely rich, the organisms grow at first almost chalk-white in light (they prefer to live heterotrophically and the chloroplast is reduced to 1/10 - 1/20 or more of the normal area); when the medium has become depleted the organisms require a normal chloroplast but even then, as shown by Myers and Graham (1956), they are very poor photosynthesizers.

In oxidation ponds fed by domestic sewage, Chlorella, Scenedesmus, Chlamydomonas and Ankistrodesmus seem to predominate. Allen (1955) found that these species utilize neither sewage nor its bacterial oxidation products efficiently enough to grow in darkness at the pH of the ponds. Other evidence, both laboratory and field, supports her conclusions that they are photoautotrophic under these conditions. Oxidizable organic matter did not decrease when this Chlorella was grown on sterilized sewage -- i.e. bacteria and other heterotrophs are responsible for the oxidation of organic matter. Chlorella grows better in sewage when the bacteria are present, but the favorable action of bacteria depends on the amount of oxidizable material present in the sewage. A similar increase in growth of Chlorella can be elicited in low BOD sewage when CO2 is supplied artificially.

Furthermore, the growth of Chlorella in the presence of adequate amounts of CO2 is neither stimulated nor inhibited by the presence of sewage bacteria -- i.e. the bacteria supply CO2 to Chlorella. The situation is highly favorable for the normal operation of the oxidation ponds. Chlorella -- and the shallowness of the ponds -- provides the oxygen necessary for the oxidative processes of the heterotrophs which in turn create a high CO2 content for Chlorella. Though the content in organics may not be high, presumably utilizable C sources and building blocks should be present in these oxidation ponds favoring the establishment of bipotential organisms (i.e. photosynthesizers with facultative heterotrophy). In the case analyzed by Allen the lack of predominance of these organisms

may be due to inhibitions; Ankistrodesmus, Oocystis, and Euglena which are also a part of this flora grewat a low rate in sewage of relatively low BOD and did not grow at all in high BOD sewage.

This emphasizes the need to consider in any environment not only the substances which may enhance growth but also inhibitory substances and metabolite concentrations which may be unfavorable for more sensitive organisms. It is well known that high concentrations of mineral fertilizers (as normally found in fish ponds) favor the growth of green algae. Pollution by duck excreta in Great South Bay (Long Island) favored growth of Nannochloris and Stichococcus and eliminated the normal flora of diatoms. Ryther (1954) found that Nannochloris and Stichococcus utilize NH4, urea, and uric acid, and that a Nitzschia isolated from the same bay grew very poorly in NH4 and organic N. The change of flora, in my opinion, is not due to inability to utilize NH4, because all algae utilize NH₄ at a pH below neutrality, but probably to inhibition. Many marine algae are inhibited or killed, depending upon the concentration, by ammonia at alkaline pH. Production of antimetabolites by the green algae is also probable.

As the quantity of organics becomes less -- between 10-100 ppm. -- the action of organics becomes subtler indeed to analyse, yet its effect is ecologically very important. These are the conditions prevailing in freshwater and bays affected by civilization. The creation of blooms and often the change in flora cannot be considered as depending principally on the heterotrophic effect. There is undoubtedly an advantage for the algae (facultative heterotrophs) which can utilize organic compounds as building blocks or carbon sources. A small body of evidence from tracer data indicates that minor syntheses may be bypassed when the synthetic products are present in the environment. Thus adenine is taken up by Ochromonas malhamensis (Hamilton, 1953), and uptake of aspartic acid suppresses endogenous synthesis of lysine in E. gracilis (Vogel, 1959). While such by-passings of synthesis may be individually minor, it is conceivable that collectively they permit a noticeable, even substantial, increase in growth. We have been concerned in heterotrophy for the most part with great stimulations and all-or-nothing effects; these smaller biosynthetic stimulations are just beginning to be explored.

A similar effect has been noted by many when a few milligrams percent of various peptones, soil extract, other infusions of natural products, and even purines and pyrimidines, are added to bacteria-free cultures of algae grown in synthetic media enriched with trace metals and vitamins. However, the stimulation of these additions is often eliminated when the mineral part and the trace metals in the medium are better adjusted to fit the particular organism. This implies a metal buffer-

ing action, or supplying of metals by the organics (peptones and infusions are rich in metals), rather than a micro-heterotrophic advantage. Other effects may also be due to pH buffering, rH poising, organic sulphur compounds -- all known to benefit the growth of some algae. In short, it is difficult to dissect the remarkable effects of minor pollution and eutrophication.

Fogg (1959) is inclined to think the heterotrophic effect of the dissolved organics is minor and he considers that the yellow acids and algal products which are a part of the organics may play an important role. Shapiro (1957) found that the organic acids of a yellow pigmented fraction present in freshwaters are stable to biological decomposition for a few months and that they chelate metal ions effectively.

The nitrogenous extracellular products given off by blue-green algae are capable of binding copper and other trace metals very strongly. Death of Anabaena cylindrica occurs at 2 mg/liter of copper in the absence of these substances while in their presence copper kills at 16 mg/liter (Fogg and Westlake 1955). "Humic acids" brought in by soil runoff act similarly. All these substances bind and solubilize trace metals making available to the algae a non-toxic continuous supply. The vitamins may play an important role because whether limiting or in ample supply they will tend to modify the flora. When limiting, the absence of auxotrophs

will leave the non-auxotrophic algae free from competition for the same nutrients; when plentiful the auxotrophs have a chance to predominate. The effluent of sewage disposal plants is likely to be rich in vitamins; sludge contains high quantities of B12 (Hoover et al. 1951; Sathyanarayama et al. 1959). Nothing is known of the vitamin content of the effluent of sewage oxidation ponds, but it would be surprising if they lacked vitamins; many bacteria and algae are high vitamin producers.

Organic enrichments whether derived from algae growing in lakes or directly from civilization have, then, a complex action:

- (a) they may be a direct source of nutrients;
- (b) they may solubilize P and trace metals;
- (c) they are vitamin sources;
- (d) as supporters of microbial growth they are likely to influence algal growth through products of microbial degradation including NH₄, NO₃, and through (b), and (c); and
- (e) they may create favorable pH and rH conditions. In seawater and freshwater unknown factors other than the usual nutrients enhance algal growth. Perhaps some of them are similar to plant harmones.

The algae in oligotrophic waters are either photoautotrophs or photoautotrophs. Blooming conditions will then mainly depend upon inorganic substances, metal solubilizers, and vitamins. Soil run-off and rivers are the most likely suppliers.

REFERENCES

Adair, E. J. and H. S. Vishniac 1958. Marine fungus requiring vitamin B₁₂. Science <u>127</u>: 147-48.

Allen, M. B. 1952. The cultivation of Myxophyceae. Arch. f. Mikrobiol. 17: 34-53.

Allen, M. B. 1955. General features of algai growth in sewage oxidation ponds. State Water Pollution Control Board. Publ. No. 13, Sacramento, Calif.

Benoit, R. J. 1957. Preliminary observations on cobalt and vitamin B_{12} in freshwater. Limnol. & Oceanogr. 2: 233-40.

Burkholder, P. R. 1959. Vitamin-producing bacteria in the sea. Inter. Oceanog. Congress. Preprints, A.A.A.S., Washington p. 912-13.

Burkholder, P. R. and L. M. Burkholder 1956. Vitamin B_{12} in suspended solids and marsh muds collected along the coast of Georgia. Limnol. & Oceanogr. 1: 202-08.

1958. Studies on Byitamins in relation to productivity of the Bahia Fosforescente, Puerto Rico. Bull. Mar. Sci. of the Gulf & Carribean 8: 201-23.

Burton, M. O., and A. G. Lochhead 1951. Studies on the production of vitamin B_{12} -active substances by microorganisms. Canad. J. Bot. 29: 352-59.

Cowey, C. B. 1956. A preliminary investigation of the variation of vitamins B_{12} in oceanic and coastal waters. J. mar. biol. Ass. U.K. $\underline{35}$: 609-20.

Cramer, M. and J. Myers 1952. Growth and photosynthetic characteristics of Euglena gracilis. Arch. f. Mikrobiol. 17: 384-402.

Daisley, K. W. 1957. Comment on article by M. R. Droop. Nature 180: 1042-43.

Daisley, K. W. 1958. A method for the measurement of vitamin B_{12} concentration in sea water. J. mar. biol. Ass. U.K. 37: 673-81.

Droop, M. R. 1955. A suggested method for the assay of vitamin B_{12} in sea water. J. mar. biol. Ass. U.K. <u>34</u>: 435-440.

Droop, M. R. 1957. Vitamin B₁₂ in marine ecology. Nature 180: 1041-42.

REFERENCES (Cont'd.)

- Droop, M. R. 1959. Water-soluble factors in the nutrition of Oxyrrhis marina. J. mar. biol. Ass. U. K. 38: 605-620.
- Droop, M. R., McLaughlin, J. J. A., Pinter, I. J., and L. Provasoli 1959. Specificity of some protophytes toward vitamin \$12-like compounds. Inter. Oceanogr. Congress, Preprints, A.A.A.S. Washington, p. 916-18.
- Ericson, L. E. and L. Lewis. 1953. On the occurrence of vitamin B₁₂-factors in marine algae. Ark. f. Kemi. 6: 427-42.
- Fogg, G. E. 1953. The metabolism of algae. Methuen & Co. Ltd. London 149 pp.
- Fogg, G. E. 1959. Dissolved organic matter in ocean and lakes. In New Biology. vol. 29, Penguin Books, p. 31-48.
- Fogg, G. E. and D. F. Westlake 1955. The importance of extracellular products of algae in freshwater. Verh. int. Ver. Limnol. 12: 219-32.
- Fries, L. 1959: Goniotrichum elegans: a marine red alga requiring vitamin B₁₂. Nature 183: 558-59.
- Hamilton, L. 1953. Utilization of purines for nucleic acid synthesis in chrysomonads and other organisms. Ann. N. Y. Acad. Sci. <u>56</u>: 961-8.
- Holm-Hansen, O., Gerloff, G. C., and F. Skoog 1954. Cobalt as an essential element for bluegreen algae. Physiol. Plantarum 7: 665-75.
- Hoover, S.R., Yaseweiez, L., Pepinsky, J.B. and N. Porges. 1951. Vitamin B₁₂ in activated sewage sludge. Science 114: 213.
- Hutchinson, E. G. 1943. Thiamine in lake waters and aquatic organisms. Arch. Biochem. 2: 143-50.
- Hutchinson, E. G. and J. K. Setlow. 1946. Limnological studies in Connecticut. VIII. The niacin cycle in a small inland lake. Ecol. 27: 13-22.
- Hutner, S. H., Bach, M. K. and G. L. M. Ross. 1956. A sugar-containing basal medium for vitamin B12-assay with Euglena; application to body fluids. J. Protozool. $\underline{3}$: $\underline{101-12}$.
- Hutner, S. H., Cury, A. and H. Baker 1958. Microbiological assays. Anal. Chem. 30: 849-67.
- Hutner, S. H. and L. Provasoli 1951. The Phytoflagellates. <u>In</u> "Biochemistry and Physiology of Protozoa" A. Lwoff edit. Vol. I Academic Press N. Y.
- 1955. Comparative biochemistry of flagellates. In: "Biochemistry and Physiology of Protozoa", Hutner and Lwoff edit. Vol. II Academic Press N. Y.
- Hutner, S. H., Provasoli, L. and J. Filfus 1953. Nutrition of some phagotrophic freshwater chrysomonads. Ann. N.Y. Acad. 56: 852-62.

- Kashiwada, K., Kakimoto, D., Morita, T., Kanazaqa, A. and K. Kawagoe 1957. Studies on vitamin B12 in sea water. II. On the assay method and the distribution of this vitamin B12 to the ocean. Bull. Jap. Soc. Sci. Fisheries. 22: 637-40.
- Kashiwada, K., Kakimoto, D. and K. Kawagoe 1957b. Studies on vitamin B₁₂ in sea water. III On the diurnal fluctuation of vitamin B₁₂ in the sea and vertical distribution in the lake. Bull. Jap. Soc. Sci. Fisheries. 23: 420-53.
- Kratz, W.A. and J. Myers. 1955. Photosynthesis and respiration of the blue-green algae. Plant Physiol. 30: 275-80.
- Krauss, R.W. 1958. Physiology of the fresh water algae. Ann. Rev. Plant Physiol. 9: 207-44.
- Lewin, R. A. 1954. A marine Stichococcus sp. which requires vitamin B₁₂. J. Gen. Microbiol. 10: 93-96.
- Lewin, J. C. and R. A. Lewin 1959. Auxotrophy and heterotrophy in marine littoral diatoms. Inter. Oceanograph. Congress, Preprints. A.A.A.S., Washington p. 928-29.
- Lochhead, A. G. and R. H. Thexton 1951. Vitamin B₁₂ as a growth factor for soil bacteria. Nature 167: 1034.
- Lwoff, A. 1943. L'evolution physiologique, Hermanm et Cie. Paris.
- Lucas, C. E. 1955. External methabolities in the sea. Papers in marine Biology and Oceanography Suppl. to Vo. 3 of Deep Sea Research p. 139-148.
- McLaughlin, J. J. A. and P. A. Zahl 1959. Vitamin requirements in symbiotic algae. Inter. Oceanograph. Congress, Preprints. A.A.A.S., Washington p. 903-31.
- Myers, J. and J. Graham 1956. The role of photosynthesis in the physiology of Ochromonas. J. Cell. comp. Physiol. 47: 397-413.
- Pringsheim, E.G. 1935. Ueber Azetatflagellaten. Naturwiss. 23: 110-114.
- 1954. Ueber Zuckerflagellaten. Naturwiss. 41: 1-3.
- _____1958. Ueber mixotrophie bei flagellation Planta 52: 405-30.
- 1959. Heterotrophie bei Algen und Flagellaten. in Handb Pflanzenphysiol. -Springer Verlag. 9: 303-26.
- Pringsheim, E. G. and O. Pringsheim 1959. Die Ernahrung Koloniebildender Volvocales. Biol. Zentralbl. 78: 937-71.
- Provasoli, L. 1958. Nutrition and ecology of Protozoa and Algae. Ann. Rev. Microbiol. <u>12</u>: 279-308.

REFERENCES (Cont'd.)

Provasoli, L. and I. J. Pintner 1953, Assay of vitamin B₁₂ in sea water. Proc. Soc. Protozool. 4:10.

Provasoli, L. and I. J. Pintner 1953b. Ecological Implications of invitro Nutritional Requirements of Algal Flagellates. Ann. N. Y. Acad. Sci. <u>56</u>: 839-51.

Robbins, W. J., Hervey, A. and M. E. Stebbins 1950. Studies on Euglena and vitamin B_{12} . Bull. Torrey Bot. Club. $\overline{77: 423-41}$.

Robbins, W. J., Hervey, A. and M. E. Stebbins 1951. Further observations on Euglena and B_{12} . Bull. Torrey Bot. Club. 78: 363-375.

Ryther, J. 1954. The ecology of phytoplankton blooms in Moriches Bay and Great South Bay, L. I., N. Y. Biol. Bull. 106: 198-209.

Samejima, H. and J. Myers 1958. On the heterotrophic growth of <u>Chlorella pyrenoidosa</u>. J. gen. microbiol. 18: 107-17.

Shapiro, J. 1957. Chemical and biological studies on the yellow organic acids of lake water. Limnol. and Oceanogr. 2: 161-79.

Sathyanarayana, R., Jastry, C. A., Bhagavan, H. N. and B. R. Balija 1959. Vitamin B_{12} in sewage sludges. Science 129: 276.

Starr, T. J. 1956. Relative amounts of Vitamin B_{12} in detritus from oceanic and estuarine environments near Sapelo Island, Georgia. Ecol. 37:658-64.

Starr, T. J., Jones, M. E., and D. Martinez. 1957. The production of vitamin B_{12} -active substances by marine bacteria. Limnol. and Oceanogr. 2: 114-19.

Vishniac, H. S., and G. Riley, 1959. B₁₂ and thiamine in Long Island Sound: patterns of distribution and ecological significance. Inter. Oceanogr. Congress, Preprints, A.A.A.S. Washington, p. 942-43.

Vogel, H. J. 1959. Lysine biosynthesis in Chlorella and Euglena: phylogenetic significance. Biochim. et Biophys. Acta 34: 282-3.

ALGAL DENSITY AS RELATED TO NUTRITIONAL THRESHOLDS*

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Definition of oligotrophic and eutrophic as poorly productive or highly productive presumes knowledge of how many organisms or how great a biomass a given body of water contains or, literally, produces in a given interval of time. Algal density is a term demanding clarification in regard to the location of the algae and whether we are talking of the numbers of algal cells, or their biomass. Algal density in the top three inches of a lake may bear little relationship to the original concept if the lake is thirty feet deep. In a moving stream the density may be uniform thruout the depth. Presumably the topic should include total numbers or biomass (of algae) in a body of water under consideration, as well as whether there is a relationship between nutrition and local aggregation of algae. Furthermore, the consideration should include aggregates, top three inches, or patches, for example, and continuity of the density in time.

Algae may be benthic or planktonic. For purposes of this seminar benthic algae may be unimportant. Unless they are great masses such as Spirogyra sometimes exhibits, and are anchored to the bottom, they tend to be confined to a thin layer, up to 5mm thick. These bottom dwelling forms neither clog filters nor produce tastes and

odors as a general thing. But we can learn much from them. In 1937, slides were hung in the Ohio River below the Stream Pollution Investigations Station, at Third and Kilgour Streets, in Cincinnati Ohio; within a few days they were colonized by various organisms. Most of these were not the organisms present as plankton in the river. This work has been repeated in Santa Fe Lake in Florida since October 1959. The same results have been obtained, but the organisms are different, Santa Fe being an acid lake.

Slides left in the lake four and five months have developed thick and dense coatings of algae and other organisms, but almost the same species occur now as were present a few days after setting out. Given a constant exposure to such nutrients as are brought to them by wind-circulated water, they do not die out, but increase, albeit slowly. Water temperatures have fluctuated between 11° and 16° C. The nutrients have not been identified or measured. But the significant thing is that species of Frustulia for example have not died out. Dominance has changed, but not the species list. The nutritional threshold has been sufficient to maintain a continuous but slow growth.

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We do not have to postulate a very sharp increase in the division rate of an algal cell to show a great increase in numbers within a short time. Birge and Juday (1922) decided that algae in the Madison (Wis.) lakes divided once or twice in 24 hours. On Aug. 31, 1956, there was a population of one Chlorella per ml. in a settling basin at Oak Ridge, Tennessee; three days later this pond was green with a population of about 20,000 per ml. This represented 15 divisions in three days rather than three -- a five-fold increase. But the biomass had increased many thousandfold. Particular attention was paid to the size of the cells. They were about the same average size throughout the three days. Since this bloom maintained itself for about two weeks, despite a heavy loss downstream every 24 hours, the necessary nutrients were evidently present during these 2 weeks in sufficient abundance to support this growth. Actually the amount could have been quite small, even for a 20,000,000/ liter population.

The nutrition of Chlorella pyrenoidosa has been carefully worked out (Burlew, 1953) and it is common knowledge that the organism requires so little mineral salts as to be a pest in laboratories by invading distilled water dispensers. Birge and Juday (1922) give the composition of the total plankton crops in the Madison lakes, a type of study which has been repeated many times for individual species of algae. Generally the dry weight is about one-tenth the wet weight, and the ash from 3.5 to 10.0 percent of the dry weight, except in diatoms where the silica makes the ash content much higher. In some prepilot-plant experiments on culturing Chlorella, Mituya et al (Burlew, 1953) set up about 1200 liters of culture medium containing roughly 8000 grams of nutrients, 3000 grams of this being urea. After ten days operation, algae had taken up.0065 percent of the available nutrients as ash content. These are rough computations from their figures, but are mass figures rather than small scale laboratory cultures. The results are a confirmation of the idea that contributed nutrients in very small amounts account for enormous growths.

It becomes of interest, then, to determine the quality of nutrients necessary for maintenance of an algal species, and the quantity necessary to produce a sharp increase in the population of that species. The first statement is that the matter is subject to very little generalization -- each species has its own optimum.

We are quite familiar with this. Thus we say that it takes one amount of copper as CuSO₄ to kill species A and a different amount for species B. All attempts to grow Spirogyra in our laboratory on spring water and distilled water were unsuccessful until glass distilled Cu-free water was used, after which the cultures grew well. The copper in the spring water is 0.01 ppm. In a long

series of tests on chlorine-and iodine-resistant algae such as Anabaena, Navicula, Gomphosphaeria, and some others of interest to swimming pool owners, it was found that after an initial inoculation with perhaps 50 species of microorganisms most were killed by extremely small amounts of these two chemicals, but it was very difficult to kill the above three algae. These, and a considerable number of other species grewwell in the controls. The questions at once arose as to whether this was a direct toxicity, or whether the ratios of nutrients were not so adjusted that other algae could live, or even if other species died because of antibiotic production. Since several nutrient substrates were used in the experiments, it seems that direct toxicity was the killing agent. But selecting a single factor is not easy.

DENSITY AS A MULTI-FACTOR RESULT

Yount (1955) concluded that "there is, in any habitat, no one factor which determines the species density of an area, but always a combination of factors." One may question how such amazing blooms as are often encountered come into being if they are dependent on a combination of factors. Certainly proper nutrients, and adequate nutrients are two of the several factors. But figures can be presented to show that these are not always effective. Thus phosphorus, supposedly contributed by the rich phosphate mining areas of central Florida has been repeatedly suggested as a cause of the blooms of Gymnodinium breve in the Gulf of Mexico. Lackey and Hynes (1955) studied the nitrogenphosphorus ratios in sea water where G. breve was present in varying numbers and could find no correlation either as to total NO3 or PO4 present, or as to N/P ratios. Samples from the vicinity of Englewood, Florida, on June 18, 1956, were centrifuged. The results of some of the 18 analyses are shown in the following tabulation:

Organisms per liter	NO3, ppm	PO ₄ ppm
4,814,000	0.507	0.
9,500	0.149	0.021
212,000	0.188	0.011
3,235,000	0.541	0.
211,000	0.160	0.

The decanted water contained virtually no particulate matter, and since there had been a marked kill of fish and other animals, with considerable decomposition, the failure to find more PO₄ was unexpected. It is unfortunate that total P was not determined. However, these figures indicate that certainly not all of it was tied up in the dinoflagellate bodies. The nitrogen figures are likewise inexplicable. The essential point here is that it is not possible to say too much or too little of either, or an improper ratio, is limiting. We have here a verification of Yount's point, mentioned above.

NEED FOR CHEMICALLY DEFINED MEDIA

Even with bacteria free cultures, the medium must be chemically defined if we are to arrive at the nutritive threshold for unlimited production. Ketchum and Redfield (1938) used sea water, enriched by the addition of 20 atoms of nitrogen to 1 of phosphorus, to secure quantities of Nitzschia closterium. The cultures were bacteria free, and by following the cell count they could determine when to add more nutrients. They found that 2 x 109 cells/liter completely exhausted the nutrients. At the end of 15 days the division rate had dropped to 0.07 per day. The only unknowns here are the substances in sea water which might have had an adverse effect on the division rate, or possibly metabolites produced by the diatoms themselves. Manifestly the division rates they obtained are far lower than those which occur in some cultures. We cannot say the same thing for populations in nature, unless we can rule out the possibility that dense blooms are aggregates, drawn together from a large volume of water, by some unknown mechanism. However, it has been shown that Entosiphon maintains a division rate of between 1 and 3 per day (Lackey, 1929; Bennett, In Press) for many generations as long as daily isolations are made, whereas in mass cultures the division rate soon shows and after ten days dead organisms as well as stunted ones are found. Where flow-through culture conditions are maintained, or under such conditions as provided by Ketchum and Redfield (1938) it is at least possible to determine the nutritive levels necessary to maintain or step up the division rate. In their cultures it was evident that nitrate and phosphate were rapidly used up, and had to be replaced.

This is a rather general assumption for algae. In a stream where there is less chance of depletion than in a lake, absorption is probably at a steady rate by users. Rice (1953) has shown this for PO₄ in the case of bacteria free strains of Nitzschia. Sawyer and Lackey (Sawyer and Lackey, 1945; Lackey, 1945) after a study of 17 lakes in southeastern Wisconsin concluded that when NO3 and PO₄ in a lake reached values of 0.3 and 0.015 ppm, respectively, a bloom appeared, sometimes one species, sometimes another, sometimes several. In Lake Santa Fe, Florida, the lowest population recorded in over a year was following a heavy rain which considerably diluted the lake, but did not bring in appreciable nutrients since there is exceedingly little run-off into the lake. The rain occurred March 31-April 1, and on April 6 the NO3 in the lake was 0.16 ppm and the PO4 0.005 ppm. Almost the entire population, aside from bacteria, consisted of Anabaena filaments, 182 per ml. Considering Anabaena as potentially able to fix its own nitrogen and therefore probably limited principally by available PO4, its lone position is understandable.

INABILITY TO DO MORE THAN GENERALIZE

Among the most frequent of the bloom forming organisms are Euglena species and their green and colorless relatives. It would seem that the nutrient thresholds for such organisms would be well known. Unfortunately, most of our knowledge pertains to their environments, rather than the nutrient substrates necessary for dense blooms. Thus we know that Euglena mutabilis favors H2SO4 acidity, that Entosiphon and Peranema form dense cultures in a medium containing autoclaved wheat, and that many genera and species of the group occur abundantly in barnyard pools. But almost all of these are either not grown in the laboratory or, if so, are grown along with other organisms so that defined substrates are not known. Astasia klebsii (von Dach, 1940) is easily grown in pure culture on an acetate medium; Euglena gracilis has been grown by many workers in bacteria-free media and its status in this respect has been reviewed by Pringsheim (1956) in a paper which indicates the need for pure culture work with this group. Cultures are easily developed and the Indiana Culture Collection (Starr, 1960) lists 55 species or strains of Euglenophyceae which they maintain either in unialgal, or bacteria-free condition. Most of these, however, are grown in media containing beef extract, yeast extract, and tryptone, viz., not a chemically defined medium. Many of their other algae are maintained in media not possible to chemically define.

When we note the conditions of blooms in the field, it is at once apparent that there is much variation of the conditions under which they appear, and that at best our knowledge of what nutrient substances and amounts are related to algal density is merely a guess. Johnson, working on raising shrimp in controlled pools at Marineland, Florida, added commercial fertilizer. The pools developed heavy concentrations of Coccolithophora, not always the same species, and occasionally another genus such as Platymonas bloomed. Nevertheless, there seems little doubt, from records of too many workers to cite, that amounts of NO3and PO4 can readily be limiting for many organisms, probably including most blooms of Euglenophyceae. However, for these last it is highly probable that undefined extractives and dissolved organic substances (often from the excreta and dung of animals) are also necessary. The use of soil extract also indicates such is the case.

We recognize the role of NO₃ and PO₄ in a practical way. Pringsheim (1949) varies the ratio of NO₃ to PO₄ between 4 and 8 to 1, in his Chu 10 formulae. He gives other formulae as Knop's solution, 11 parts NO₃ to 2 parts PO₄; Pringsheim's own medium is 10 parts NO₃ to 1 PO₄. The formulae of Ketchum and Redfield (1938) are by weight, 9 to 1 and 6.8 to 1; by atoms, 20 and 15 to 1.

BLOOMS DURING AND FOLLOWING WASTE TREATMENT

Anyone familiar with the accepted methods of biological treatment of sewage and trade wastes knows that eventually certain blooms are inevitable; in trickling filters, only on the top of the medium; in lagoons and oxidation ponds, on the surface; and after treatment, almost invariably in the receiving water. These blooms may be blue greens or filamentous greens on trickling filter: small Chloroeoccales such as Chlorella, Schenedesmus, and Golenkinia, or Volvocales such as Chlorogonium or Chlamydomonas on oxidation ponds. Sometimes Euglena is a heavy contributor to oxidation pond blooms. Trade waste lagoons may vary a great deal in bloom composition, depending apparently on specific substances in the waste. Thus the lagoons at Plymouth, Florida, which handled citrus wastes bloomed heavily with Euglena gracilis, E. pisciformis and Chlorogonium euchlora. Receiving water blooms are frequently mixed, but with numerous Euglenophyceae. Sometimes such waters fail to bloom, as is often the case with the polishing ponds at the University of Florida campus treatment plant.

Assuming that the above methods of treatment have produced a satisfactory reduction in BOD and in bacterial numbers, what can we say regarding the algal densities produced, either coincident, or subsequently? What do they — at least the subsequent densities—indicate regarding eutrophication of receiving waters?

It is well known that lakes in the vicinity of cities, if they receive urban drainage, may become eutrophic. There seems little doubt that this is due to an increased addition of phosphate and nitrate, and, what we are prone to forget, other necessary growth promoting substances. This is not a case of secondary BOD contributions which, if they oc-

cur at all, lag far behind the algal blooms. There has been considerable discussion of the possibility of removing PO₄ from the effluents of conventional treatment plants. It is suggested here, that this may change only the composition of the bloom. There are algae whose PO₄ requirements are extremely low, below the amounts we might remove. In short, it would seem that conventional treatment has solved the major waste treatment problems. While algal blooms are often a nuisance, and sometimes highly objectionable, they are not necessarily a menace to health. Perhaps we had better concentrate investigation on means of lowering algal densities in receiving waters, by biologic means, such as predator-prey relationships or by harvesting the algae.

SUMMARY

In summary, we can say that for some species of algae, density is directly related to the amounts of nitrate and phosphate present, and that minima of these for producing blooms are 0.3 and 0.015 ppm. However, it should also be determined whether the algal bloom is merely a local aggregate due to some probably unknown factors, or whether it is present in a great volume of water or is widespread.

We are not ready to set absolute values on nutrients, because we still know too little about the requirements of individual species. This is especially true of those species which are not obligate autotrophs, as seems to be the case for many Euglenids and Coccolithophora or Chlorophyceae. We also know too little about the production of antibiotic metabolites by other species in the environment. But we have reached the stage where it is possible to generalize, just as the agriculturist can generalize about the particular fertilizer needs, if we realize that sometimes he, too, is not successful despite following a pattern.

REFERENCES

Bennett, Carrie F. In Press., Sublethal effects of Co⁶⁰ Gamma radiation on Entosiphon sulcatum. Paper delivered before the 21st annual meeting of the Assn. of Southern Biologists, New Orleans, April 22, 1960.

Birge, Edward A. and Chancey Juday. 1922. The inland lakes of Wisconsin. The plankton. I. Its quantity and chemical composition. Bull. 64, Scientific Series 13. Wisconsin Geological and Natural History Survey.

Burlew, John S., Editor. 1953. Algal culture from laboratory to pilot plant. Publication 600, Carnegie Inst. of Washington.

Ketchum, Bostwick H. and Alfred C. Redfield. 1938. A method for maintaining a continuous supply of marine diatoms by culture. Biol. Bull. 75, 1: 165-169.

Lackey, James B. and Jacqueline A. Hynes. 1955. The Florida Gulf Coast Red Tide. Bull. Series No. 70, Florida Eng. and Ind. Exp. Station. 1-24.

Lackey, James B. 1945. Plankton productivity of certain southeastern Wisconsin lakes as related to fertilization. II. Productivity. Sew. Wks. J., 17(4): 795-802.

Lackey, James B. 1929. Studies in the life histories of Euglenida. II. The life cycles of Entosiphon sulcatum and Peranema trichophorum. Archiv. fur. Protistenkimde. 67(1): 127-156.

Pringsheim, Ernst Georg. 1956. Contributions toward a monograph of the genus Euglena. Nova Acta Leopoldina. 18(125): 1-168.

Pringsheim, E. G. 1949. Pure cultures of algae. Cambridge University Press. XII - 119.

Rice, Theodore R. 1953. Phosphorus exchange in marine phytoplankton. Fishery Bull. 80, U.S. Fish and Wildlife Service, 54: 1-89.

Sawyer, C. N. and J. B. Lackey. 1945. Investigation of the odor nuisance occurring in the Madison Lakes, particularly Monona, Waubesa, Kegonsa, from July 1943 to July 1944. Report to the Governor's Committee, Madison, Wisconsin. 1-92.

Starr, Richard C. 1960. The culture collection of algae at Indiana University. Am. J. Bot. $\underline{47}(1)$: 67-86.

von Dach, H. 1940. Factors which affect the growth of a colorless flagellate, Astasia klebsii, in pure culture. Ohio J. Sci. 40: 37-48.

Yount, James L. 1955. Factors that control species numbers in Silver Springs. Productivity of Florida Springs. NR 163-106 (NONR 580⁰2). Third Annual Report to Biology Branch, Office of Naval Research. Jan. 1 to Dec. 31, 1955. Unpaged.

ZoBell, Claude E. 1946. Marine Microbiology. Chronica Botanica Co., Waltham, Mass. XV - 239.

Productivity and How to Measure It

METHODS OF MEASUREMENT OF PRIMARY PRODUCTION IN NATURAL WATERS*

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ABSTRACT

To estimate primary production of natural plant populations it is necessary to measure the rate of photosynthesis of the populations, or portions of them, under natural environmental conditions. Measurements may be carried out in the field or under simulated natural conditions in the laboratory. Some of the methods by which this has been done are the following:

- 1. Small samples of the natural plant community are confined in transparent vessels, and the rate of change of some product or raw material of photosynthesis is measured. Changes in dissolved oxygen, pH, or uptake of C¹⁴-labeled carbon may be followed.
- 2. Changes in the concentration of a product or raw material of photosynthesis are measured in unconfined natural waters.
- 3. By measuring the chlorophyll content of a system and the radiant energy reaching the plant populations the rate of photosynthesis may be deduced empirically.
- 4. Plant material may be harvested periodically to deduce the photosynthetic rate from changes in plant biomass.

The accuracy of most of these methods is limited by the methods of sampling the plant populations or parameters of photosynthesis. Improved sampling accuracy can be achieved by the use of proper statistical methods and by making many more observations. To take a significant number of observations, techniques of automation can be used. One possibility is to continuously record the oxygen tension, either invessels or in nature, with polarographic electrodes. Another is to use automatic sample-collecting devices.

In principle the measurement of photosynthesis is the same in the laboratory and in the field. In practice complexities and uncertainties creep in

when we attempt to estimate the photosynthetic rate of natural populations under natural conditions. While laboratory studies of photosynthesis are likely to be concerned with defined conditions, field studies seek to find the rate of photosynthesis under existing conditions in nature. These conditions may be difficult to define and are variable with time.

Several recent reviews (Lund and Talling, 1957; Steele, 1959; Strickland, 1960) and symposia (Cons. Int. Explor. Mer, Rapp. et Proc. Verb., Vol. 144; Second all-Union Conference on Photosynthesis: translation by Office of Technical Services, 123 pp, 1957) have dealt with problems of measuring primary production.

I shall briefly review the more promising methods in the light of these critiques, and point out what seem to me to be the important shortcomings of our present methods and the most likely means of making improvements. Four general approaches have been made in estimating primary production, and I shall review each briefly.

1. Small samples of the natural community are confined in transparent vessels where the rate of change of concentration of some product or raw material of photosynthesis can be measured. Examples of this approach are the light-and-dark-bottle method (Gaarder and Gran, 1927) and the C¹⁴ method (Steeman-Nielsen, 1952). Similar methods have been used with benthic populations as well as phytoplankton (Odum, 1957; Pomeroy, 1959; 1960a).

This general approach has some important limitations.

- a) The samples are small and may not be representative. This difficulty may be overcome in part by the use of integrating sampling methods (Griffith, 1957) or by abundant replication of observations and statistical treatment.
 - b) Natural eddy diffusion is lacking in small

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vessels. This may result in poor exchange of materials. It certainly prevents vertical movement of the cells. Experiments on the effect of stirring on photosynthesis in closed vessels are conflicting (Talling, 1960), and further experimental work is needed to clarify this point.

c) Small closed vessels may become depleted of essential materials. Especially in the study of blooms or macrophytes (cf. Emerson and Green, 1934) the investigator is pressed by possible latent periods on one side and by depletion of CO₂ or nutrients on the other. The exposure period must be chosen with care.

Closed-vessel methods usually involve the exposure of the vessels in nature at the depths from which the samples were collected. This is laborious and expensive. It has become increasingly popular to place the bottles instead in some sort of chamber, in the laboratory or aboard ship, that duplicates the natural physical environment. The chambers vary in sophistication from washtubs to illuminated constant-temperature baths with rolling or shaking devices. The principal difficulty in using such chambers is the duplication of the radiant energy spectrum and energy flux found in nature (Strickland, 1958; 1960). Daylight flourescent lamps may have energy spectra that depart widely from that of sunlight (MacAdam, 1958). The use of simple light meters to estimate radiation may lead to both qualitative and quantitative errors. Furthermore, there is some evidence that flourescent lights inhibit the growth of some algae (Kain and Fogg, 1960).

In spite of all these limitations and others, closed-vessel methods are useful. The ${\rm C}^{14}$ method potentially offers much greater accuracy than the light-dark-bottle method, but this accuracy is largely potential rather than actual at present. Interpretation of the results of C¹⁴ uptake studies is in a state of controversy (cf. Steeman-Neilsen, 1952; 1959; Ryther, 1956). Corrections for isotope exchange and for respiration vary. Ryther (1956) found that newly-respired carbon is used preferentially for photosynthesis in cultures of Dunaliella (see also Myers and Johnston, 1949). If this is true under all natural conditions, it will greatly improve the accuracy of the C14 method. However, more experimental work under more varied conditions is needed to see if this is true under all natural conditions. At present the method offers little advantage except in very oligotrophic conditions where no other method is sufficiently sensitive.

The light-dark-bottle method, used with proper precautions (cf. Lund and Talling, 1957; Steeman-Nielsen, 1957; Strickland, 1960), is satisfactory in situations of moderate to high productivity, where observations need not be longer than 12 hours. This method is not fashionable at present,

but it requires a minimum of equipment and can be made to yield satisfactory results in many situations.

2. Changes in the concentration of some product or raw material of photosynthesis may be measured in unconfined natural waters, to estimate net photosynthesis of all plant populations in the system. The difficulties of confined samples are avoided, and if the system is well mixed, the sampling problem is less. However, we must contend with the estimation of import and export of the substance measured from an open system.

A useful application of this type of approach is the measurement of diurnal changes in dissolved oxygen (Sargent and Austin, 1949; Odum, 1956; 1957). The method works well in highly productive, flowing systems. The limit on accuracy would seem to be the estimation of the oxygen exchange coefficient and, in unproductive or incompletelymixed systems, this is a serious limitation.

It is not certain whether the diurnal curve method is comparable with the light-dark-bottle method (cf. Jackson and McFadden, 1954; Talling, 1957; Odum and Hoskins, 1959; Pomeroy 1960a). Talling suggested that both methods were rather crude and that close agreement should not be expected. Probably results within an order of magnitude should be looked upon as in agreement.

Changes in pH can be used in much the same way as changes in oxygen (Verduin, 1956a; 1956b). The gas exchange difficulties are less. The method is less sensitive in sea water, and in any case requires a very good pH meter.

Long-term changes in phosphorus have been used to estimate production in the sea and coastal waters (Riley, 1951; 1956; Steele, 1956). In its present form it is only suitable for relatively simple systems, such as the sea, where interchange of phosphorus with the land and the bottom is minimal. Short-term changes in phosphate concentration cannot be correlated with photosynthetic rates, because the turnover of phosphate is rapid and partly independent of photosynthesis (Pomeroy, 1960b).

- 3. The measurement of chlorophyll content and available energy offers a rapid, inexpensive procedure (Ryther and Yentsch, 1957). It would not appear to offer great precision, regardless of refinements in technique. The accuracy of the method, aside from problems of sampling and pigment analysis, depends on the estimation of the assimilation number of the plant populations. The assimilation number may vary widely (Steeman-Nielsen, 1959; Strickland, 1960), probably even within a given system.
- 4. Harvest methods may be used to estimate production where much of the plant material pro-

duced accumulates in situ over a considerable period. Such methods have been used to estimate production of seaweed (Blinks, 1955), sea grasses (Grntved, 1958), salt-marsh grasses (Smalley, 1959; Odum and Smalley, 1959) and some other aquatic plants (Penfound, 1956). The scientist inevitably is harvesting side by side with the fishes, snails or grasshoppers. To estimate net production, it is necessary to know the food consumed by the herbivores. This is possible (Teal, 1958) but laborious.

In selecting a method for estimating primary production, it is necessary to consider the kind of system to be studied and the questions to be asked about it. The use of the C¹⁴ method is very promising, although its present widespread use does not seem to be justified by our present knowledge of the path of newly-respired carbon under diverse natural conditions. With more complete knowledge of these details the C¹⁴ method may permit quite accurate work with rather simple field equipment.

Probably the most serious problems in estimating production are the interpretation of the physiological requirements and responses of the plants, and the proper sampling of natural populations of aquatic plants. Much attention has been given to the physiological problems, and such as remain for the ecologist may soon be solved. The sampling problem has received considerably less attention and, in my opinion, it is the greatest limitation on accuracy of most current methods of estimating production.

Natural plant populations typically are not randomly distributed, and methods of statistical analysis suited for clustered, over-dispersed distributions must be used. Such methods exist, and they have been used occasionally in plankton studies (Barnes and Marshall, 1951; Barnes and Hasle, 1957; Bliss and Calhoun, 1954) but seldom

if ever in production studies. The chief impediment to their application to production studies is the need for many replications of the observations. This becomes impossibly laborious and expensive. The best way to surmount this difficulty would seem to be by the use of techniques of automation. The necessary equipment need not be more expensive than that needed for some of the methods currently popular.

The use of polarographic electrodes makes it possible to obtain a continuous record of changes in oxygen tension in nature or in closed vessels (Carritt and Kanwisher, 1959; Kanwisher, 1959). Much more detail can be obtained in this way, and it should be possible to incorporate oxygen recorders in buoys, so a number of samples or stations can be studied concurrently with a single ship or field party. By the use of time switches the input from several pairs of electrodes can be fed into one recorder, or a multi-channel recorder can make continuous records of all inputs. Incident radiation and water temperature can be recorded simultaneously, often on the same recorder.

A different automation technique is employed by H. T. Odum, who has in operation an automatic sampling buoy that collects a 24-hour series of samples for Winkler determinations. While this may lack some of the advantages of a continuous record, it permits sampling of a wide spectrum of chemical information. Similar installations along the shoreline are now fairly common.

These, then, are some suggestions for improved estimates of primary production: randomized or otherwise statistically planned sampling programs, data collected automatically over broad segments of space and time, and statistical treatment of the observations to give both an estimate of production and a parameter of reliability. While it is not always possible to set up a program such as this, it is a goal we might try to attain.

REFERENCES

Barnes, H., and G. R. Hasle. 1957. A statistical examination of the distribution of some species of dinoflagellates in the polluted inner Oslo Fjord. Nytt Mag. Bot., $\underline{5}$: 113-124.

Barnes, H., and S. M. Marshall. 1951. On the variability of replicate plankton samples and some applications of 'contagious' series to the statistical distribution of catches over restricted periods. J. Mar. Biol. Assoc. U.K., 30: 233-263.

Blinks, L. R. 1955. Photosynthesis and productivity of littoral marine algae. J. Mar. Res., <u>14</u>: 363-373.

Bliss, C. I., and D. W. Calhoun. 1954. An outline of Biometry. New Haven, Yale Co-operative Corp. 272 pp.

Carritt, D. E., and J. W. Kanwisher. 1959. An electrode system for measuring dissolved oxygen. Anal. Chem., 31: 5-9.

Emerson, R., and L. Green. 1934. Manometric measurements of photosynthesis in the marine alga Gigartina. J. Gen. Physiol., <u>17</u>: 817-843.

Gaarder, T., and H. H. Gran. 1927. Investigations of the production of plankton in the Oslo Fjord. Cons. Int. Explor. Mer, Rapp. et Proc.-Verb., $\underline{42}$: 3-48.

- Griffith, R. E. 1957. A portable apparatus for collecting horizontal plankton samples. Ecology, 38: 538-540.
- Grntved, J. 1958. Underwater macrovegetation in shallow coastal waters. J. du Cons., 24: 32-42.
- Jackson, D. F. and J. McFadden. 1954. Phytoplankton photosynthesis in Sanctuary Lake, Pymatuning Reservoir. Ecology, <u>35</u>: 1-4.
- Kain, J. M., and G. E. Fogg. 1960. Studies on the growth of marine phytoplankton. III. <u>Prorocentrum micans Ehrenberg</u>. J. Mar. Biol. Assoc. U.K., 39: 33-50.
- Kanwisher, J. W. 1959. Polarographic oxygen electrode. Limnol. Oceanogr., 4: 210-217.
- Lund, J. W. G., and J. F. Talling. 1957. Botanical limnological methods with special reference to the algae. Bot. Rev., 23: 489-583.
- MacAdam, D. L. 1958. Continuously recording spectroradiometer. J. Opt. Soc. Amer., <u>48</u>: 832-840.
- Myers, J., and J. A. Johnston. 1949. Carbon and nitrogen balance of <u>Chlorella</u> during growth. Plant Physiol., <u>24</u>: 111-119.
- Odum, E. P., and A. E. Smalley. 1959. Comparison of population energy flow of a herbivorous and a deposit-feeding invertebrate in a salt marsh ecosystem. Proc. Nat. Acad. Sci., 45: 617-622.
- Odum, H. T. 1956. Primary production in flowing waters. Limnol. Oceanogr., $\underline{1}$: 102-117.
- Odum, H. T. 1957. Trophic structure and productivity of Silver Springs, Florida. Ecol. Monogr., 27: 291-320.
- Odum, H. T., and C. M. Hoskins. 1959. Comparative studies on the metabolism of marine waters. Pub. Mar. Sci. (Univ. Texas). In press.
- Penfound, W. T. 1956. Primary production of vascular aquatic plants. Limnol. Oceanogr., 1: 92-101.

- Pomeroy, L. R. 1959. Algal productivity in salt marshes of Georgia. Limnol. Oceanogr., $\underline{4}$: 386-397.
- Pomeroy, L. R. 1960a. Primary productivity of Boca Ciega Bay, Florida. Bull. Mar. Sci. Gulf and Carib. In press.
- Pomeroy, L. R. 1960b. Residence time of dissolved phosphate in natural waters. Science. In press.
- Riley, G. A. 1951. Oxygen, phosphate, and nitrate in the Atlantic Ocean. Bull. Bingham Oceanogr. Coll., 13: 1-126.
- Riley, G. A. 1956. Oceanography of Long Island Sound. IX. Production and utilization of organic matter. Bull. Bingham Oceanogr. Coll., <u>15</u>: 324-344.
- Ryther, J. H. 1956. Interrelation between photosynthesis and respiration in the marine flagellate <u>Dunaliella euchlora</u>. Nature, <u>178</u>: 861-862.
- Ryther, J. H., and C. S. Yentsch. 1957. The estimation of phytoplankton production in the ocean from chlorophyll and light data. Limnol. Oceanogr., 2: 281-286.
- Sargent, M. C., and T. S. Austin. 1949. Organic productivity of an atoll. Trans. Amer. Geophys. Union, 30: 245-249.
- Smalley, A. E. 1959. Comparison of energy flow in two invertebrate populations in a salt marsh ecosystem. Doctoral thesis, University of Georgia.
- Steele, J. H. 1956. Plant production on the Fladen Ground. J. Mar. Biol. Assoc. U. K., 35: 1-33.
- Steele, J. H. 1959. The quantitative ecology of marine phytoplankton. Biol. Rev., <u>34</u>: 129-158.
- Steeman-Nielsen, E. 1952. The use of radio-active carbon (C^{14}) for measuring organic production in the sea. J. du Cons., <u>18</u>: 117-140.
- Steeman-Nielsen, E. 1957. Experimental methods for measuring organic production in the sea. Cons. Int. Explor. Mer, Rapp. et Proc.-Verb., <u>144</u>: 38-46.

Steeman-Nielsen, E. 1959. Untersuchungen uber die Primarproduktion des Planktons in Einigen Alpenseen. Osterreichs. Oikos, 10: 26-37.

Strickland, J. D. H. 1958. Solar radiation penetrating the ocean. A review of requirements, data and methods of measurement, with particular reference to photosynthetic productivity. J. Fish. Res. Bd. Canada, 15: 453-493.

Strickland, J. D. H. 1960. Measuring the production of marine phytoplankton. Bull. Fish. Res. Bd. Canada. In press.

Talling, J. F. 1957. Diurnal changes of stratification and photosynthesis in some tropical African waters. Proc. Roy. Soc. London, <u>B147</u>: 57-83.

Talling, J. F. 1960. Comparative laboratory and field studies of photosynthesis by a marine planktonic diatom. Limnol. Oceanogr., 5: 62-77.

Teal, J. M. 1958. Energy flow in the salt marsh ecosystem. Proc. Conf. Salt Marshes, pp. 101-107.

Verduin, J. 1956a. Primary production in lakes. Limnol. Oceanogr., 1:85-91.

Verduin, J. 1956b. Energy fixation and utilization by natural communities in Western Lake Erie. Ecology, 37: 40-50.

FACTORS WHICH REGULATE PRIMARY PRODUCTIVITY AND HETEROTROPHIC UTI LIZATION IN THE ECOSYSTEM*

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In this paper algae are considered as components of the ecosystem and the algal bloom is viewed as an unbalance between production and consumption of organic matter in the ecosystem. We might even go so far as to think of the noxious bloom as a sort of cancer in the metabolism of Nature. Abnormal growths, whether at the cellular or at the ecosystem level, result in the final analysis from the failure of, or at least the overtaxing of, regulatory processes. In the case of the metropolitan waste problem the excess growth results, of course, from the overloading of the system with growth-promoting inorganic and/or organic substances. If control is to be more than a trial and error proposition we must study and understand not only the growth itself (i.e. the "bloom") but also the system in which the growth occurs (i.e. the body of water).

A couple of other general points may well be emphasized before we get down to cases. First, descriptive information alone rarely solves practical problems; functional information is also usually needed. Dr. Pomeroy in the previous paper has discussed some of the methods of measuring

one important rate function, that is, primary production. It was evident from his paper that procedures such as the diurnal oxygen curve method, which measures function of the whole ecosystem, are potentially of greater practical value than methods which are restricted to small samples in bottles. Much remains to be done to establish the reliability of total system measurements, but until we do have such measurements we will be greatly handicapped in attempts to understand any specific component within the system. As will be pointed out later there are important functions other than primary production which must be considered.

A second point is that in applied research we observe again and again that no single method of control can be counted on to work or to be feasible under all of the varied conditions of Nature. The control of algal blooms will certainly prove to be no exception to this rule. Consequently, we should not restrict our thinking to one or a few approaches just because a measure of success has been achieved with them. We need to have other possibilities ready in case the recommended method

^{*}Contribution No. 20 from the University of Georgia Marine Institute, Sapelo Island, Georgia.

does not work. We must also be prepared for unforeseen changes in the nature of wastes resulting from social and economic development beyond the control of the mere scientist. Sooner or later, at some time or place, any theoretically sound approach may prove practical. There should be no limit to the arsenal of the mind!

At this point it would be well to review briefly the basic theory of ecosystem function. Figure 13 illustrates in a simplified manner what I like to call "the two basic principles of functional ecology", namely, the one-way flow of energy and the cycling of materials (nutrients, etc.). The "boxes" in the diagram represent the standing crops of functional autotrophs and heterotrophs in terms of weight or calories per unit area. The "pipes" represent energy flow in terms of calories per unit time. The "stippled circle" represents movement of nutrients and other materials from one biological unit to another, or to and from a pool within the system. A point to emphasize is that while materials circulate and inorganic nutrients may be used over and over (if not lost from the system) the flow of energy is one-way; once utilized in respiration the energy is dispersed as heat and lost from the system. Imports and exports of both materials and fixed energy are also shown on the diagram; such imports and exports, of course, are of primary concern in waste disposal situations.

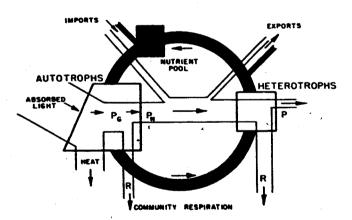


Figure 13. SIMPLIFIED FLOW DIAGRAM OF AN ECOSYSTEM SHOWING THE ONE-WAY FLOW OF ENERGY AND THE RECYCLING OF MATERIALS. The "boxes" represent the standing crops of autotrophic and heterotrophic components, the open "pipes" the flow of energy and the stippled circuit the flow of materials (nutrients, etc). PG = gross production; PN = net primary production; P = heterotrophic production which may be consumed within the system (by secondary consumers, i.e. carnivores, etc., which are not shown on the diagram). It may be stored or exported from the system.

Another point to emphasize is that there are two kinds of primary production but only one kind of secondary production. In the diagram, total phytosynthesis is designated as gross production while that portion of the fixed energy not used in plant respiration and ending up as "growth" is designated as net production. At the heterotrophic level the only thing "produced" is "growth". In this broad sense "growth" as a phenomenon includes not only additions to the biomass but production of stored food and soluble organic materials which may leak out or be excreted from the organisms. A source of much confusion is that the various methods of measuring primary production, as described in the preceding paper, do not measure the same thing; some methods measure gross primary, some net primary, others apparently estimate a quantity in between, while still other methods measure net community production (i.e. gross production minus community respiration). Thus, it is often difficult to compare different situations where different methods have been employed. Finally, as clearly shown in Figure 13, energy flow at any level, or for any specific species or population, is the sum of production and respiration. If the population is growing rapidly production may be a major part of energy flow. but if the population is not growing then all of the energy flow may be respiration. In our preoccupation with production and its measurement we must not forget that consideration of respiration is equally important. None of these important functions can be determined by merely describing the composition of the standing crop or by making single estimates of its size.

Diagrams such as that of Figure 13 can be made quantitative to represent a particular situation. The area of the "boxes" can be made proportional to the standing crops, and the diameter of the energy flow "pipes" and the nutrient "pipes" can be made proportional to the rates of flow. Above all, however, such graphic representations of energy flow enable us to visualize ways in which unbalance between production and consumption may come about, and, by the same token, we may visualize various ways in which the unbalance might be corrected. For example, if the inflow of nutrients is increased gross production will increase unless the inflow is counteracted by (1) loss from the system or into an unavailable pool or (2) a decrease in the available light energy (as might result from increased turbulence, increased turbidity or shading). Increase in gross production will result in an increase in standing crop of autotrophs and perhaps the development of a bloom situation unless there is a compensatory energy flow through the community respiration "pipes" (i.e. plant respiration plus heterotrophic respiration) or unless there is immediate and efficient conversion of net production into herbivore biomass. Thus, quickly visualize that there are several quite different ways by which a bloom resulting from excess inorganic nutrients might be controlled, as for example: (1) reducing inflow of nutrients (i.e. "stripping"), (2) diverting nutrients into a pool (as by converting into unsoluble or unavailable forms), (3) reducing effective light, (4) increasing respiration of the algae themselves (i.e. "self destruction"), (5) increasing direct consumption of net production by herbivores (i.e. increasing grazing), and (6) exporting (i.e. removing) organic matter (and with it some of the nutrients) either as primary (algae) or as secondary production (for example, fish). How effective any of these approaches would be depends, of course, entirely on quantitative considerations, that is, how much or how fast materials or energy can be moved along the routes in question.

Increasing imports of organic matter result in a somewhat different situation as can be visualized in the diagram. Nature develops several mechanisms to deal with the situation but, of course, these mechanisms are commonly overtaxed by the magnitude of metropolitan wastes. Increased consumption by microorganisms and other heterotrophs, and increased export downstream or storage within the system are the usual direct responses of the ecosystem. The increased community respiration, of course, puts critical demands on the oxygen supply which becomes the major limiting factor. As organic matter piles up between the autotrophic and heterotrophic "boxes" (see diagram) some of it may flow backwards, as it were, and the autotrophs may become heterotrophs. From what little we know about this at present it would appear that this route cannot be very effective as a means of reducing the organic matter. From the standpoint of the ecosystem as a whole it would appear that extensive shifts from autotrophic to heterotrophic nutrition (in those species of algae which are capable of doing so) is a sort of last-ditch regulatory mechanism. Certainly, organisms adapted to photosynthetic metabolism can hardly be expected to be as efficient consumers of organic matter as obligatory heterotrophs, or can they?

One of the reasons metropolitan wastes present such difficult problems is that they consist of complex mixtures of inorganic nutrients and energy-containing organic materials (plus poisons of various kinds!). It is a striking but little understood phenomenon that blooms resulting from organic nutrients seem to be more resistant to consumption by the community than are those resulting from inorganic fertilization. The kinds of algae (bluegreens or filamentous types, for example) which respond to organic nutrients often seem to be unpalatable to consumers. Or perhaps antibiotics are produced which inhibit consumers. Either possibility produces the same thing; something about

the chemical composition of the bloom favors delayed consumption often resulting in a big pile up of algae followed by decomposition of dead matter and depletion of oxygen. The well documented situation at Moriches Bay in Long Island Sound (Ryther. 1954) illustrates what often seems to happen. In this example when the nutrient flow shifted from inorganic to organic form (as a result of duck farming along the shore) previously rare and unknown species of algae, which were able to use organic phosphorous and nitrogen, produced large blooms. Unlike the diatoms and other phytoplankton previously present in the bay the new forms were not readily eaten by zooplankton and the shellfish. As a result both the human bathers and the oysters suffered. We certainly need to know a great deal more about why certain types of blooms are more resistant to natural consumption than are others. And of course we would like to know if by regulating the type of inflow we can avoid encouraging the resistant-type of bloom.

In discussing the functional processes in the ecosystem it would be well to keep in mind some orders of magnitude. The world distribution of primary production has been summarized by E. P. Odum (1959) and Ryther (1959). The quantitative relationships between production and consumption (i.e. Community P/R ratios) have been considered from the theoretical standpoint by H. T. Odum (1956, 1960), and with reference to lakes by Verduin (1956), Ohle (1956) and others, and with reference to estuaries by Odum and Hoskins (1958). The most naturally fertile parts of the biosphere are the shallow waters, areas such as marshes, shallow (eutrophic) lakes and ponds, estuaries and shallow coastal seas (especially with "upwhelling") as well as the alluvial plains, deltas and coastal terraces. Gross primary production in such areas may average 3-10 grams of dry matter per square meter per day for extended periods (to visualize these figures in approximate pounds per acre, multiply by 10) with even higher rates occurring during the most favorable seasons. In these fertile ecosystems the autotrophic and heterotrophic components (the "boxes" of Figure 13) are close together physically (thus promoting rapid regeneration of nutrients) while light, water and other conditions of existence tend to be favorable. In lakes, productivity is inversely related to depth (Rawson 1952) or to put it another way, is inversely related to distance between major autotrophic and heterotrophic components. Production rates may rise as high as $60 \text{ gms/M}^2/\text{day}$ in bloom situations. Some of the highest sustained production rates so far "officially" recorded have been found in sewage ponds (see Bartsch and Allum, 1957). At the other extreme, deep lakes, the deep oceans and land deserts may average less than 0.5 gm/M²/day. A large portion of the earth is in the low production

category where even temporary blooms are rare, unless, of course, production is augmented by man.

In most naturally fertile aquatic ecosystems production tends to get ahead of respiration, at least seasonally, with the result that some organic matter is either stored or exported. However, there are many fertile aquatic ecosystems which rarely develop blooms in the sense of a large excess of standing crop accumulating at any one time. Such systems need to be carefully studied for clues as to how man might handle situations where he inadvertently boosts fertility (i.e. "artificial eutrophication"). In our program at the Marine Institute of the University of Georgia we are intensely interested in one such fertile system which comprises the vast salt marshes and estuaries of the Georgia coast. At present we are promoting a multi-disciplined study in which ecologists, biogeochemists, microbiologists and geologists are taking part. Primary production in the marshestuary system may reach 5-10 gms/M²/day for a good portion of the year, yet except for an occasional outburst of dinoflagellates in limited areas (Pomeroy, Haskins and Ragotzkie, 1956) blooms are rare. Strong tidal mixing and export to the sea, of course, are important factors, but both macro-consumers (fiddler crabs, mussels, snails and fish) and micro-consumers (bacteria) are very active. Algae living on or near the surface of the vast intertidal sediments have a surprisingly high productivity which is maintained throughout the year (Pomeroy, 1959), yet the standing crop remains small. What portion of the net production is removed by currents and what portion is utilized by the abundant heterotrophs associated with the sediments has not yet been determined.

The coral reef is another very fertile natural system where community respiration is well balanced against community production. In our work on Eniwetok reefs (Odum and Odum, 1955) we were impressed not only with the importance of water movement but also with the following: (1) the intimate association of algae with corals and many other animals, (2) the highly developed symbiosis between autotrophs and heterotrophs in general, and (3) the large standing crop of consumers including large numbers of herbivorous fish which were capable of consuming algae as fast as produced. The important point I would like to make is that there is nothing in nature to indicate that high fertility must necessarily generate noxious blooms. Nor is there any reason to assume that poisons or other chemical controls are the only possible approaches to artificial eutrophication. It ought to be possible to establish reasonable balance by physical manipulations of water movement and by stimulating heterotrophic utilization. It may be necessary, however, to get man into the food chain. We

seem to have a paradox here. In many parts of the world people are crying for more production, while at this seminar we seem to be crying equally loudly for less production! Perhaps it would be well to begin thinking in terms of making use of the increased productivity created by cities rather than attempting to destroy it.

The diagram in Figure 13 is over simplified in that only one broad energy flow is shown. Actually, between the first and second trophic levels in most ecosystems the energy flow of the community is divided into two broad streams resulting in two types of primary consumption: (1) direct and immediate utilization of living plant tissues by herbivores and plant parasites, and (2) delayed utilization of dead tissues and stored food by other consumers. In our work on the Georgia estuaries we have been impressed with the fact that the ratio of detrital consumers to direct herbivores apparently is an important factor in determining the pattern of heterotrophic utilization (Odum and Smalley, 1959). If detrital consumers (bottom fauna and microorganisms, for example) predominate then a pulsating energy flow may be expected with the likelihood of periodic pile-ups of primary production assuming little or no export. If herbivores (i.e. "grazers" such as zooplankton) predominate then a more nearly steady-state may be maintained. In many natural waters, such as Long Island Sound, grazing by zooplankton provides an important check on spring blooms (Riley, 1956). As already indicated, waste disposal situations do not favor grazing by microcrustacea. The relative size of autotrophs and heterotrophs and the "size-metabolism law" (i.e. higher rates of metabolism per grams are small as compared to large organisms) are important considerations. To be effective, herbivores must be large enough individually to consume the algae (or else capable of breaking them down to "bite size") yet have a high enough rate of metabolism per gram to bring about a high rate of consumption. Also the question of antibiotic effects comes up again. There is no doubt that algae often produce antibiotics which affect their growth (Proctor, 1957). What is not clear is whether algae may produce in significant quantities metabolites which inhibit herbivores. Harvey's (1955) "exclusion theory" (to explain patchy distribution of phyto - and zoo-plankton in the sea) is based on such an assumption, but there is yet no proof. While we are thinking along these lines it would be well to point out that Nature rarely works only one side of the street. Thus, there may be substances which stimulate the herbivores to graze. Perhaps in the future we can look forward to the time when we may be able to apply an "environmental hormone" to a body of water to bring about increased heterotrophic consumption of the growth in the same manner as hormone therapy might be employed to correct unbalance in the human body. While we are letting our imagination run wild, it might also be possible some day to shift the enzyme systems in the algal blooms so that plant respiration is increased at the expense of photosynthesis thus bringing about "self destruction" of the excess growth. Although sufficient data are not yet available, it would seem that the autotrophic component in many subtropical and tropical ecosystems exhibits a rather low net production because a high rate of respiration counteracts high gross production.

To return to the more immediately practical mere conversion of net production into herbivores may not provide the answer if the rate of importation of fertilizer continues high. Some sort of harvest (i.e. "export") must be worked out so that some of the nutrients as well as the organic matter can be removed from the system or that part of the system which is causing trouble. Harvest of large organisms which turn over slowly and accumulate energy over periods of time (i.e. long periods of individual growth) will likely be most practical. Harvesting algae or small herbivores on a large scale requires frequent or continuous operations, whereas harvest of larger units can be periodic. Even if filters or screens can be developed, maintenance problems would be great in "gunky" environments. Therefore, it appears that truly herbivorous species of fish which consume algae directly and are adapted to tolerate organically fertile waters provide very definite candidates for a "conversion-harvest" solution. We know very little about such fish in this country. Fish management or fish culture in fresh water in the United States is almost exclusively concerned with sport fishes which are grown on a long food-chain (usually through bottom fauna) involving delayed utilization of primary production and consequent low yields per acre. Sports fish would be completely ineffective, and, in fact, could not survive in many waste disposal situations. As I understand it there are species of fishes in India, in the Orient and in the Philippines which eat algae, even blue-greens and filamentous types (which as already indicated are resistant to direct consumption). Yields of 1000 pounds or greater, per acre per year, are common in oriental ponds stocked with herbivores (Hickling, 1948). Such yields would amount to a secondary production energy flow of 100 gms/M²/year or about 0.3 gms/M²/day. If the assimilation efficiency (ratio production to total assimilation) of the fish population were no more than one-third, which is to say that the respiratory energy flow would be at least twice the production, then the fish would consume at three times their production rate or about one gram dry weight of algae per square meter per day. Referring to the primary production levels previously discussed it can be seen that such a

herbivorous fish population could be effective except in the more explosive bloom situations. I shall not attempt to estimate the "biological stripping" effect which an annual harvest of 1000 pounds per acre would have on the nutrients, but it would seem that the effect could be important, at least with reference to micronutrients and perhaps to phosphorus.

The big ecological point that I am making is this: When large amounts of organic wastes, etc., are introduced into natural waters essentially a new ecosystem is produced which may lack some biological components simply because there are no adapted species available. A definite "open niche" is certainly present in sewage ponds which contain no fish or other grazers. Under such conditions the possibilities of filling the niche are worth investigating. We might find what we need in some other part of the world where organic pollution has been in effect for a longer period of time; or we might breed a strain especially adapted for the situation. Such a procedure would amount to "biological engineering" in that we seek to adapt a population to a function. The game bird situation in the Middle West will illustrate the point. When natural grasslands and forests were converted by man into corn fields, the productivity was not changed, but the habitat was. No native species of gallanaceous bird could adapt and take advantage of the high productivity of such lands. The ring-necked pheasant, which had a long history of adaptation in the intensive grain farming regions in Europe and Asia, filled the niche very well when it was introduced. We are all leary, of course, of introducing exotics because they sometimes become pests. In my opinion the "open niche" situation is the only situation where we should consider introductions, and we should make very certain that our introduction is very specialized for the intended niche. If specialized for the 10b it will not only be more efficient but it will be less likely to spread into waters where it is not wanted.

One other factor which may affect heterotrophic utilization should be mentioned. Species diversity may be very important in determining the pattern of energy flow. Our studies on organic production and turnover in old-field succession (Odum, 1960) as well as the work of Margalef (1958) on phytoplankton succession indicate that the process of diversification which usually occurs during ecological succession favors stability of the ecosystem often at the expense of total production at primary level.

Typically, early stages of succession are characterized by a small number of species, which, however, have rapid growth rates. As the number of species increases, production is divided among

more kinds and is thus spread out more uniformly in both time and space. Since many of the later invaders may be low-yielding types, the total community production may be reduced, but from the functional standpoint the ecosystem is more stable and is better able to regulate itself and to adapt to changing climate or other conditions imposed from without. Seasonal succession, as well as the slower annual changes, seems to conform to this pattern. Anything which can be done to increase diversity at both autotrophic and heterotrophic levels should help stabilize productivity. Talling (1951) has pointed out that the element of chance may play a large part in determining what species become dominant in new ponds. It would be interesting to experiment with seeding new waste stabilization ponds with a variety of species of algae to see if the composition of the community could be regulated and the pattern of primary production stabilized.

To summarize, the basic theory of ecosystem function, as graphically illustrated by the energy flow diagram, points to a number of approaches to control of the unbalance between production and consumption which is inherent in the noxious bloom situation. Some possibilities are: (1) reducing inflow of nutrients, (2) converting nutrients to unavailable form, (3) poisoning production machin-

ery, (4) reducing effective light, (5) increasing respiratory energy flow in autotrophs, (6) increasing "grazing" by herbivores, and (7) harvesting net production (and with it some of the nutrients) either as primary or secondary produc-The first three possibilities were not discussed in this paper, since they are considered in detail in other papers in the symposium. Increased depth and increased vertical turbulence do reduce productivity in natural waters. While the first three or four approaches are the ones being currently investigated, they are essentially "negative" in that attempts are made to prevent or destroy natural productivity. The viewpoint of this paper has been that accumulation of undesirable organic materials following algal blooms may be as much a problem of delayed or incomplete utilization as it is a problem of increased primary production. Consequently, attention was focused on the utilization side of the picture, with the suggestion that we should at least be thinking about using the fertility of cities rather than doing away with it. A consumer-harvest approach involving herbivorous fish was suggested as deserving practical consideration. Estimation of population energy flow based on recorded yields of herbivorous fish in the Orient indicates that such an approach could be effective.

REFERENCES

Bartsch, A. F. and M. O. Allum, 1957. Biological factors in the treatment of raw sewage in artificial ponds. Limnol. and Oceanogr., 2: 77-84.

Harvey, H. W. 1955. The chemistry and fertility of sea water. Cambridge Univ. Press, Cambridge, England.

Hickling, C. F. 1948. Fish farming in the Middle and Far East. Nature, <u>161</u>: 748-751.

Odum, H. T. 1956. Efficiencies, size of organisms, and community structure. Ecol, <u>37</u>: 592 - 597.

1960. Ecological potential and analogue circuits for the ecosystem. Amer. Sci., 48: 1-8.

Odum, H. T. and C. M. Hoskins. 1958. Comparative studies on the metabolism of marine waters. Publ. Inst. Mar. Sci., Univ. Texas, 5: 16-46.

Odum, H. T. and E. P. Odum. 1955. Trophic structure and productivity of a windward coral reef community on Eniwetok Atoll. Ecol. Monogr., 25: 291-320.

Odum, E. P. 1959. Fundamental of Ecology, Second Edition. W. B. Saunders Co., Philadelphia. pp 1-546.

1960. Organic production and turnover in old-field succession. Ecol, 41: 34-49.

Odum, E. P. and A. E. Smalley, 1959. Comparison of population energy flow of a herbivorous and a deposit-feeding invertebrate in a salt marsh ecosystem. Proc. Nat. Acad. Sci., 45: 617-622.

Ohle, W. 1956. Bioactivity, production and energy utilization of lakes. Limnol. and Oceanogr., 1: 139-149.

REFERENCES (Cont'd.)

Margalef, Ramon. 1958. Temporal succession and spatial heterogeneity in phytoplankton. Perspectives in Mar. Biol. Univ. California Press, pp 323-349.

Pomeroy, L. R., H. H. Haskins, and R. A. Ragotz-kie. 1956. Observations on dinoflagellate blooms. Limnol. and Oceanogr., 1: 54-60.

Pomeroy, L. R. 1959. Algal productivity in salt marshes of Georgia. Limnol. and Oceanogr., 4: 386-397.

Proctor, Vernon W. 1957. Studies on algal antibiosis using <u>Hematococcus</u> and <u>Chlamydomonas</u>. Limnol. and Oceanogr., <u>2</u>: 125-139.

Rawson, D. S. 1952. Mean depth and fish production in large lakes. Ecol., 33: 513-521.

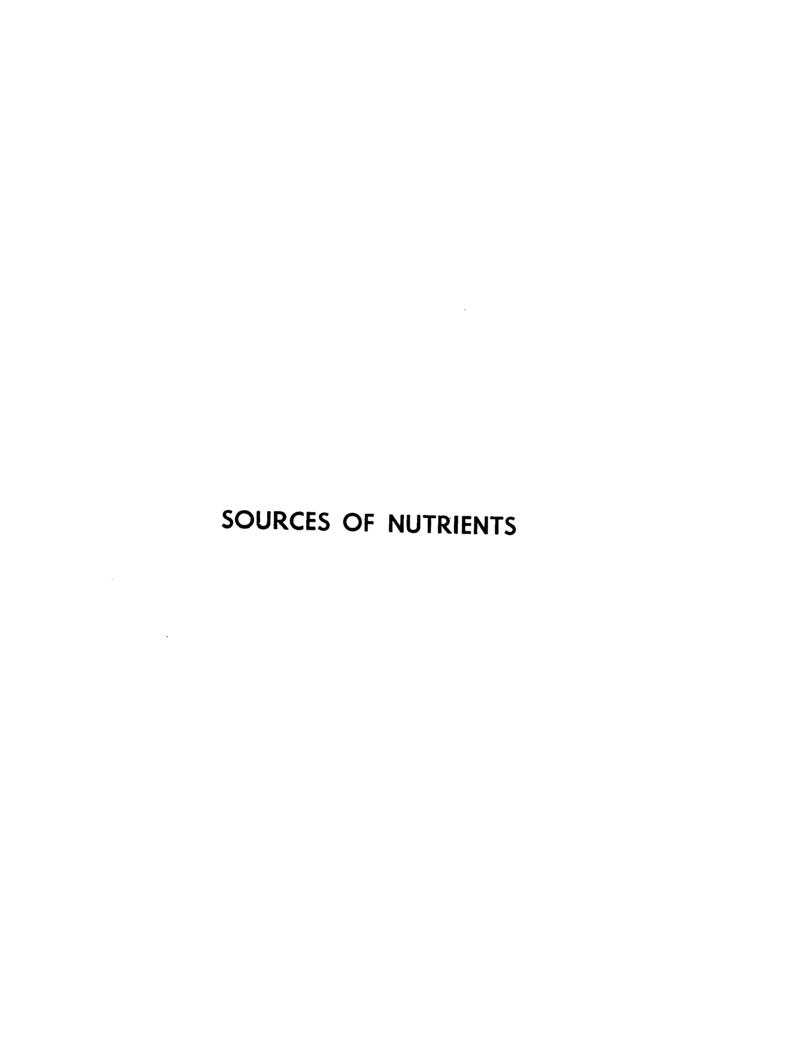
Riley, Gordon A. 1956. Production and utilization of organic matter. Bull. Bingham Oceanogr. Coll., 15: 324-344.

Ryther, John H. 1954. The ecology of phytoplankton blooms in Moriches Bay and Great South Bay, Long Island, New York. Biol. Bull., 106: 198-209.

1959. Potential productivity of the sea. Science, 130: 602-608.

Talling, J. R. 1951. The element of chance in pond populations. The Naturalist (London); Oct. - Dec.: 157-170.

Verduin, Jacob. 1956. Energy fixation and utilization by natural communities in western Lake Erie. Ecol, 37: 40-50.



Land Drainage

LAND DRAINAGE AS A SOURCE OF PHOSPHORUS IN ILLINOIS SURFACE WATERS*

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INTRODUCTION

Phosphorus in surface waters may stem from a variety of sources. Major sources of phosphate substances are human wastes, chemicals employed in water conditioning, synthetic detergents, certain industrial wastes, and drainage of agricultural lands. The purpose of this paper is to discuss some of the factors which bring about various concentrations of phosphate substances in the drainage water from agricultural lands, and to present the results obtained in stream surveys of several Illinois surface waters, particularly the Kaskaskia River. These studies indicate the importance of land drainage as a source of orthophosphate, hydrolyzable phosphate, and organic phosphate in streams which drain highly cultivated lands.

PHOSPHORUS IN AGRICULTURAL LAND

Bennett (1939) reported that analyses of 389 samples of surface soils throughout the United States showed an average phosphoric acid content of 0.15 per cent, as P₂O₅. Waggaman (1952) stated that phosphoric acid is the main fertilizer element used in American agriculture, and the predominant ingredient of nearly all mixed fertilizers. Waggaman observed a phosphoric acid content of 0.07 to 0.25 per cent for the first nine inches of good productive soils, or 1750 to 6250 pounds per acre, as P2O5. Only a small fraction of this total is available as soluble phosphoric acid. Melsted (1960) has indicated that the available P2O5 of farm lands in the Kaskaskia River basin of Illinois varied from 40 to 50 pounds per acre. It is a well known fact that the readily soluble phosphates of commercial fertilizers soon revert in the soil to less soluble phosphoric acid compounds.

TRANSPORT OF PHOSPHORUS TO SURFACE WATERS

Phosphorus carried to surface waters may be in the simple orthophosphate form or as soluble hydrolyzable phosphate, or it may be adsorbed on clay particles. It is known that much of the soil phosphate exists in an adsorbed form on soils and clays. According to Kurtz (1945), as these adsorbed forms of phosphate increase in amount their solubility in water increases rapidly. Silvey (1953) reports that clays act as ion exchange compounds and that certain phosphates in calcium bearing soils would be converted to calcium phosphate and remain in the humus or the sub-soil until the pH closely approaches 7.0, at which time the soluble phosphate would be eluted by runoff, percolation, and subsurface seepage.

Kohnke and Bertrand (1959) suggest that typical surface runoff water is high in solid soil particles, especially clay and organic matter, high in adsorbed phosphorus, and low in soluble salts. If runoff occurs quickly after the start of a rain, soluble salts, accumulated in the surface soil during a dry period, may be washed off before infiltration carries them into the body of the soil. Percolation water contains a relatively high concentration of soluble salts. Thus, for an area which is drained by tile, surface waters might be expected to receive significant amounts of both adsorbed and soluble phosphorus in times of high rainfall and runoff.

Concentrations of available phosphate in seepage waters from agricultural soils were determined in lysimeter studies conducted by the Department of Agronomy at the University of Illinois. Melsted (1960) reported P_2O_5 concentrations in percolation water of from 0.2 to 0.7 mg/1, depending upon the rate of percolation. A concentration of approximately 0.5 mg/1 P_2O_5 per acre-inch of water was typical of the results obtained.

The contribution of phosphate to surface waters by land drainage in the Madison, Wisconsin, area was investigated by Sawyer (1944). He reported total phosphorus from land drainage of approximately 1.6 lb P₂O₅/day/sq. mile, of which about 75 per cent was in the organic form.

PHOSPHATES IN ILLINOIS SURFACE WATERS

The Sanitary Engineering Laboratory of the University of Illinois conducted surface water surveys in 1956 and 1957 to determine concentrations of phosphates in Illinois surface waters. The meth-

^{*}This investigation represents part of a research project supported by the Association of American Soap and Glycerine Producers, Inc.

od used in the analyses of samples for orthophosphate and hydrolyzable phosphate was that reported by the Association of American Soap and Glycerine Producers Subcommittee on Phosphates (Anon, 1958).

Figure 14 is a map of Illinois, showing the locations of lakes and reservoirs, streams, and sewage treatment effluents sampled during the surveys.



Figure 14. SAMPLING LOCATIONS IN ILLINOIS

The overall results of the various surveys and the details of sampling and analysis have been reported by Engelbrecht and Morgan (1959). Table 16 presents typical results for P2O5 concentrations found in different surface waters. It is seen that phosphate concentrations in surface waters vary considerably.

One of the samples from a relatively pollution-free reservoir, Lake Bloomington, showed an ortho plus hydrolyzable P2O5 concentration of 0.23 mg/1, of which only 10 per cent was orthophosphate. This reservoir has a drainage area of 60 square miles. In contrast to the low phosphate concentrations found in reservoirs were those observed in a small stream receiving the industrial

Table 16. TYPICAL LEVELS OF DIFFERENT SURFACE WATER SOURCES, ILLINOIS, 1956

Source	Ortho P ₂ 0 ₅	Ortho + Hydrolyzable
Lakes and reservoirs,		- 2-5,6, -
8 samples.	0.04	0,09
Major drainage basins, moderate domestic pollution, 25 samples.	0.37	0.63
Streams with high domestic pollution, 33 samples.	6-15	7–16
Stream with high industrial pollution, 7 samples.	77	81

waste from a metal finishing plant, where levels of 80 mg/1 P₂O₅ were noted.

KASKASKIA RIVER BASIN SURVEY

In order to assess the relative amounts of phosphate from land drainage and domestic sources, one major drainage basin was selected for an intensive survey. Phosphorus and stream flow data were then analyzed according to the scheme outlined in Table 17. The Kaskaskia River was chosen

Table 17. STREAM PHOSPHATE BALANCE

(1)	S = L + W + 1
	or
(2)	$Q \cdot c = A \cdot a + P \cdot p + 1$
S =	Q·c = Pounds in stream
	Q = Runoff
	c = Concentration
L =	A·a = Pounds from land drainage
	A = Area drained
	a = Pounds per unit area
W =	P·p = Pounds from domestic sources
	P = Total population
	p = Per capita quantity
	1 = Industrial quantity
	1

for this survey because it was convenient to the laboratory, because of the existence of a system of rain gages and USGS stream gaging stations along its length, and because of the availability of hydrological and agricultural data for the watershed area.

The location of the Kaskaskia River is shown in Figure 14, while the locations of the various stream sampling points and sewage treatment effluents sampled are shown in Figure 15. Six stream sampling points are shown. Descriptive data for each stream sampling point are given in Table 18. Farm lands in the Kaskaskia basin are drained mainly by tile drain placed at a depth of 24 inches.

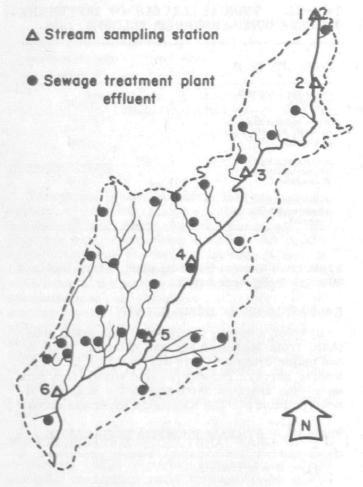


Figure 15. KASKASKIA RIVER BASIN, SHOWING SEWAGE TREATMENT EFFLUENTS AND STREAM SAMPLING STATIONS

Table 18. DESCRIPTIVE DATA, KASKASKIA RIVER BASIN

Stream station	Mile	Tributary population	Total Drainage area, sq. mi.	Per cent cultivated
1	273	0	12	86
2	245	820	125	86
3	170	6,670	1030	82
4	120	12,850	1980	76
5	65	18,320	2680	69
6	0	90,240	5220	77

Phosphate from Domestic Sources

There were no known phosphates discharged separately as an industrial waste along the Kaskaskia River. Therefore it was only necessary to account for phosphate contributions from sewage treatment plant effluents in order to estimate the magnitude of the phosphate contributions from land drainage above each of the stream sampling points. Figure 16 shows the variation in ortho and ortho plus hydrolyzable P2O5 for three sewage treatment plant effluents in the Kaskaskia basin. Per capita values of ortho plus hydrolyzable P2O5 in the three effluents ranged from 0.003 to 0.045 pounds per day.

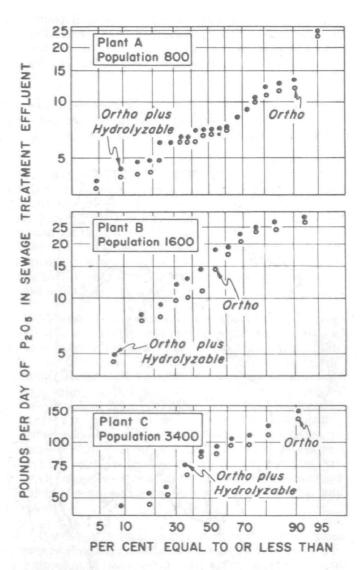


Figure 16. DOMESTIC P₂O₅ IN 3 KASKASKIA BA-SIN SEWAGE TREATMENT EFFLUENTS, 1956

Variation of Runoff and Stream Phosphates

Stream samples were collected approximately once each week at three points in the upper Kaskaskia, from April through September, 1956. Samples were collected at the stations in the lower basin in April and May of the same year. Figure 17 describes the variations in stream runoff, calculated pounds per day of P2O5 in the stream runoff, and concentration at Station 1. This station received no domestic P2O5, and received runoff from a cultivated drainage area of 11 square miles. Thus, all phosphate found in the stream at this station is attributed to land drainage. Concentration of ortho plus hydrolyzable P2O5 varied from 0.03 to 0.96 mg/1 in the 27 samples collected. Pounds per day of ortho plus hydrolyzable P2O5 ranged from 0.1 to 7.5, with an average value of 1.1 pounds per day. This corresponds to a value of 0.1 pound of P2O5 per day per square mile of drainage area.

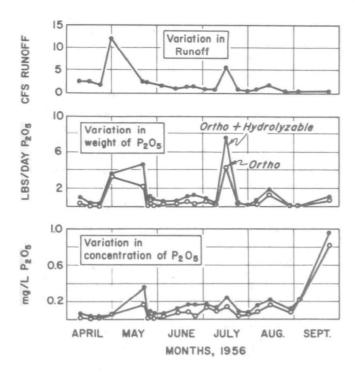


Figure 17. VARIATION IN RUNOFF AND ORTHO AND ORTHO PLUS HYDROLYZABLE P₂O₅. Station 1, Kaskaskia River. 11 Sq. miles cultivated. No domestic P₂O₅.

Total phosphate values may be 20 to 30 per cent higher, because only a portion of the organic phosphate is accounted for by the method of analysis used.

Figures 18, 19 and 20 describe variations in phosphate and stream runoff for the remainder of the Kaskaskia sampling stations. Both domestic and agricultural phosphates are expected to be present at these locations. A line corresponding to the maximum 90 per cent value of domestic phosphate has been shown on the plots describing variation in weight of P_2O_5 at each of the stations. It is apparent that the quantity of stream phosphate attributable to land drainage varies with the stream runoff. Similar correlations with rainfall have been observed, as would be expected.

Overall Results of Kaskaskia Survey

For each of the sampling locations on the Kaskaskia River, Table 19 presents mean values of phosphate concentrations and the approximate per cent of the ortho plus hydrolyzable P2O5 in the samples collected which could be attributed to land drainage. These per cents are based on assumed average contributions of P2O5 from domestic sources. Orthophosphate concentrations are seen to average from 0.11 to 0.39 mg/1 P2O5, while ortho plus hydrolyzable phosphate averages range from 0.16 to 0.68 mg/1 P2O5.

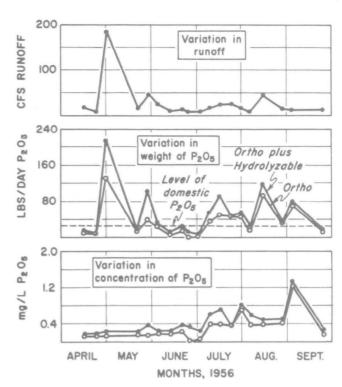


Figure 18. VARIATION IN RUNOFF AND ORTHO AND ORTHO PLUS HYDROLYZABLE P₂O₅. Station 2, Kaskaskis River. 109 sq. miles cultivated. No domestic P₂O₅.

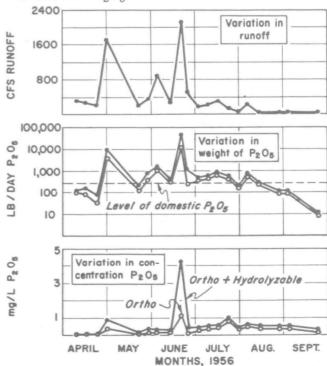


Figure 19. VARIATION IN RUNOFF AND ORTHO AND ORTHO PLUS HYDROLYZABLE P₂O₅. Station 3, Kaskaskia River. 842 Sq. miles cultivated. Maximum domestic P₂O₅, 260 lb/day.

DISCUSSION

The stream survey results obtained for the Kaskaskia Riyer, though subject to considerable variation, do indicate that land drainage is a significant source of phosphates in streams which drain agricultural lands. The Kaskaskia basin, as shown in Table 18, is extensively cultivated. The farm lands contain in the order of 40 to 50 pounds of available P2O5 per acre, and are drained by tile drains. High rainfalls and resultant high rates of percolation and runoff might be expected to carry some portion of both soluble and adsorbed phosphorus forms to the stream. This expectation is borne out by the presence of measurable phosphate concentration at a stream location receiving no domestic phosphate, and by the presence at other

stream locations of phosphates in excess of those expected from domestic sources alone.

The amount of agricultural phosphate transported to streams undoubtedly depends upon a number of factors: nature and amount of phosphates in the soil, mode of drainage, topography, intensity and distribution of rainfall, rates of infiltration and percolation, and probably others. For the 100 samples reported here, the calculated pounds of P_2O_5 per day per square mile varied from 0 to 58 for ortho plus hydrolyzable. The mean pounds of P_2O_5 per square mile for all samples was 1.4, which is on the same order of magnitude as that reported by Sawyer (1944).

Table 19.	RESULTS OF	KASKASKIA	STREAM SURVEY.	1956

Stream station	Drainage area, sq. mi.	No. of samples	Mean Ortho P ₂ 0 ₅ , mg/1	Mean Ortho + hydrolyzable P ₂ 0 ₅ , mg/l	Mean per cent Ortho + hydrolyzable P ₂ 0 ₅ , land drainage
1	12	27	0.11	0.16	100
2	125	24	0,29	0.40	55
3	1030	25	0.39	0.68	45
4	1980	10	0.14	0.34	25
5	2680	7	0.13	0.24	55
6	5220	7	0.20	0.43	35

REFERENCES

Anon. 1958. Determination of orthophosphate, hydrolyzable phosphate, and total phosphate in surface waters. AASGP Committee Report. Jour. Amer. Water Works Assoc., 50: 1563-1574.

Bennett, H. H. 1939. Soil Conservation. 1st Ed. McGraw-Hill Book Co., N. Y., pp. 9-10.

Engelbrecht, R. S. and J. J. Morgan. 1959. Studies on the occurrence and degradation of condensed phosphates in surface waters. Sew. and Ind. Wastes, 31: 458-478.

Kohnke, H. and A. R. Bertrand. 1959. Soil Conservation. McGraw-Hill Pub. Co., N. Y., pp. 91-92.

Kurtz, L.T. 1945. Adsorption and release of phos-

phate ions by soils and clays. Unpublished Ph.D. Thesis, University of Illinois, Urbana, Illinois.

Melsted, S. W. April, 1960. Personal communication. Agronomy Department, University of Illinois, Urbana, Illinois.

Sawyer, C. N. 1944. Fertilization of lakes by agricultural and urban drainage. Jour. New England Wat. Works Assoc., <u>61</u>: 109-127.

Silvey, J. K. G. 1953. Relation of irrigation to taste and odor. Jour. Amer. Water Works Assoc., 45: 1179-1186.

Waggaman, W. H. 1952. Phosphoric acid, phosphate, and phosphatic fertilizers. 2nd Ed., Reinhold Pub. Corp., N. Y., pp. 20-24.

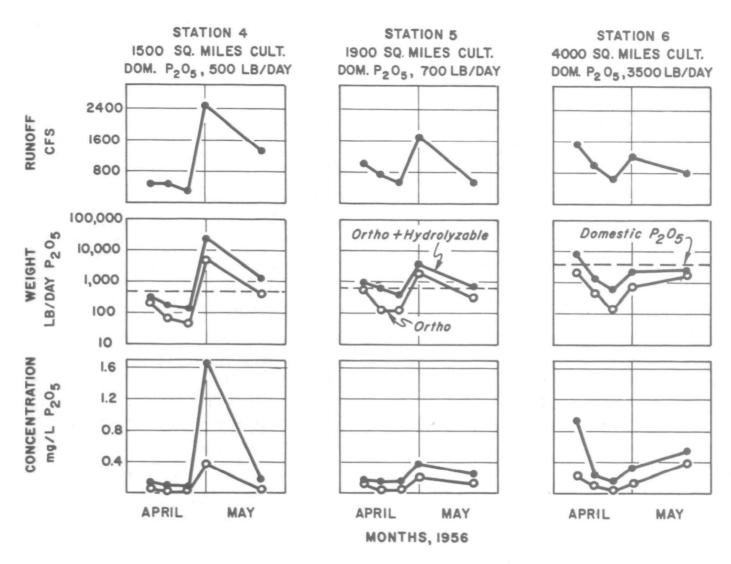


Figure 20. VARIATION IN RUNOFF AND ORTHO AND ORTHO PLUS HYDROLYZABLE P_2O_5 , KASKASKIA RIVER.

NUTRIENT CONTENT OF DRAINAGE WATER FROM FORESTED, URBAN AND AGRICULTURAL AREAS

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SYNOPSIS

Nutrient concentrations are presented from field observations on drainage waters originating in timbered areas of little habitation and land use; from urban street drainage; sub-surface and surface drains carrying irrigation return flows; urban streams; a eutrophic lake, and a comparison is made of the nutrient change in a stream of multipurpose usage. The effect of land and water use on nutrient concentrations is clearly shown. Nutrient loss through sedimentation and incorporation in the bottom sediments and loss to littoral and other attached vegetation is indicated.

INTRODUCTION

Nutrient data in the form of soluble and total phosphorus, nitrates and total Kjeldahl nitrogen, were obtained during field studies of a eutrophic lake and its environment (Sylvester and Anderson, 1960) and from a study now in progress on the character and significance of irrigation return flows in a large river basin. Nitrite values were found to be very low and are not included for the sake of brevity. Ammonia is included in the total Kjeldahl nitrogen.

Natural drainage or runoff waters contain varying quantities of nutrients (fertilizers) depending upon their source. These natural nutrient concentrations become altered through man's multipurpose land and water usage. This alteration normally consists of an increase in the phosphorus' and nitrogen content of the drainage water. An increase in the nutrient content of a water, along with a sufficiency of other elements, results in an increased plant productivity in the water that may reach such proportions that normal water uses are harmed or jeopardized. This plant productivity may be in the form of algae; emergent or nonemergent sessile plants, such as willows, cattails, water weeds; or it might consist of slime growths, such as the extensive Sphaerotilus beds of the lower Columbia River (Anon.) that have been attributed to unnatural increases in several nutrients, among which are wood sugars. An increase in the primary producers, or phytoplankton, will usually result in an increase in the zooplankton and thus a better habitat is provided for fish production. When this algae production exceeds the fish food needs, the water may become undesirable as a source of water supply or as a medium for recreation. The stimulated growth of water weeds and other vegetation increases the cost or difficulty of ditch maintenance, shoreline maintenance and of insect control.

Nutrient concentrations necessary to produce nuisance water growths will vary, depending upon the concentrations of other substances, the water temperature, light penetration in the water, and the rate at which nutrients can be supplied, once growth commences. The temperature of and the light penetration in the aquatic environment are normally subject to change only from natural phenomena (with a few exceptions), leaving nutrients as that requirement for plant growth that has been altered by man. Many waters have become highly productive of luxuriant plant growths in the absence of man's machinations and many others would naturally reach this stage in the course of time (witness the many eutrophic lakes). Man's water and land uses have usually served to hasten this process of natural maturing by his addition of nutrients to the water through land cultivation and fertilization and through the discharge of his spent or waste waters into the natural water bodies of his environment. Sawyer, in his study of Wisconsin lakes (1947) reports that large phytoplankton blooms may be supported when phosphorus concentrations are in or above the range of 10 ppb PO₄ P and nitrogen concentrations are above 300 ppb NO, N. Hutchinson (1957) reports mean nitrate nitrogen values of 50 to 86 ppb in three Wisconsin lakes that produce regular nuisance blooms of algae. Some lakes have high nitrogen values but no regular nuisance blooms because of a deficiency in some other factor. Nuisance algal blooms were observed (Sylvester and Anderson, 1960) to commence in Seattle's Green Lake (a very soft-water lake) when nitrate nitrogen levels were generally above 200 ppb and soluble phosphorus was greater than 10 ppb. These blooms usually stopped when the soluble phosphorus dropped to zero. On one occasion, the bloom was attenuated when the nitrate concentration was depleted and the soluble phosphorus was still above 10 ppb.

DATA PRESENTED

The information presented herein consists of nutrient data (summarized) that were obtained incidental to comprehensive water quality studies. Data are generally presented by showing the range of values observed together with a weighted-aver-

^{1. &}quot;Character and Significance of Irrigation Return Flows", N. I. H. Research Grant (RG6412C1) to University of Washington, study in progress.

age value. Whenever possible, other factors are given, such as nutrient ratios, rate of water flow, soil types and concentration of nutrients discharged per acre. Weighted-average values (to account partially for flow variations) are obtained by multiplying each individual determination by the flow rate at the time of the determination, summing these individual products and then dividing by the summation of the flow rates. Table 26 gives mean rather than weighted average values since flow rates were not available. In most cases, the mean value was reasonably close to the median value.

Phosphorus concentrations are reported as the element phosphorus, and nitrogen as the element nitrogen. Soluble phosphorus was determined by the stannous chloride method (Anon, 1955) and total phosphorus by evaporating and igniting a sample containing magnesium chloride followed by the stannous chloride method. Nitrate nitrogen was determined by the phenoldisulfuric acid method (Anon, 1955). Samples were collected at frequencies of from once a week to about once a month.

Urban Street Drainage

Table 20 shows the nutrient content in street gutter drainage water collected from major highways, arterial and residential streets, under varying conditions of antecedent rainfall. Samples were dipped from the gutters anywhere from 30

Table 20. NUTRIENTS IN URBAN STREET DRAINAGE 1 , MAY - NOVEMBER, 1959^2

Type of	Antecedent		- mg/1 N	Phosphorus	as P, ppo
street	rainfall, ³ inches	Total Kjeldahl	Nitrates	Soluble	Total
Arterial st.	0.21		0.185		10
Arterial st.	0.21	i	0.10		49
Arterial st.	0.01	0.44	0.56		104
Residential st.	0.01	1.01	0.16		328
Major highway	0.01	1.4	0.86	1 1	
Major highway	0.01	0.4	0.52]	228
Major highway		0.45	0.22	i i	
Residential st.	0.01	0.37	0.17		228
Residential st.	0.01	0.32	0.17		440
Residential st.	0.78	2.78	1.10	Tr	154
Residential st.	0.78	1.31	0.52	16	308
Arterial st.	0.78	1.34	0.29	Tr	144
Arterial st.	0.78	0,57	0.23	2	90
Arterial st.	0.78	1.43	0.29	20	108
Arterial st.	0.78	1.84	0.23	Tr	126
Residential st.	0.0	6,68	0.65	14	166
Major highway	0.0	9.06	2,24	54	352
Major highway	0.0	7.45	2.80	72	404
Major highway		5.36	0.96	30	102
Major highway	0.0	7.21	0.53	28	162
Arterial st.	0.0	8.01	0.52	14	81
Arterial st.	0.22	0.38	0.65	22	146
Arterial st.	0.22	0.60	1.00	32	198
Arterial st.	0.22	0.41	1.00	156	300
Arterial st.	0.22	0.88	0.80	784	1400
Arterial st.	0.0	1.67	0.48	280	280
Arterial st.	0.0	1.54	0.36	154	182
Arterial st.	0.55	0.22	0.12	20	21
Major highway		0.62	0.03	10	13
Major highway		0.37	0.03	10	14
Major highway		0.91	0.03	8	21
Residential st.		0.39	0.02	70	98
Residential st.	0.55	0.39	0.10	78	78
Arterial st.	0.07	0.17	0.42	58	212
Arterial st.	0.07	0.24	0.11	48	280
Median		0.41	0.42	22	154
Mean	l	2.01	0.53	76	208

minutes to several hours after a rainstorm had commenced and they were dipped so as not to include particulate matter that would be retained by a catch basin. Since most of the area sampled was served by combined sewers, the gutter water samples did not generally include roof drainage, but did include all types of surface drainage. As one would expect, low antecedent rainfall conditions gave the highest nutrient concentrations. Since these samples were not all collected at the very beginning of rainstorms nor at the end of prolonged rainstorms, they can be considered quite representative of urban surface runoff under varying conditions of street sweeping and flushing, antecedent rainfall, and the presence and absence of deciduous vegetation. The major highway (not a limited access freeway) had the greatest nitrogen values; the arterial streets contained the most soluble phosphorus; and the residential streets the highest total phosphorus concentrations.

Forested Areas

Nutrient values in three streams as they emerge from forested areas are given in Table 21. Each

NUTRIENTS IN STREAMS FROM Table 21. FORESTED AREAS

Characteristics	Yakima River at Easton (12 months)	Tieton River (7 months)	Cedar River ² at Landsberg (12 months)
Mean annual flow, cfs	587	559	690
Mean flow for data period	630	520	587
Drainage area, sq. mi.	182	239	125
Phosphorus as P, pph			
Total		1	i
Range of values	19-140	32-200	15-85
Weighted average	70	115	22
Soluble		i	1
Range of values	0-23	0-23	2-7
Weighted average	9	8	4
Nitrates as N, mg/1			
Range of values	0.05-0.50	0.03-0.18	0.018-0.154
Weighted average	0,20	0.126	0,065
Total Kieldahl N. mg. 1			
Range of values	0-0.22	0-0.13	
Weighted average	0.08	0.068	
Total nitrogen as N, mg/1	0.28	0.194	
Total phosphorus, lbs./acre/year	0.74	0.77	0.32
Total nitrogen, lbs./acre/year	2.96	1.30	
Datie Total N	4	1.7	
Ratio Total P	4	1 *	

Areas subject only to logging and road construction - large reservoirs
present on all headwaters.

watershed contains large reservoirs, roads and some logging but no human habitation that would contribute any significant amount of waste water to the streams. The Cedar River originates on the western slope of the Cascade mountains, is the source of Seattle's water supply, and it is generally a less turbid river than the other two. The Yakima River at Easton is the point of diversion for the 70,000 acre Kittitas irrigation project while the Tieton River at the point of sampling is just below the diversion structure for the 28,000 acre Tieton project (see figure 21). Discharge of phosphorus and nitrogen from the eastern slope streams is over twice as great as that from the western slope streams.

Samples collected from gutters on Seattle streets.
From Sylvester and Anerson (1960).
Antecedent rainfall in inches during week prior to sample collection.

^{2.} Seattle Engineering Department data.



Figure 21. YAKIMA RIVER BASIN. 1. Yakima River at Easton; 2. Yakima River at Parker; 3. Yakima River at Kiona; 4. Tieton River; 5. Wilson Creek; 6. Granger Drain; 7. Main Reservation Drain; 8. Sunny-side 35.4 Drain; 9. D-2 Sub-surface Drain; 10. D-4 Sub-surface Drain; 11. D-5 Sub-surface Drain; 12. Sub-surface Drain; 13. D-14 Sub-surface Drain.

Irrigation Return Flow Drains

About 450,000 acres of land are irrigated in the arid Yakima River Basin. Water application ranges from 2.5 to 11 acre-feet per acre per year, averaging around 3.7. From 20 to 75 percent of this irrigation water is returned to the main river each year as return flow. Irrigated land must be well drained to prevent an accumulation of salts in

the surface portion of the soil. A favorable salt balance is attained when the drainage water contains a higher salt load than the applied water. Return flow drains may consist of ditches (surface) or buried pipe that is porous or has open joints (sub-surface). The water in these return flow drains is highly mineralized. For example, the Yakima River in receiving the return flow from

some 450,000 irrigated acres between Easton and Kiona (see figure 21) has its specific conductance increased from 30 to 240 micromhos during the height of the irrigation season.

Tables 22 and 23 give the nutrient variations in typical surface and sub-surface drains and table 24 shows the difference in the soil characteristics. The study from which tables 22 and 23 were prepared 1/ is still progressing and it is too early to definitely speculate on what causes or does not cause the differences in the nutrient content of the drain water. Variables herein involved include the soil types (surface and sub-surface); quality and quantity of water applied to the land; presence of irrigation waste (excess) water; nature of crops being grown; fertilizers applied; and the depth of the drain and water table. The principal difference between the nutrient content in the surface and subsurface drains is the higher mineralized nutrient content of the sub-surface drains. Nitrates are twice as high in the sub-surface drains and the N/P ratio of the sub-surface drains averages 2.5 times as high as the surface drains.

In the surface drains, the total phosphorus contained in the drainage water varied from 0.9 to 3.9 lbs./acre/year while the total nitrogen varied from 2.5 to 24 lbs./acre/year. Sub-surface drains re-

moved total phosphorus at the rate of 2.5 to 8.9 lbs./acre/year and total nitrogen at the rate of 38 to 166 lbs./acre/year. Sawyer has reported (1947) Wisconsin agricultural lands as discharging 0.398 lbs. of phosphorus and 7.03 lbs. of nitrogen per acre per year. With the limited data now available on fertilizers applied to the land (Blake, 1960), it would appear that a considerable portion of them are being carried off in the drainage water.

Multi-Purpose Water Usage

The Yakima River below Easton receives the treated sewage discharge from some 80,000 persons, irrigation return drainage from 450,000 acres and industrial waste effluents having a population equivalent of over 100,000 (Sylvester, Weston, Suzuki and Dailey, 1951; and Anon., 1952). The drainage area below the mountains is quite arid (average rainfall is 7 inches) so that most of the runoff in the area comes from the irrigated land. Changes in nutrient concentrations in the river between Parker and Kiona (see figure 21), where there are no significant year-around tributaries other than spent waters, are given in Table 25. Between Parker and Kiona, a distance of 72 river miles, the tributary irrigated acreage is 250,000 acres and the sewered population plus industrial equivalent is about 93,000 persons. The

Table 22. NUTRIENTS IN SURFACE IRRIGATION RETURN FLOW DRAINS YAKIMA VALLEY, MARCH 1959 - MARCH 1960

Characteristics	Main drain Wapato Project	Granger drain Sunnyside Project	Drain 35.4 Sunnyside Project	Wilson Creek Kittitas Project
Approx. drainage area, sq. mi.	150	13	2	252
Approx. mean flow, cfs	379	47	7	340
Predominate soil type	Naches soils	Sagemoor sandy loam	Esquatzel fine sandy loam	Naches fine sandy loam
Total phosphorus, P ppb				
Range of values	105-240	120-380	130-650	135-300
Weighted average	165	260	360	220
Soluble phosphorus, P ppb				
Range of values	75-255	88-300	115-295	72-192
Weighted average	127	210	180	130
Nitrates as N, mg/1				
Range of values	0.03-4.2	0-5.70	0-5.0	0-1.40
Weighted average	1.19	1.54	1.90	0.38
Total Kieldahl N. mg/1				
Range of values	0.05-0.47	0-0.34	0.08-1.30	0.02-0.48
Weighted average	0.15	0.13	0,33	0.21
Total Nitrogen as N, mg/1	1.34	1.67	2.23	0.59
Total phosphorus, lbs./acre/year	1.28	2.88	3.88	0.92
Total Nitrogen, lbs./acre/year	10.45	• 18.5	24.0	2.45
Ratio N/P	8.1	6.4	6.2	2.7

^{1.} All drain areas of diversified farming except for Wilson Creek which drains areas of hay, corn and grains.

Table 23. NUTRIENTS IN SUB-SURFACE IRRIGATION RETURN FLOW DRAINS YAKIMA VALLEY, MARCH 1959 - MARCH 1960

Characteristics	D-2 Kittitas Project	D-4 Kittitas Project	D-5 Tieton Project	D-12 Sunnyside Project	D-14 Tieton Projec
Characteristics					(8 months)
Soil type	Naches loam	Esquatzel and Selah loam	Ahtanum loam	Sagemoor loam	Varied
Avg. discharge, gpm	67	157	275	830	42
Crops grown	Sweet corn	Wheat	Barley & pasture	Hops, grapes, wheat, hay, mint	Apples
Fertilizers used	Commercial	Barnyard & commercial	Gypsum & commer.	All types	
Approx. water applied - Ac.Ft./Yr.	4.1	5	3	4.3	
Type irrigation	Furrow	Furrow	Furrow	Furrow	Sprinkler
Approx. area drained, acres	15	27	35	100	10
Drain				0000	400
Length - Feet	960	2400	2500	8000	400
Diameter - inches	8_	8-10	10-15	10	8
Depth - Ft.	3-7	5-8	4-10	4-10	4
Age	1.5	2	1	43	2
Total Phosphorus, P, ppb				75~405	82-173
Range of values	73-276	195-460	175-465		0
Weighted average	173	320	260	195	133
Soluble Phosphorus				40 170	
Range of values	29-156	120-460	175-350	49-170	82-127
Weighted average	89	320	260	150	103
Nitrates as N, mg/1				0.10-9.0	0.01-5.9
Range of values	0.40-5.0	0.42-4.1	0.20-6.5		1.77
Weighted average	2.73	2.03	2.55	4.37	1.77
Total Kjeldahl N, mg/1				0.100	0.08-0.83
Range of values	0-0.58	0-0.32	0-0.32	0-1.86	0.08-0.83
Weighted average	0.15	0.09	0.13	0.18	0.31
Total Nitrogen, N, mg/1	2.88	2.12	2.68	4.55	2.08
Total Phosphorus, lbs./acre/year	3.4	8.1	8.9	7.1	2.5
Total Nitrogen, lbs./acre/year	56	54	92	166	38
Ratio N/P	16.7	6.7	10.3	23	15.6

Table 24. SOIL CHARACTERISTICS (from Anon., 1958; and Smith, Dwyer and Schafer, 1945)

Soil type	Surface soil	Subsoil	Inherent fertility	Internal drainage	
Ahtanum loam	Pale-brown; strongly alkaline; calcareous	Greyish-brown loam over lime hardpan; strongly alkaline; calcareous	Very low	Medium to very slow	
Esquatzel silt loam	Brownish-gray; neutral to mildly alkaline; non- calcareous	Pale-brown; mildly alkaline; calcareous in lower part	Moderate to high	Medium	
Naches loam	Brown; neutral; non- calcareous	Brownish gravelly clay; neutral; non-calcareous	Moderate	Medium to high	
Sagemoor loam	Light brownish-gray; netural to mildly alkaline; non-calcareous	Light brown to gray sandy loam; alkaline; strongly calcareous	Moderate	Medium	
Selah loam	Light brown; neutral to mildly alkaline; non-calcareous	Brown, compact, sandy clay; lime-silica hardpan in lower part alkaline; calcareous	Moderate	Very slow	

Table 25. CHANGE IN RIVER NUTRIENTS FROM VARIOUS SPENT WATERS YAKIMA RIVER, WASHINGTON¹ -- MARCH 1959 - MARCH 1960

•		
Characteristics	Yakima River at Parker	Yakima River at Kiona
Mean annual flow - cfs Mean flow for data period - cfs Drainage area - sq. mi. Runoff drainage area - sq. mi. ²	2,529 3,180 3,650 2,290	3,916 4,550 5,600 2,930
Irrigated land tributary - acres Sewered population tributary - persons Industrial waste tributary - pop. equiv. 3 Industrial waste flow - mgd3	200,000 59,260 39,160 4.1	450,000 80,340 111,510 6.0
River miles from headwaters	110	182
Total Phosphorus as P, ppb Range of values Weighted average	49-210 70	55-240 135
Soluble Phosphorus as P, ppb Range of values Weighted average	29 -75 43	23-94 51
Nitrates as N, mg/1 Range of values Weighted average	0.04-0.45 0.25	0-0.78 0.32
Total Kjeldahl Nitrogen as N, mg/1 Range of values Weighted average	0-0.23 0.12	0-0. 73 0.175
Total Nitrogen as N, mg/1	0.37	0.495
Phosphorus - lbs/acre/year4	0.30	0.64
Nitrogen - lbs./acre/year4	1.575	2.35
Phosphorus - tons discharged/year	219	602
Nitrogen - tons discharged/year	1,150	2,210
Ratio N/P	5.3	3.7

- 1. 85% of drainage basin is relatively uninhabited -- 1,064,100 acrefeet of storage on headwaters.
- 2. Below the mountains, the land is arid and runoff comes principally from the irrigated areas.
- Food processing wastes are mostly seasonal.
 Using "runoff drainage area".

river flow at Parker frequently becomes very low during the irrigation season (less than 150 cfs) while at downstream Kiona, the flow will be over 1700 cfs, this additional flow coming from irrigation drains. Because of these spent waters, one would expect the river to show a very large increase in nutrients between Parker and Kiona with nutrient values that would approach the levels of those observed in the drains. Although all nutrient values did increase in this river stretch, only the Kjeldahl nitrogen values approached the concentration found in the drains. The readily assimilable phosphates and nitrates showed little increase indicating their uptake in the stream plant life. This plant life is abundant and is sufficient to frequently cause dissolved oxygen saturation values of 120 percent in the daytime and 60 percent just before

sunrise (Sylvester, 1958). River turbidity has a median value of about 9 units. Much of this plant growth is flushed from the stream bed during the autumn and spring freshets. The nitrogen-phosphorus ratio dropped in this stream stretch indicating a proportionately greater uptake of nitrogen.

Eutrophic Lakes

Table 26 gives some mean nutrient values for surface waters in the City of Seattle. Green Lake has regular nuisance blooms of algae in the spring. summer and early autumn. This lake had reached a trophic state long before it was affected by man. The Lower Woodland and Densmore drains shown in table 26 are typical of those feeding Green Lake. Nutrient concentrations in the lake water are much less than in the source water. Calculation of a nutrient budget (Sylvester and Anderson, 1960) indicated that 55 percent of the phosphorus was lost to the sediment (permanently), through deposition of algae and particulate matter, and through the thousands of fish taken from the lake by fishermen. $\frac{2}{3}$ Phosphate or nitrate values will reach zero during periods of heavy algal blooms. The algae will then die and promptly settle to the lake bottom. The inflow of nutrient-rich water and regeneration of nutrients from the mud permit another algal pulse within a short period.

Thornton Creek drains into Lake Washington. This nutrient-rich stream is typical of many tributaries to the Lake that are, along with sewage inflows, cause for concern as to the future trophic state of the Lake.

SUMMARY

Nutrient concentrations from various water sources have been presented. A summary of these values is given in Table 27 with a visual comparison presented in Figure 22. Although "clean" streams from forested areas contain the lowest assimilable nutrients, their concentrations are close to the mean concentrations in a eutrophic lake and it would appear from Table 27 that an increase in phosphate (soluble phosphorus) is all that would be needed to create a productive stream out of the "clean" stream.

The greatest concentrations of total phosphorus were found in surface irrigation return flow drains: of soluble phosphorus and nitrates, in sub-surface irrigation return flow drains; and of total Kjeldahl nitrogen, in urban street drainage.

^{2/}It is estimated that approximately 100,000 fish, averaging about 0.3 lb. each, were taken from Green Lake in 1959 by fishermen. According to Professor Lauren Donaldson, these fish would contain about 292 mg of phosphorus per 100 grams of fish. The phosphorus thus removed from the lake in the form of fish flesh might amount to 88 lbs. per year which is but 14 percent of the mean phosphorus content of the lake water.

ACKNOWLEDGMENTS

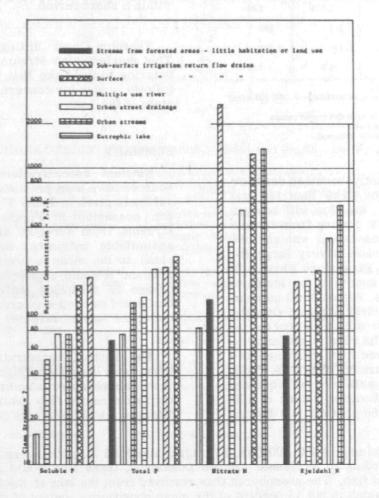
The study of Green Lake was supported by the City of Seattle and the irrigation water study is being supported by research grants from the National Institutes of Health, Grateful acknowledgment is given George C. Anderson, Research Assistant Professor of Oceanography, and Robert W. Seabloom, Assistant Professor of General Engineering, both of the University of Washington, for their assistance in data collection and analysis and for their review of this manuscript.

Table 26. NUTRIENTS IN MISCELLANEOUS WATERS, SEATTLE URBAN (from Sylvester and Anderson, 1960)

Location	Nitrates - mg/1 N		Total Kjeldahl N mg/1 N		Soluble phosphorus ppb		Total phosphorus ppb	
20cation	Range of values	Mean	Range of values	Mean	Range of values	Mean	Range of values	Mean
Green Lake ¹ Lower Woodland Drain ² Densmore Storm Drain ³ Thornton Creek ⁴	0-0.47 0.20-0.85 0.53-2.02 1.26-1.70	0.084 0.46 1.24 1.48	0.02-1.0 0.01-0.88 0.10-1.34	0.34 0.32 0.57	0-58 23-112 58-128 45-85	16 75 85 66	38-178 49-292 69-300 74-213	76 103 136 110

- 1. 256 acre lake in central Seattle receiving nutrient rich drainage samples April to January, 1959.
- 2. Subsurface drain in park; discharging to Green Lake some surface water samples April to Jan., 1959.
- 3. Small creek in urban area with duck ponds.
- Creek in urban Seattle area with mean annual flow of about 23 cfs draining 13.5 sq. mi. (Data from Seattle Engineering Department).

Figure 22. SOURCE COMPARISON OF MEAN NUTRIENT CONCENTRATIONS



Source	Total phosphorus ppb P	Soluble phosphorus ppb P	Nitrates ppb N	Total Kjeldahl Nitrogen ppb N
Streams from forested areas - little habitation or land use.	69	7	130	74
Sub-surface irrigation retrun flow drains	216	184	2690	172
Surface irrigation return flow drains	251	162	1250	205
Urban street drainage Urban street drainage	208 154*	76 22*	527 420*	2010 410*
Multiple - use river ¹	135	51	320	175
Urban streams ²	123	76	1360	570
Eutrophic lake ³	76	16	84	340

Table 27. SOURCE COMPARISON OF MEAN NUTRIENT CONCENTRATIONS (See Fig. 22)

- 1. Yakima River at Kiona
- 2. From table 26
- 3. Green Lake, Seattle
- Median values

REFERENCES

Sylvester, R. O. and G. C. Anderson. 1960. An engineering and ecological study of Green Lake. Seattle Park Department (In press), February.

Anon. Columbia River study, 1956-1958. Progress Report by the Washington Pollution Control Commission and Crown Zellerbach Corp.

Sawyer, C. N. 1947. Fertilization of lakes by agricultural and urban drainage. J. N. E. W. W. A. 61: 2, June.

Hutchinson, G. E. 1957. A Treatise on Limnology. Vol. I, New York Wiley. 1015 pp.

Anon., 1955. Standard methods for the examination of water, sewage and industrial wastes. A.P. H.A., 10th Edition.

Anon., 1958. Soil survey, Yakima County, Washintion, U.S.D.A., Soil Conservation Service Series 1942, No. 15, Issued April 1958.

Smith, L. H., C. H. Dwyer, and G. Schafer, 1945. Soil survey, Kittitas County, Washington, U.S. G. P.O., Series 1937, No. 13, Issued January.

Blake, Larry J. 1960. Water quality studies of irrigation sub-surface return flows and influencing factors. M.S. Thesis, University of Washington.

Sylvester, R. O., M. J. Westin, J. T. Suzuki, and M. G. Dailey, 1951. An investigation of pollution in the Yakima River Basin. Washington Pollution Commission Technical Bulletin No. 9.

Anon., 1952. A comprehensive pollution control program for the Yakima River Basin, Washington Pollution Control Commission Bulletin, March.

Sylvester, R. O. 1958. Water quality studies in the Columbia River Basin. U.S.F. & W. S. Special Scientific Report - Fisheries No. 239.

Wastes

METROPOLITAN WASTES AND ALGAL NUTRITION

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INTRODUCTION

Organic wastes rank high among the many sources of nutrients which increase the fertility of natural waters. Computations indicate that in the United States alone approximately 20 million tons of agricultural, food processing, domestic, and industrial wastes must annually be deposited directly or indirectly into natural waters. The quantity of these wastes is increasing not only in direct proportion to the population increase of our country. but also in proportion to the current tendency for people to spend a greater percentage of their recreational time near, on, or in the water. Algal growths in natural waters may be either heterotrophic, autotrophic, or a combination of the two. Heterotrophic algal growth is not considered in this paper except to note that such growth in competition with bacteria must be small in magnitude when compared with autotrophic algal growth which is benefited by bacterial activity. As wastes added to water undergo bacterial decomposition, carbon dioxide, ammonia, phosphate, sulfate, nitrate and other elementary substances are liberated and become available to the autotrophic algae, which in the presence of adequate light and suitable temperatures, tend to grow in direct proportion to concentration of added decomposable nutrients.

Autotrophic algal growth, which occurs as a result of addition of organic wastes to natural waters, is of interest not only because it may lead directly or indirectly to gross nuisances in lakes, streams, or reservoirs, but also because it may be employed in controlled processes to produce oxygen and eliminate odors in stabilization ponds, to remove the bulk of nutrients from wastes prior to dilution in receiving waters thus controlling unwanted algal growth, and under special circumstances to accomplish both nutrient recovery and water reclamation (Oswald, Golueke and Gee, 1959). While each of these applications is of sufficient importance to be considered individually, they involve the same basic questions, namely; to what extent will a given organic waste support autotrophic algal growth, and what factors govern the magnitude of algal growth that will occur in a given waste? An approach to the answers to these basic questions will be the subject matter of this

paper.

Autotrophic growth in a given waste and body of water is a function of temperature, light and nutrient. Although taste and odor nuisance may occur at low temperatures, algal blooms reach nuisance growth proportions mainly when nutrients have accumulated and water temperature is between 20 and 30° C. Light has an important influence on autotrophic algal growth because it is the energy source for such growth. During periods of optimum temperature the amount of light per unit of area is rarely ample so that nutrient seldom becomes a predominantly important factor in determining the instantaneous magnitude of algal growths in natural waters. However, if light is abundant on a sustained basis, repetitive crops of algae may occur until some nutrient becomes exhausted. Thus it is important to know the nutrient limiting magnitude of algal growth in a given water.

Control of mutrients entering natural waters may have considerable impact on the problem of nuisance blooms. Such control may stem from excluding wastes from particular waters, from specialized processing of wastes to remove bloomproducing nutrients before they enter natural waters, or from the use of compounds which sequester critical nutrients in an unavailable form. Thus to accomplish control we become concerned with the nature of bloom-producing wastes and their nutrient composition. One such bloomproducing waste is domestic sewage. From the standpoint of its quality, domestic sewage has been known for 60 years or more to encourage algal growth, since it was incorporated into media used by many of the pioneers in algal culture. The elementary chemical composition of an average domestic sewage is shown in Table 28. The elementary analysis of an artificially constituted algal nutrient is also shown. This artificial nutrient was used by Fisher (1953) for pilot plant experiments on Chlorella cultures also shown in Table 28. It is evident from a comparison of the data for the two types of nutrient that although domestic sewage contains all of the essential macro nutrients for Chlorella growth, these are much more dilute than in the pilot plant nutrient, and are present in different forms and ratios. On the basis of macroele-

Table 28 - COMPARISON OF SEWAGE AND AN INORGANIC MEDIUM FOR ALGAL GROWTH

	Concentration, ppm			
Item	Sewage	Medium		
Total Solids	574.0			
Volatile	247.0			
Ash	327.0			
Total N	61.3	350.00		
Organic N	26.6	Nil		
Ammonia N	33.6	0.05		
Nitrate N	1.4	350.00		
Nitrite N	0.0			
Phosphorus	10.7	296.00		
Sulphur	9.5	322.00		
Potassium	13.0	1330.00		
Magnesium	18.0	246.00		
Calcium	6.0	15.00		
Sodium	72.0	5.50		
Iron	0.4	0.15		
Alkalinity	240.0			
BOD	168.0			
рH	9.3	6.00		

mentary composition in comparison with pilot plant media, one might conclude that domestic sewage is a poor nutrient for <u>Chlorella</u>. However, out-of-doors and due mainly to light limitations, domestic sewage will produce a standing crop of <u>Chlorella</u> practically equal to that in pilot plant media.

In the work reported here there has been no effort to evaluate the nutrient value of the various wastes on the basis of elementary composition. Rather, we have sought to establish quantitative relationships between waste concentration as measured by total solids and B.O.D., and algal growth employing the waste, diluting water, bacteria and algae in simple bioassays in which no factor other than the nutrient species relationship was limiting to algal growth.

In the assays we have sought to measure the potential of wastes to produce algal growth in excess of that which occurs in dilution waters without wastes added. We have also sought to determine the nutrient-limiting magnitude of such growth. On a short-time practical basis the magnitude of a bloom may not be the most significant factor since nuisance blooms are not always large, but on a long-time basis eutrophication is a function of bloom magnitude. Another feature of the assays reported here is interpretation of the data in terms of B.O.D. and oxygen production. Theoretically, a waste which will support a growth of algae of such magnitude that the oxygen produced during algal growth exceeds the B.O.D. of the waste, can be considered to be "treatable"; that is, it should be possible to remove the fertility compounds from the waste by means of intensive algal culture.

MATERIALS AND METHODS

Selection of Wastes

The wastes used in the assays were selected on

the basis of convenience as well as their significance to receiving streams. The list of wastes assayed is certainly far from complete and included milk, abattoir, reduction plant, packing house, cattle-holding pen, chicken pen, ground garbage, vegetable cannery, winery, tomato cannery, masonite, monosodium glutamate, glutamic acid, domestic sewage, and high rate domestic sewage pond supernatant. Glutamic acid, domestic sewage supernatant, domestic sewage and ground garbage were included in the assays because these have been subjected to analyses by ourselves and other workers.

Dilution Water

The concentration of certain essentials to algal growth, such as phosphorus, potassium, calcium, magnesium and trace elements is influenced or sometimes determined by the composition of dilution waters. Thus, for a valid assay of a waste it was considered important that the potential receiving water be incubated with the waste in determining maximum probable algal growth.

Normally the concentrated waste was diluted with the type of water used for dilution in the receiving stream and endogenous bacteria comprised the bacterial seed. At times, sub-dilutions were made with distilled water or with water of special interest and sewage seed was introduced separately.

Selection of Algal Species

Since our procedure called for determination of the maximum algal crop which could be grown in a given waste and at a given dilution, it was important to minimize the loss of algal nutrients, such as carbon dioxide and ammonia which sometimes escape during the early stages of decomposition. This was done by selecting algae which would grow well in actively decomposing wastes. E. gracilis was used in the early experiments, but a comparison of growth of E. gracilis with that of C. pyrenoidosa showed that the latter attained a higher growth rate than the former. Since it will grow so rapidly, the extent of the growth of Chlorella in a given waste indicates more accurately than does the growth of Euglena the maximum standing crop to be attained in a water receiving the waste. Consequently, Chlorella was the alga used in most of the succeeding studies.

Methods of Evaluating Growth

Algal growth was evaluated through cell counts used as a base for estimates of culture weight as a function of dilution of wastes. Because of the large volume of material normally required, determinations of culture weight, volume and light penetration, though commonly used to determine growth, were not used for these small cultures.

In these studies, cell counts were taken daily and the weight of <u>Euglena</u> or of <u>Chlorella</u> cells was estimated from earlier studies correlating cell count with cell weight for culture of various ages.

Figure 23 shows the culture weight in micro-

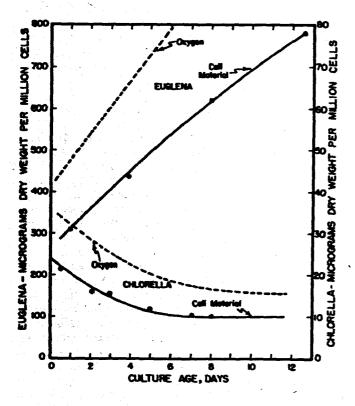


Figure 23. WEIGHTS OF ONE MILLION CHLO-RELLA OR EUGLENA CELLS AS A FUNCTION OF CELL AGE; WEIGHT OF OXYGEN EVOLVED BY GROWING ONE MILLION CELLS OF CHLORELLA OR EUGLENA.

grams per million <u>Euglena</u> and <u>Chlorella</u> cells as a function of cell age as determined from large continuous culture in which data on cell weight and cell age were accurately obtained (Ludwig, Oswald, Gotaas and Lynch, 1950). Also shown is the equivalent weight of oxygen determined by multiplying the weight of algae by 1.6, the ratio of oxygen produced to algal cell material synthesized, a factor also determined in continuous cultures (Oswald, Ludwig, Gotaas and Lynch, 1953). The cell count in millions of cells per liter was multiplied by the cellweight in milligrams per million cells obtained from Figure 23. This gave cell weight in milligrams per liter. This weight was then multiplied by 1.6 to estimate oxygen production.

Cell counts are quickly made, require little experimental material, and are not subject to interference by the presence of extraneous materials which do interfere seriously with gravimetric, volumetric, and spectrophotometric determina-

tions. A disadvantage of the cell count method is the relatively low reproducibility of algal counts and the uncertainty regarding the weight of algal cells invarious cultures. Enumeration with a haemocytometer of a large number of replicate algal samples gave information on the accuracy of our algal counts. An analysis of variance made on the repetitive algal counts showed that 70% of the Euglena counts in excess of 10⁵ cells per milliliter were within 20 per cent of the stated values, and that 70% of the Chlorella counts in excess of 106 cells per milliliter were within 10 per cent of the stated value. The same order of reproducibility is to be expected then for the dry weight estimations. With regard to the accuracy of the assumed weights of algal cells in confirmatory experiments, a large number of replicate algal cultures were grown on filtered wastes and their cell weights determined volumetrically by determining centrifuged packed volume and the relationship between packed volume and cell weight. Good correlation was obtained between the cell count and volumetric methods for growth evaluation.

Incubation Apparatus

A further advantage of the cell count method of growth evaluation is the simplicity of culture apparatus. In Figure 24 a cross-section of the simple incubator used for the assays is shown. It consists of several wooden shelves (A) and is illuminated by a 30-watt daylight fluorescent lamp (B) mounted parallel to each shelf. A window glass shield (C) reduces the amount of heating within the sample culture (D), and also decreases the magnitude of the ultraviolet component of the fluorescent spectrum to a point where it has little effect on the cultures. Their radiance within a 200 milliliter Erlenmeyer flask in place in the incubator is about 10 calories per liter per minute, and under continuous illumination, the total energy available to a 75 milliliter culture within a flask is about 14,000 calories per liter of culture per day. Because of their high surface area to volume ratio, the flasks dissipate waste heat to the surrounding air during incubation and therefore maintain a uniform temperature of about 25°C.

Performance of Tests

In the actual test procedure, 75 milliliter samples of serial dilutions made of the wastes under study were inoculated with unialgal cultures of Chlorella and of Euglena and, if required, of sewage bacteria. The cultures were then incubated under the standard conditions previously described until the daily algal growth increment became negligible. Daily cell counts were made with a haemocytometer and total growth computed as previously described. Growth rate constants were also computed from some of the cell counts. The average growth rate kg, was computed from the

expression $k_g = \frac{1}{t} \log \frac{C_2}{C_1}$ in which C_2

is the population at the end of the time period and C_1 , the population at the beginning of the time period. These rates were determined merely as a matter of interest. The wastes were analyzed initially for total solids, pH, alkalinity, and for B.O.D. as determined either according to Standard

S'-10°

BALLAST

VENT

VENT

12°

Figure 24. INCUBATION APPARATUS FOR BATCH CULTURES OF ALGAE IN ORGANIC WASTES. A - shelf; B - lamp; C - window glass; D - Sample flask.

Methods or with a Warburg respirometer. In the confirmatory experiments, packed solids were analyzed for initial and final B.O.D., pH, alkalinity and daily algal counts. The B.O.D. of the final effluent was determined by centrifuging the culture at 500 times gravity for 10 minutes and determining the B.O.D. of the decanted supernatant.

Values of the deoxygenation constant k were computed on the basis of B.O.D. data from the oxygen versus time curves using the simplified method for analysis of B.O.D. data as described by Moore, Thomas, and Snow (1950). A value for algal reoxygenation termed $\mathbf{k_a}$ was also computed in certain cases using the Moore, Thomas, and Snow method for analyzing data on oxygen produced.

RESULTS

The Influence of Time and Algal Species

Figure 25 shows the growth curves of Euglena

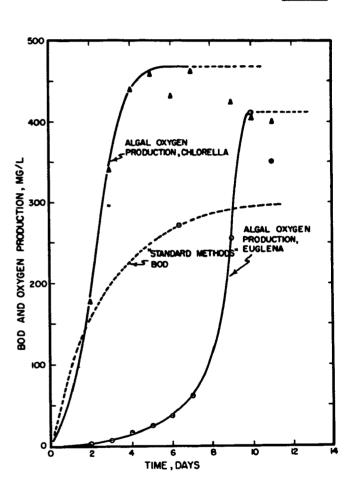


Figure 25. COMPARABLE GROWTH CURVES FOR EUGLENA AND CHLORELLA ON FRESH CHICKEN-PEN WASTE.

and <u>Chlorella</u> cultured on fresh chicken pen waste which had been diluted first with tap water to 1,000 ppm, and then with distilled water to obtain a B.O.D. of about 275 milligrams per liter. The growth curves are given in terms of total oxygen produced, i.e., 1.6 times the dry weight as determined from Figure 23, in milligrams per liter. According to the figure, <u>Chlorella</u> had a growth rate sufficient to result in the production of oxygen

at a rate greater than that required to meet the B.O.D. of the waste. Given sufficient time, however, the maximum dry weight of algal material produced in a given culture was approximately the same for both Chlorella and Euglena. Nevertheless, it was decided that although Euglena are characteristically polysaprobic, their generation time is too long to permit their use as valid indicators of maximum potential algal production. It is considered likely that, had it been measurable, the exertion of B.O.D. by bacteria in some of the cultures with Euglena would have been retarded by a lack of oxygen. Chlorella produced more total oxygen than Euglena. In fact, within five days Chlorella produced more than 1.6 times as much oxygen as required to meet the five-day B.O.D. of the waste, although, over a period of ten days Euglena produced more than 1,4 times as much oxygen as was required.

The Influence of Waste Dilution

In Figure 26 is shown the influence of time and solids dilution upon the B.O.D. of chicken pen

wastes and upon oxygen production by Chlorella. According to Figure 26(A) algal oxygen production exceeded the B.O.D. of the wastes at the beginning of the experiment and was the equivalent of twice the B.O.D. after five days. At a solids concentration of 250 ppm (Figure 26(B), algal oxygen production did not meet oxygen demand until 3.5 days had elapsed after initiating the experiment. However, after six days, oxygen production was 1.7 times the B.O.D. As shown in Figure 26(C), at a waste concentration of 500 ppm, almost five days elapsed before oxygen production by Chlorella was equal to the B.O.D. of the waste. The curve in Figure 26(D) shows that when the concentration of the waste was 1,000 ppm the B.O.D. of the waste was exerted at a rate much more rapid than that of oxygen production by Chlorella.

The Influence of Dilution Water

In Figure 27 is shown the five-day Standard Methods B.O.D. of reduction plant wastes as a function of waste concentration. Dilution of the wastes for B.O.D. determination was made with dilution

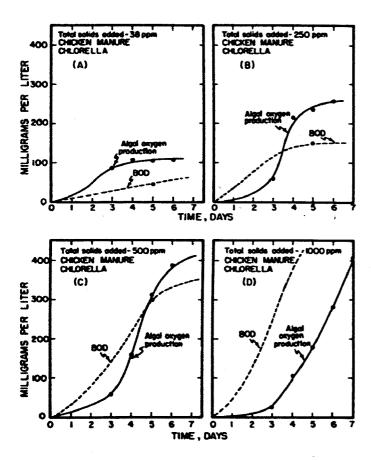


Figure 26. THE INFLUENCE OF SOLIDS DILUTION UPON THE B.O.D. OF CHICKEN-PEN WASTES AND UPON OXYGEN PRODUCTION BY CHLORELLA.

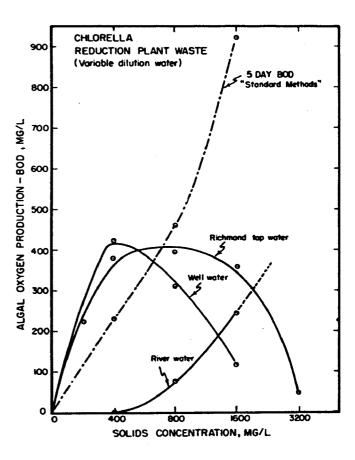


Figure 27. THE INFLUENCE OF WASTE DILUTION AND OF DILUTION WATER TYPE ON GROWTH OF CHLORELLA IN REDUCTION PLANT WASTE.

water prepared according to Standard Methods. Oxygen production by Chlorella grown in reduction plant wastes diluted with three types of dilution water to the solids concentration indicated are shown in curves identified according to the type of water used. The curves show that with the exception of river water, the oxygen produced by Chlorella increased with waste concentration between 0 and 400 or 500 milligrams per liter, and then decreased. In the case of river water, no algal growth occurred at a waste concentration of 200 milligrams per liter, and growth was barely detectable at a waste concentration of 400 milligrams per liter. Growth was enhanced when the concentration of the waste was increased to 816 milligrams per liter. When either well water or Richmond tap water was used, the oxygen produced during five days of growth exceeded the five-day B.O.D. up to a waste solids concentration of about 650 ppm. Since growth was plentiful when either well water or Richmond tap water was used as a diluent, it is evident that some factor in the river water inhibited the growth of algae and consequently, the production of oxygen.

Influence of Type of Waste

In Table 29, the various wastes subjected to

Table 29. ALGAL GROWTH AND OXYGEN PRO-DUCTION

	Relative (2) Equivalent		(1) 5 day BOD ppan		
Waste	Growth	conc. ppm.	Initial	Fina	
Muk	++	None	T		
Abattoir	+++	150	100	_	
Reduction plant	++++	650	400	20	
Packing house	+++	300	200	10	
Cattle holding pen	++	150 (2)		-	
Chicken pen	++++	500	300	50	
Ground garbage	++	350	50		
Vegetable cannery	++	150 (2)	100		
Winery	+	None (2)	None	_	
Tomatoe cannery	+++	100	90		
Magonite	•	None	1 -	_	
Monosodium glutamate	+	None	i		
Glutamic acid	+++	400	200	40	
Domestic sewage	+***	370	300	30	
Domestic sewage supernatant	•		35	10	

(1) Dry weight concentration of the waste at which BOD is equal to aigal oxygen production.

- (2) +

assay are listed, together with data on the equivalent concentration of the waste at which the B.O.D. of a particular dilution equalled the oxygen produced by the algae grown at that dilution, and data on the initial and final B.O.D. It is evident that, in most cases, at a concentration of waste less than the listed equivalent concentration, algal oxygen production usually exceeded the oxygen demand of the waste. A waste was labeled "none", when the extent of algal growth was insufficient to produce the oxygen necessary to meet the estimated B.O.D. at any of the several dilutions tried.

The fourth column in Table 29 lists the fiveday 20°C B.O.D. of the wastes at their equivalent concentration. A waste for which no B.O.D. is

listed either was not tested or gave highly erratic results. Determinations were made of the final B.O.D. of reduction plant, packing house, chicken pen wastes, and of domestic sewage and glutamic acid. In all cases, the final B.O.D. was low, an indication of the exhaustion of the readily available organic matter in the wastes. The supernatant from domestic sewage was reinoculated with Chlorella and incubated under standard conditions for 20 days. No detectable growth of algae was observed during this period, an indication that only one crop of algae could be grown in this dilute waste. It is evident from the table that algal growth was favored by the more nitrogenous wastes, since relatively poor growths occurred in vegetable wastes. Although pigmented and acid wastes only permitted poor growths, the extent of the algal growth observed at the higher dilutions of these wastes indicates their potential for producing longtime nuisance blooms.

In Table 30 are presented the results of experiments referred to in the introduction as confirmatory, conducted primarily to verify the results listed in Table 29 which were obtained on the basis of cell counts. As noted previously, at the time a given sample was incubated in the light, after being inoculated with algae, a replicate was placed in the Warburg to determine its Warburg B.O.D. The

Table 30. ALGAL OXYGEN PRODUCTION IN COMPARISON TO WASTE BOD

	BOD	Algal O2 production	(1) k _a	(2) k	(3) kg
Non-fat milk	180	60	.25	.21	.45
Reduction plant*	220	350	.30	.30	.60
Chicken manure*	100	130	.09	.10	.60
Fresh sewage*	160	375	.075	.15	.50
Monosodium glutamate*	150	Nil			

- (1) k2 = Oxygen production.
- (2) k = Oxygen utilization.
- (3) kg = Average population growth rate.
- · Filtered.

Warburg B.O.D. and the packed volume of the algal cell material were determined daily. Inasmuch as the wastes had been filtered or were highly dilute, most of the extraneous material was excluded, and consequently the centrifuged packed volume gave a relatively valid approximation of the dry weight of cells produced. It is seen from the table that filtered reduction plant waste, chicken manure, and fresh sewage sustained an algal growth sufficient to produce oxygen in excess of the equivalent Warburg B.O.D. whereas the simulated milk wastes supported little algal growth even when highly dilute. Few algae could grow in wastes containing monosodium glutamate end-liquor, probably because light could not penetrate the dark liquid. Its high solids content may have been a contributing inhibitory factor. Values of k are presented in Table 30 to give an indication of the relative rates

of 'algal oxygen production and bacterial oxygen utilization in the waste study. Comparable to the k values normally used in B.O.D. analyses, the larger the value of k and k_g , the more rapid the rate of oxygen utilization or oxygen production respectively. It is interesting to note that algal oxygen production was rapid in milk wastes but that the total amount was so small it never exceeded B.O.D. Hence, although algal growth occurred, the concentration was never such that oxygen production equalled oxygen utilization, as was the case with other wastes studied.

DISCUSSION

The criterion that the nutrient quality of a waste for algal growth is indicated by the algal production of oxygen in excess of the B.O.D. of the waste. is open to some discussion. Its validity certainly may be considered to be proved with respect to domestic sewage but the amount of research done in culturing algae on other types of wastes has been so limited as to exclude all but the most preliminary of conclusions. It seems reasonable to believe, however, that a waste having sufficient nutrients in balanced form to produce a strong surge of algal growth in a laboratory growth unit is also likely to promote a comparable growth in any body of natural water, providing that light and temperature are within permissible limits as to magnitude. It is unlikely that a more concentrated growth of algae would occur outdoors than under the laboratory condition used, because the total light energy available in the laboratory incubator exceeded severalfold that available under usual outdoor conditions.

In some of our earlier studies, attempts were made to culture algae in sterile organic wastes. Algae did grow well in many such wastes but the growth was much less intense under sterile conditions than when the waste inadvertently became contaminated. This led to the adoption of the practice of intentional bacterial inoculation with endogenous organisms which was followed in this study. It now appears certain that bacterial decomposition of the waste just prior to or simultaneously with the onset of algal growth liberates essential nutrients and leads to a more extensive algal growth than would otherwise occur. In natural waters many of the bacterial sludges which form during decomposition find their way into benthal deposits where decomposition to methane decreases the amount of carbon available for photosynthesis.

Direct utilization of compounds by algae has been a subject of great interest, and there is much documentary evidence that certain soluble substances are assimilated by the cells in the dark. Nevertheless, there is little question that during times when significant increases in algal concentration do occur, photosynthesis is the major mechanism for increase. Under such conditions ammonium is the principal energy containing com-

pound incorporated into cell material. Such rapid incorporation of ammonia into algal cells prevents its oxidation to nitrate, and inasmuch as algae eventually settle, this portion of the nitrogen may be removed from soluble form.

Each of the wastes in which strong algal growth occurred, also showed an optimum concentration for maximum algal growth. Such concentrations probably were much higher than those which would be encountered in natural waters and therefore algal growth occurring naturally would be more comparable to that obtained at the higher dilutions studied. The decrease observed in the algal concentration obtained at waste concentrations above a certain maximum, probably was due to a decline in the total available light. Some of the stronger wastes were extremely turbid. This turbidity absorbed light and inhibited algal growth to the extent that less oxygen was produced than was required to satisfy the B.O.D. Several of the strong wastes became foul smelling and acid conditions persisted, with an apparently detrimental effect on Chlorella. After long periods of incubation these foul cultures also became fresh smelling as a result of algal-bacterial stabilization.

Perhaps the most interesting, if not the most expected finding in this study, is the strong influence of diluting water upon the growth of algae attained in reduction plant wastes. For example, wastes diluted with water from a river in the disposal area strongly inhibited algal growth. This inhibition could be attributed to some nutrient present in well water and in Richmond tap water, but missing in the river water, or it could be attributed to some toxic substance in the river water. A more extensive study of the phenomenon is now under way.

Highly pigmented wastes supported algal growth only at highest dilutions. Because some of the wastes, such as masonite waste, also contained substances toxic to bacteria, such as phenols, their nutrient content was released only at the higher dilutions. In natural lakes or streams the slow release of nutrient from such wastes, despite their toxicity, could cause nuisance blooms over a long period of time in spite of the fact that there was little evidence of such tendency in the laboratory.

Milk wastes, because of their acidity, do not favor algal growth, but under conditions where oxidation is favored, algae do grow in their presence. For example, a small rapidly flowing stream at Novato, California, receives the waste from a cheese factory and courses over sandstone for several hundred yards. A one millimeter thick layer of Chlorella grows upon the submerged sandstone surface, where it is protected from the strong sunlight and perhaps from whey acids by a thin layer of aerobic bacteria growing upon its surface. The little stream is highly aerobic in

spite of a B.O.D. load which, on the basis of the atmospheric reaeration theory, should render it anaerobic.

SUMMARY

A series of bioassays was performed for the purpose of determining the nutritional value of the more common organic metropolitan wastes for the algae, Euglena gracilia, and Chlorella pyrenoidosa. The algae were seeded into a series of flasks containing the whole and diluted wastes of known B.O.D., which previously had been seeded with bacteria and were undergoing active bacterial decomposition. In each experiment a standard sewage was used as a reference and control. Inasmuch as the cultures were incubated at a temperature of about 20°C, in fluorescent light of optimum wave length, moderate intensity and long duration, the magnitude of algal growth obtained was mainly a function of the quality and availability of nutrients in the waste and its dilution water undergoing assay.

Any algal growth in excess of that which occurred in the dilution water was assumed to indicate the potentiality of long term problems. It was assumed that wastes in which oxygen production by algae exceeded the B.O.D. of the waste could be expected to produce heavy algal blooms under natural conditions, whenever dilution, light and temperature permitted, and when bacterial oxidation of the waste was in progress.

The metropolitan wastes studied were: milk, abattoir, reduction plant, packing house, cattle

holding-pen and chicken pen wastes, ground garbage, mixed vegetable cannery wastes, tomato cannery wastes, masonite manufacturing wastes, monosodium glutamate end liquor, glutamic acid, winery waste and domestic sewage. Analytical work was carried out to determine: (1) the influence of waste type on algal growth; (2) the influence of waste concentration on algal growth; (3) and the influence of algal growth on stabilization of the wastes as indicated by the relationships between algal growth, oxygen production and B.O.D.

It was found that Chlorella because of its high growth rate was of greatest value in waste assays. Each of the wastes would support some algal growth. However, milk, monosodium glutamate, winery waste and masonite did not support a sufficient growth of algae to produce the oxygen needed to meet the B.O.D. requirements of the diluted wastes and therefore it is possible that these wastes would cause long term nuisance problems. Algal growth in each of the other wastes was sufficient to satisfy the B.O.D. at some specific dilution. These wastes could, therefore, be expected to support heavy algal growths under natural conditions with odorless natural removal of nutrients occurring, providing the waste is properly diluted, and the waste pond correctly designed.

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REFERENCES

Fisher, A. E. 1953. Pilot plant studies in the production of Chlorella, Chapter 17, page 248 in Algal Culture from Laboratory to Pilot Plant, Carnegie Institution of Washington Publication 600.

Ludwig, H. F., W. F. Oswald, H. B. Gotaas and V. H. Lynch. 1950. Algae symbiosis sewage oxidation ponds. University of California IER Series 44, 11.

Moore E. W., H. A. Thomas and W. B. Snow. 1950. Simplified method for analysis of B.O.D. data. Sewage and Ind. Wastes 10: 1343. (1959)

Oswald W. J., C. G. Golueke and H. K. Gee. 1959. Waste water reclamation through production of algae. Contribution 22, Water resources center, University of California, Berkeley.

Oswald W. J., H. F. Ludwig, H. B. Gotaas, V. H. Lynch. 1953. Photosynthetic oxygenation. Sewage and Industrial Wastes 25, 6, 692.

Limnological Relationships

THE ROLE OF LIMNOLOGICAL FACTORS IN THE AVAILABILITY OF ALGAL NUTRIENTS *

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ABSTRACT

The availability and utilization of algal nutrients in lentic habitats are influenced by a complex of factors that vary from one body of water to another. In a broad consideration, these factors are related to the morphometry of the basin and the nature of the surrounding drainage area. Climatic conditions such as wind and solar radiation are also involved inasmuch as they affect the distribution and utilization of nutrients once they enter the basin.

The relative availability of nutrient materials is closely linked with the shape of the basin and its mean depth. The cyclic relations involved in plant growth, decomposition and nutrient regeneration are more efficient when the zone of active plant growth is in close proximity to the zone of most active decomposition of organic matter and regeneration of nutrients. The extent of water movement and the influence of water temperature on the metabolism of the biota are also important considerations in these cyclic phenomena.

Biotic factors are chiefly involved with utilization of nutrients and their regeneration from organic matter produced within the habitat or derived from its drainage area. The rate and thoroughness of the decomposition of organic matter in the water and the release of inorganic and organic materials from the bottom sediments are important factors in the cycle of some nutrients. Dissolved organic matter may function as a nutrient or accessory growth factor for planktonic algae; it can also act as a toxin, or in the chelation of trace metals.

In reviewing some of the major limnological factors that influence the availability and utilization of algal nutrients, it is apparent that the history of limnology and knowledge of the factors associated with the growth of phytoplankton have been so completely interwoven that the two can not be conveniently separated. Much of the early work in limnology involved attempts at lake classification based on their ability to produce phytoplankton and other organic matter. Naumann (1919) estab-

lished the nutrient-poor "oligotrophic" and the nutrient-rich "eutrophic" lake types. He also added a third type, the "dystrophic", in which humic substances were regarded as important limiting factors in the availability and utilization of nutrients. Welch (1941; 1952) and Findenegg (1955) have provided extensive reviews of the general subject of lake classification. Despite the many attempts, it remains to be demonstrated that a generally satisfactory system of lake classification based on nutrients or other indices of productivity can be derived owing to the milieu of interrelated factors.

In attempting to evaluate the physical and chemical parameters that influence the dynamic processes in lake metabolism, Rawson (1939; 1955; 1958) has considered three primary groups:

- a. Morphometric factors which relate to the size and shape of the basin, and which may affect the availability of nutrients.
- b. Edaphic factors which affect the supply of dissolved nutrients in the water.
- c. <u>Climatic factors</u> which affect the utilization of nutrients and, indirectly, their availability.

Depending on conditions, <u>human influence</u> may be considered a fourth group. These primary groups consist of many related factors which interact to determine the lake's ability to produce and circulate organic matter; depending on specific conditions, each of these may have a large or small effect on the lake.

As a background to the more pertinent limnological factors, it may be well to review some of the physical and chemical conditions which occur in lakes and reservoirs, as they relate to this discussion.

During the spring when the temperature is uniform throughout the lake, the effect of increased solar radiation is to warm the water at all depths, but decreasingly so at increasing depths. The den-

^{*}Contribution No. 22 from the University of Georgia Marine Institute, Sapelo Island, Georgia.

sity differences thus produced eventually lead to the formation of three relatively distinct layers of water: the upper layer or epilimnion and the lower layer or hypolimnion, separated by the thermocline or discontinuity layer. Under most conditions, the temperature is approximately the same at all depths in the epilimnion; the temperature within the discontinuity layer falls rapidly with increased depth and the resultant density gradient functions in separating the epilimnion from the hypolimnion. In the hypolimnion, the temperature is lower than the overlying water, and it does not change appreciably with increasing depth. When meteorological conditions result in reduced air temperatures for prolonged periods, the stratification is destroyed by alteration of the density structure and by action of strong winds on the lake surface. The lake is then considered to enter the period of overturn and the entire water mass is freely circulated. An inverse stratification may be effected by the occurrence of an ice cover, and is followed by another overturn during the spring.

It is generally considered that the primary synthesis of organic matter takes place in the epilimnion (trophogenic zone) and these materials ultimately find their way into the hypolimnion and to the bottom (tropholytic zone). Here the processes of decay and mineralization result in the release of various inorganic and organic compounds, and the utilization of dissolved oxygen. The latter may result in oxygen depletion during periods of prolonged stratification.

It is known that the chemical composition of plankton is usually higher in protein and fat content than organic matter derived from terrestrial vegetation. Its incomplete mineralization is evidenced by the chemical composition of upper lakebottom sediments of eutrophic lakes which invariably contain considerable amounts of nutrient elements in the form of resistant organic compounds. It has been supposed that these materials are mineralized in bottom sediments to a considerable extent, with the nutrients thus released becoming available in the trophogenic zone during periods of overturn. The existence of extensive mineralization has not been supported by chemical analyses of the bottom sediments, however (Kleerekoper, 1953). Mortimer (1942) described the existence of an oxidized microzone at the mud surface which he considered to be maintained by molecular diffusion of oxygen into the mud. It was demonstrated to exert a profound influence upon the exchange of substances across the mud-water interface. Recently, Gorham (1958) has presented evidence indicating that the thickness of the oxidized microzone may depend upon the turbulent displacement of the uppermost sediments into the overlying aerated water, as well as upon the reducing power of the sediments themselves.

In general, the redox potentials in the organic

matter determine the extent to which organic substances can be hydrolyzed by bacterial enzymes or decomposed by abiotic chemical processes. In eutrophic lakes, these processes are limited by the temporary or permanent lack of oxygen in the tropholytic zone. Under anerobic condition, the course of decomposition is different and less wellknown; decomposition does not proceed to complete mineralization, but stops short at intermediate organic stages such as methane (Ruttner, 1953). Studies by Kleerekoper (1953) on the mineralization of plankton suggest that most of the decomposition of the sinking planktonic detritus may take place in the epilimnion with the resultant liberation of much of the nitrogen; the liberation of phosphorus appeared to be slower, and both phosphorus and silica accumulated in the surface bottom sediments.

The availability of certain nutrients such as iron, manganese, nitrogen and phosphorus is closely associated with the cyclic chemical conditions which exist during periods of stratification and overturn. Under certain conditions, bicarbonate salts of iron and manganese are precipitated in the presence of oxygen; phosphorus may behave similarly, though it is also made available in a dissolved form through bacterial putrifaction or by activities of protozoa (Hooper and Elliott, 1953). These materials may thus be relatively abundant in the dissolved state in the oxygen depleted hypolimnion, along with silicic acid which occurs in a dissolved or colloidal form. Nitrogen may be present as nitrate in the epilimnion, but it appears chiefly as ammonia, derived from breakdown of protein, in the oxygen-free hypolimnion.

It has already been pointed out that increased solar radiation in the spring eventually may result in the production of a stratified condition. Of the sunlight which strikes the lake surface, a certain fraction is reflected and never enters the water. The remainder penetrates to varying depths, depending upon the concentration of dissolved colored substances and the absorbing and scattering materials such as plankton and inorganic and organic particulate matter. All of the light is eventually absorbed by the water and phytoplankton, except for that reflected at the surface or lost by back-scatter.

Thus, the amount of light which penetrates in different bodies of water is variable, depending upon prevailing conditions; it is also altered in spectral composition by differential absorption of certain wave lengths. In stained water, for example, the short wave lengths of the violet and blue portion of the spectrum are absorbed more readily than the orange and red. In distilled water, essentially the reverse is true. Since the rate of photosynthesis of algae is controlled by both the intensity and spectral composition of light, and by temperature, the extent to which solar radiation

penetrates the water influences the utilization of nutrient materials. Specific relationships between photosynthesis and various components of the visible spectrum are not well recognized. It is known, however, that the light and temperature relations are not the same for all algae but the same general limiting factor relation applies; namely, at any given temperature, photosynthesis is directly proportional to the light intensities at low light, but at high intensities, it is independent of light intensity and can be increased only by a rise in temperature (Lund, 1958). The seasonal variation in light and temperature has been used to explain the shift in composition of phytoplankton from spring to fall; some investigators attribute the observed changes to the availability of nutrients and/or the production and effect of metabolites.

The cyclic relations involved in the utilization, regeneration, and circulation of nutrients is most efficient when the zone of active plant growth is in close proximity to the zone of most active decomposition of organic matter and regeneration of nutrients. From this viewpoint, one would anticipate the most efficient system to be that in which there was a continual circulation, with light and dissolved oxygen being present to the bottom. In this situation, there would be relatively little lag between regeneration of nutrient material and its utilization in plant synthesis. These are essentially the conditions that exist in the western end of Lake Erie and other shallow lakes and reservoirs where there is little or no stratification owing to a reduced mean depth and a favorable exposure to prevailing winds. The mechanisms involved are also those which function in the sewage stabilization ponds employed in some regions of the United States.

From the physical point of view, the absorption of the major portion of the incident solar radiation in the surface water, owing to suspended materials or water color, may result in the establishment of an appreciable density difference that can not be destroyed by prevailing wind conditions. In such instances, a relatively thin epilimnion could develop. The nature and extent of the thermocline may vary greatly, but in general the depth tends to increase as the summer advances and the ratio of the volume of epilimnion to volume of hypolimnion therefore increases.

The availability of nutrients in bodies of water that have become stratified is dependent on the water circulation patterns and basin morphometry. The density structure that is established by increased solar radiation and by wind energy can be visualized as a series of circulating cells, with the surface wind activity providing the driving force. Laminar flow exists along the boundaries of the circulating cells only when relative current movements along the density interface are below a certain velocity. Depending on the existing conditions

of viscosity and density, a velocity may be reached at which the laminar flow becomes turbulent: wave-like disturbances develop on the interface and grow in amplitude, and finally break into vortices, resulting in a great increase in friction and mixing. The production of waves and vortices increases the area along the density interface many times, and the mixing is completed by microturbulence and molecular motion. It is this phenomenon which results in the erosion of the thermocline and the increase in the volume of the epilimnion as the summer progresses.

The mixing caused by turbulence and eddy formation not only functions in the transfer of heat, but also results in the movement of nutrients into the trophogenic zone where they may again take part in organic matter synthesis. The extent to which the waters of the hypolimnion are involved in such circulation is not well known. The work of Gorham (1958) on bottom sediments and that of Mortimer (1951) on water movements suggests that hypolimnetic circulation may be appreciable and may provide an explanation for the development of oxygen depletion near the bottom sediments at a rate more rapid than would be expected on the basis of molecular diffusion alone.

In shallow lakes where the volume of the epilimnion is large in comparison to the volume of the hypolimnion, one could expect a more rapid and efficient circulation of regenerated nutrients. In shallow lakes, particularly those with irregular shore lines, the intimate contact of the shore and bottom sediments with the productive volume of the lake should not be discounted in considerations of nutrient availability. When the volume of the hypolimnion is appreciably greater than that of the epilimnion, as is often the case in deep lakes, circulation of nutrients would be expected to be much slower and less efficient. The general relationships between mean depth and organic matter production of lakes have been studied by Rawson (1955). His findings show an excellent correlation between average lake depth and the unit area production of plankton, benthic organisms, and fish, and provide evidence for the efficient circulation and utilization of nutrients in shallow bodies of water.

Only a few of the many factors associated with the availability of nutrients have been briefly discussed. It should be pointed out that the products of mineralization are not the only important components in algal nutrition. Dissolved organic matter is known to exert its effects on phytoplankton in four general ways (Saunders, 1957). Investigations have demonstrated that certain algae are capable of utilizing organic substances as an energy source or as a nutrient. In some instances, dissolved organic matter may supply accessory growth factors which are required for or stimulate growth of phytoplankton, whereas toxic substances are known to be produced and inhibit growth. Organic complexes

may be formed with various trace metals and, depending on circumstances, may function to remove a particular nutrient from the trophogenic zone or cause it to be retained. Recent investigations conducted in some of Michigan's hard-water lakes (Schelske, 1960) demonstrated that the addition of certain organic compounds can enhance algal production by apparently chelating iron and perhaps other nutrients so as to prevent their chemical precipitation as insoluble carbonates.

In summary, it may be stated that the availability and utilization of algal nutrients are associated with many physical, chemical and biological factors that are related to the basin's morphometry, as well as the edaphic and climatic conditions that prevail. Since these factors vary in importance from one body of water to another, and from time to another, anything more than simple generalizations is difficult; however, information is available which will permit detailed evaluation of particular situations if desired.

REFERENCES

Findenegg, V. I. 1955. Trophiezustand und Seetypen. Schweizarische Zeitschrift Für Hydrologie. 17: 87-97.

Gorham, E. 1958. Observations on the formation and breakdown of the oxidized microzone at the mud surface in lakes. Limnol. and Oceanogr. 3: 291-298.

Hooper, F. F., and A. M. Elliott. 1953. Release of inorganic phosphorus from extracts of lake mud by Protozoa. Trans. Am. Micro. Soc. 72: 276-281.

Kleerekoper, H. 1953. The mineralization of plankton. J. Fish. Res. Bd. Can. 10: 283-291.

Lund, J. W. G. 1958. Primary production in inland waters. In the Biological Productivity of Britain, Publ. Inst. of Biol., London: 1-6.

Mortimer, C. H. 1942. The exchange of dissolved substances between mud and water in lakes. J. Ecol. 30: 147-201.

1951. Water movements in stratified lakes, deduced from observations in Windermere and model experiments. Union Geodes et Geophys. int., Assoc. int. d'Hydrol. scientif., Assemblee gen. de Bruxelles. 3: 336-349.

Naumann, E. 1919. Nagra sympunkter angaende planktons okologi. Svenske. Bot. Tidskrift 13.

Rawson, D. S. 1939. Some physical and chemical factors in the metabolism of lakes. In Problems of Lake Biology, Publ. Am. Assoc. Adv. Sci., No. 10: 9-26.

1955. Morphometry as a dominant factor in the productivity of large lakes. Proc.Int. Assoc. Theor. and Appl. Limn. 12: 164-175.

1958. Indices of lake productivity and their significance in predicting conditions in reservoirs and lakes with disturbed water levels. In Invest. of Fish-Power Problems. H. R. MacMillan Lectures in Fisheries. Univ, of British Columbia: 27-42.

Ruttner, F. 1953. Fundamentals of limnology. Univ. of Toronto Press. 242 pp.

Saunders, G. W. 1957. Interrelations of dissolved organic matter and phytoplankton. Bot. Rev. 23: 389-410.

Schelske, C. L. 1960. Preliminary report of the influence of iron, organic matter and other factors upon primary productivity of a marl lake. Rept. No. 1586, Inst. for Fish. Res., Mich. Dept. Cons., Ann Arbor.

Welch, P. S. 1941. Dissolved oxygen in relation to lake types. In a Symposium on Hydrobiology. Univ. of Wisc. Press: 60-70.

1952. Limnology. McGraw-Hill Col, New York. 538 pp.

NITROGEN FIXATION IN NATURAL WATERS UNDER CONTROLLED LABORATORY CONDITIONS

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The subject of nitrogen fixation in aquatic areas and its relationship to the productivity of lakes and impoundments appears to be a sorely neglected topic of investigation, particularly in view of the fact that the productivity of so many aquatic areas seems to be unrelated to the gross contribution of fertilizing matter in tributary waters. Clarke (1954) in his book on "Elements of Ecology" states -- "the supply of nitrogen in the soil and in the water may be further augmented as the result of nitrogen fixation by specialized bacteria belonging to the genera Azotobacter and Clostridium which are free living..." He also goes on to say -- "Free nitrogen can also be fixed by certain photosynthetic bacteria, sulfate reducing bacteria, and bluegreen algae." The significance of this phenomenon appears to be left to the reader's imagination. however.

Hutchinson (1957) discusses nitrogen fixation in somewhat greater detail and points out that a considerable number of bacterial species including Azotobacter, Clostridia, Azotomonas, Aerobacter, Methanomonas, and Pseudomonas are capable of fixing nitrogen. He lists a number of blue-green algae that have been reported to fix nitrogen. These include such well known forms as Anabaena, Gloeocapsa, and Nostoc.

Allen (1956) points out that the nitrogen fixing ability of blue-green algae has been established since 1928 and discusses the macro and micro nutrient requirements in considerable detail. Molybdenum, cobalt, and sodium have been found to be necessary items in the nutrition of blue-green algae, and the significance of domestic sewage as a source of these elements is well correlated with the stimulation of blue-green algae blooms that occur in aquatic areas fertilized by such waste waters.

STUDIES AT MADISON, WISCONSIN

The senior author's interest in nitrogen fixation in aquatic areas stems from personal observations made during the conduct of the Madison Lakes Survey, 1942-44 (Sawyer, 1944; and Sawyer, 1945). A part of the survey method consisted of obtaining visual evidence to demonstrate to a committee composed of laymen as well as scientists that the productivity of the aquatic areas under consideration was related to the degree of fertilization. For this demonstration, samples of waters collected from the various lakes and streams were kept in loosely stoppered, partially filled, glass containers under natural light conditions in a green house.

The visual evidence obtained was quite dramatic and perhaps, had more weight in convincing some members of the committee of the significance of fertilizing matters than all the analytical data obtained.

During the course of the above-mentioned studies it was noted that many of the lightly fertilized waters showed a productivity greatly in excess of what would have been predicted, particularly when the exposure period was continued for several weeks. In addition, it was noted that blue-green algae often came into dominance. This led to a series of studies in which the nitrogen content of the specimens was placed under close surveillance. The results of these studies are presented in Table 31 and show that nitrogen fixation occurred in all waters with the exception of the specimen from Lake Wingra.

Table 31. NITROGEN FIXATION IN NATURAL WATERS UNDER LABORATORY CONDITIONS (NATURAL LIGHT)

		Tot	al nitrogen,	mg/L
	Days	Start	Finish	Δ
Lake Waters				
Mendota	58	0.48	0.79	0.31
Mendota.	167	0.51	2.61	2.10
Monona .	41	1.03	2.03	1.00
Monona	61	0.58	1.17	0.59
Waubesa	115	1.72	5.35	3.63
Kegonsa	116	0.82	5.80	4.98
Wingra	45	1.88	1.58	-0.30
Streams				
Six Mile Creek	151	1.97	7.26	5.29
Crawfish River	167		5.45	?
Yahara River	116	2.00	7.83	5.83

Nitrogen fixation appeared to be most prolific in those lake waters receiving significant amounts of fertilization from domestic sewage or drainage from farm lands. An experiment was designed, therefore, in an attempt to show the significance of available nitrogen and phosphorus as factors in nitrogen fixation. These studies were not made in replicate and interpretations drawn from them were considered tentative. The experiment consisted of adding normal sewage treatment plant effluent, effluent minus its nitrogen and phosphorus, and effluent minus its nitrogen to Lake Mendota water. The results of the study are shown in

Table 32 and indicate that phosphorus is a key element in the stimulation of nitrogen fixation.

Table 32. NITROGEN FIXATION IN LAKE MENDOTA WATER FERTILIZED WITH SEWAGE EFFLUENTS

	i	Total nitrogen, mg/L		
Mixture	Days	Start	Finish	Δ
Lake water, control	167	0.51	2.61	2.10
Plus 10% effluent Plus 10% effluent	181	2.67	4.39	1.72
minus its N & P Plus 10% effluent	170	0.67	2.17	1.50
minus its N	188	0.67	11.85	11.18

STUDIES AT BOSTON, MASSACHUSETTS

Taste and odor problems related to algal growths are becoming more and more common and, oftentimes, a logical explanation of why the problem occurs is not always evident. It is always embarrassing to a consulting engineer to have to resort to some of the stock answers that have been used in the past for, to him, there is a cause for every effect.

In an attempt to explore more fully the significance of nitrogen fixation in the productivity of aquatic areas, our laboratory undertook a series of experiments. These studies were designed to evaluate the significance of nitrogen, phosphorus and increased alkalinity, all items of considerable concern insofar as fertilization by human wastes is concerned.

The experiments were performed in the following manner: Pond waters were brought to the laboratory, fortified with ammonium and/or phosphate salts to increase the level significantly and the alkalinity was adjusted as desired by adding sodium bicarbonate. The use of calcium salts was avoided because of the known stimulatory effect of calcium ion (Allen, 1956).

Two pond waters were selected for the initial studies and plans were made to make repetitive runs around the calendar in order to evaluate seasonal changes. The samples were kept under fluorescent lighting at an intensity of 500 foot-candles for 12 hours of each day.

Spy Pond. This pond has an area of 129 acres and is located in Arlington, Mass. It is not used as a public water supply since it is used for recreational purposes and is fringed by homes. It is subjected, therefore, to some street drainage and possibly some human wastes. Algae blooms of moderate intensity do occur on occasion, in spite of the fact that nutrient levels of nitrogen and phosphorus are quite low.

Samples of Spy Pond water were fortified with 0.2 mg/L of phosphorus and/or 0.5 mg/L of ammonia nitrogen. The aklalinity was increased

when desired by adding 200 mg/L of NaHCO₃, equivalent to 120 mg/L of CaCO₃. Three series of studies have been completed and the results are given in Tables 33, 34, and 35. The data for the

Table 33. NITROGEN FIXATION IN SPY POND WATER (FLUORESCENT LIGHTING - 12 HR/DAY) JULY 21 - AUGUST 25, 1959

İ	Total P.	Total nitrogen, mg/L		
	mg/L	Start	Finish	Δ (35 days)
Control	Tr.	0.57	0.78	0.21
Control + Alk.*	Tr.	0.57	0 77	0.20
Plus N	Tr.	1.07	1.38	0.31
Plus N + Alk.	Tr.	1.07	1.04	-0.03
Plus P	0.2	0.57	1.69	1.12
Plus P + Alk.	0.2	0.57	1.60	1.03
Plus N + P	0.2	1.07	1.86	0.79
Plus N + P + Alk.	0.2	1.07	1.61	0.54

*200 mg/L NaHCO3

Table 34. NITROGEN FIXATION IN SPY POND WATER (FLUORESCENT LIGHTING - 12 HR/DAY) AUGUST 27 - NOVEMBER 25, 1959

	Total P,	Total nitrogen, mg/L		
	mg/L	Start	Finish	∆(90 days)
Control	Tr.	0.92	1.23	0.31
Control + Alk.*	Tr.	0.92	1.30	0.38
Plus N	Tr.	1.42	2.26	0.84
Plus N + Alk.	Tr.	1.42	1.94	0.52
Plus P	0.2	0.92	2.43	1.51
Plus P + Alk.	0.2	0.92	2.58	1.66
Plus N + P	0.2	1.42	2.81	1.39
Plus N + P + Alk.	0.2	1.42	3.00	1.58

*200 mg/L NaHCO3

Table 35. NITROGEN FIXATION IN SPY POND WATER (FLUORESCENT LIGHTING - 12 HR/DAY) DECEMBER 3, 1959 — FEBRUARY 17, 1960

	Total P.	Total nitrogen, mg/L		
	mg/L	Start	Finish	Δ (70 days)
Control	Tr.	0.98	1.55	0.57
Control + Alk.*	Tr.	0.98	1.32	0.34
Plus N	Tr.	1.48	1.55	0.07
Plus N + Alk.	Tr.	1.48	1.72	0.24
Plus P	0.2	0.98	2.02	1.04
Plus P + Alk.	0.2	0.98	1.95	0.97
Plus N + P	0.2	1.48	1.70	0.22
Plus N + P + Alk.	0.2	1.48	1.79	0.31

*200 mg/L NaHCO3

first two periods are summarized graphically in Figures 28 and 29. From these data we conclude that phosphorus plays a key role in nitrogen fixation and that the bicarbonate ion is relatively unimportant, since Spy Pond water has a .natural alkalinity of only 30 mg/L.

Hagar's Pond. Hagar's Pond is a natural impoundment of about 27 acres in Hagar's Brook and the water is heavily fertilized by the sewage plant effluent from the city of Marlboro, Mass. The pond water, as brought to the laboratory, contained over

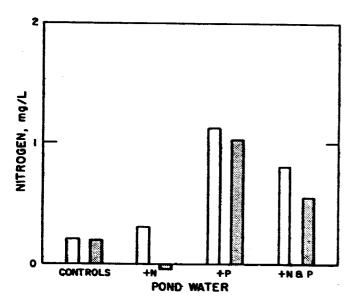


Figure 28. NITROGEN FIXATION IN SPY POND WATER. (35 days, July-Aug.). Shading indicates alkalinity added.

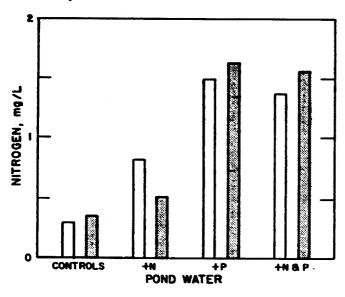


Figure 29. NITROGEN FIXATION IN SPY POND WATER. (90 days, Aug.-Nov.). Shading indicates alkalinity added.

3 mg/L of total phosphorus and in excess of 5 mg/L of total nitrogen. It was considered useless to attempt studies by fortifying this water with phosphorus and nitrogen. Instead a study was made to determine the nitrogen fixing ability of the water when diluted with a high quality water. Boston tap water was used for this purpose. A series of dilutions were made as shown in Table 36. The samples were kept under laboratory conditions for 72 days and then analyzed. The data are given in Table 36 and Figure 30. Only the mixture containing 100 percent and 20 percent of pond water showed significant increases in nitrogen fixed. The experiment was not repeated so no explanation except biological variation can be offered at this time.

Table 36. NITROGEN FIXATION IN HAGAR'S POND - BOSTON TAP WATER MIXTURES - JULY 29 - October 9, 1959

Hagar's Pond	Total P.	Total nitrogen, mg/L			
water, %	mg/L	Start	Finish	∆ (72 days)	
100	3.3	5.38	6.74	1.36	
50	1.65	2.92	3.46	0.54	
20	0.66	1.45	4.55	3.10	
10	0.33	0.95	1.61	0.66	
5	0.17	0.71	1.03	0.32	
2	< 0.10	0.56	1.09	0.53	
1	< 0.10	0.50	0.97	0.47	
0	< 0.10	0.46	0.81	0.35	

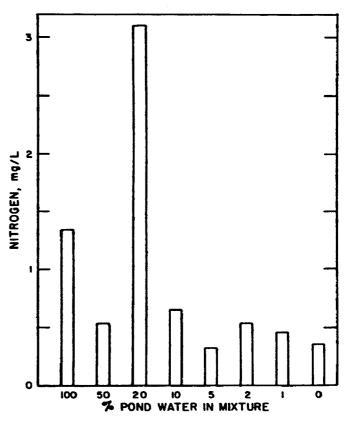


Figure 30. NITROGEN FIXATION IN HAGAR'S POND. Boston tap water mixtures. (72 days, Aug. -Oct.).

DISCUSSION

In the laboratory studies conducted to date, no attempt has been made to ascertain whether nitrogen fixation was due to algae or bacterial action. It seems important to note, however, that whenever unusual amounts of nitrogen were fixed bluegreen algae were found in the test specimens. The dominant genus noted was Aphanizomenon.

The studies conducted in the relatively soft waters of Massachusetts have confirmed the importance of phosphorus as a key element in nitrogen fixation. This points up another important facet

of the effect of human wastes in determining the productivity of receiving bodies of water. Domestic wastes, particularly since the advent of synthetic detergents, are extremely rich in phosphorus in relation to nitrogen. Biological utilization of the available nitrogen leaves a water that is abundantly rich in phosphorus and ripe for exploitation by nitrogen fixing forms. It is quite likely that this condition accounts for the explosive blooms of blue-green algae which occur in many of our lakes and reservoirs.

CONCLUSIONS

1. Phosphorus is a key element in nitrogen fixa-

tion.

- 2. Fertilization of aquatic areas by domestic wastes stimulates biological productivity.
- 3. Sewage plant effluents contain phosphorus in excessive amounts.
- 4. The excess phosphorus can stimulate extensive blooms of nitrogen fixing blue-green algae.
- 5. The productivity of most aquatic areas is probably related to their phosphorus budgets.

REFERENCES

Allen, M. B. 1956. Photosynthetic nitrogen fixation by blue-green algae. Scientific Monthly, p. 100, August.

Clarke, G. L. 1954. Elements of ecology. John Wiley & Sons, N.Y.C.

Hutchinson, G. E. 1957. A treatise on limnology. John Wiley & Sons, N.Y.C.

Sawyer, C. N. 1944. Investigation of the odor nuisance from Madison Lakes. July 1942-July 1943.

Sawyer, C. N. 1945. Investigation of the odor nuisance from Madison Lakes. July 1943-July 1944.

RECENT OBSERVATIONS ON NITROGEN FIXATION IN BLUE-GREEN ALGAE

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INTRODUCTION

The fixation of N₂ by organisms occupies one facet of an extremely complex and interesting biogeochemical cycle. An excellent review of the steps through which unequivocal proof was obtained of the ability of certain of the blue-green algae to fix nitrogen has been made by Fogg and Wolfe (1954). In that article, a list showing the known distribution of the ability to fix nitrogen in the My-xophyceae is given; although several species have been added since then, the early conclusion that the family Nostocaceae is most important is still supported.

With the ability of blue-green algae to fix nitrogen definitely established, speculation has naturally arisen regarding the role of the process under natural conditions. Hutchinson (1941) noted a very considerable increase in the combined nitrogen content in Linsley Pond during a bloom of blue-greens dominated by Anabaena circinalis and suggested that biological fixation by the algae was probably responsible. Early recognition of the possible importance of this process was made by Saw-

yer, et al (1945) whose work on the Madison lakes has already been reported to this conference. In a recent appraisal, Hutchinson (1957) concludes that this source of combined nitrogen can probably be expected to be important when <u>Anabaena</u> is found in heavy concentrations. Allen (1956) also concluded from laboratory culture studies that fixation of nitrogen by blue-green algae could, under optimum conditions, amount to about 480 pounds per acre per month, more than double the quantity which would be fixed by a legume-<u>Rhizobium</u> combination for an entire crop.

A serious hindrance to an understanding of the role of biological fixation in the aquatic environment has been the lack of a suitable method for direct measurement of this contribution. Although a balance-sheet can be constructed, as e.g., Rohlich and Lea (1949) have done, the possibility of concomitant denitrification makes the magnitude of the fixed component uncertain even if it were possible to determine accurately the loss to the sediments over the study period.

The N¹⁵ Method for Measuring Fixation Rates

Investigations in progress at the University of Wisconsin and at the University of Pittsburgh employ the heavy isotope of nitrogen, N¹⁵, as a tracer, the method having been adapted from that used by Burris, et al (1943). The procedure involves primarily the removal of atmospheric dissolved N2 from the water sample, held in specially designed 1-liter vessels, and the subsequent replacement with a quantity of nitrogen gas heavily enriched in N^{15} (95 atom-percent in most of these experiments). The sample is then incubated by suspending it from a buoy in the lake or in a tank in the laboratory. Following a suitable incubation period, usually 24 hours or less, the sample is boiled down, Kjeldahl-digested, distilled, and titrated with acid. After converting the ammonia to nitrogen gas by the method of Rittenberg, et al (1939) the final isotope ratio is determined with a mass spectrometer. The total amount of N2 incorporated during the experiment is given by the simplified expression:

N fixed = atom-percent excess N^{15} x total N in

atom-percent N¹⁵ supplied sample

if the increase in total N is reasonably small. The entire procedure has been carefully calibrated; the least significant enrichment in N 15 has been found to be 0.03 μg of N2. Ammonium sulfate is used as a standard; excess N 15 is computed by subtracting the atom-percent N 15 of untreated lake water, drawn from the same lot as that used in the experiment, from that of the incubated sample.

Results Obtained from the N¹⁵ Method

One full year of work on Sanctuary Lake, the uppermost portion of Pymatuning Reservoir in northwestern Pennsylvania, has been completed. Sanctuary Lake has a surface area of about 2,000 acres, a maximum depth of 3 meters and a mean depth of 2 meters. The lake has been shown by Tryon and Jackson (1952) and by Hartman (in press) to exhibit extremely dense blooms of blue-green algae. Essentially no light penetrates to the lake-bottom.

In Figure 31, the rate of nitrogen fixation, expressed as a percentage fixed per day of the existing total combined nitrogen, concentration of Anabaena spp., nitrate, nitrite, and ammonia, is plotted. The data refer to fixation at the lake surface and represent a composite of samples incubated in the lake and in the laboratory. Significant fixation rates appeared concurrently with the presence of Anabaena in numbers. Following a decline which coincided with a short-lived decrease in the Anabaena population, the fixation rate rose and remained at about 1 percent newly fixed nitrogen per day until late August when a very considerable increase in both Anabaena and fixation rate took place. Two features are especially noteworthy.

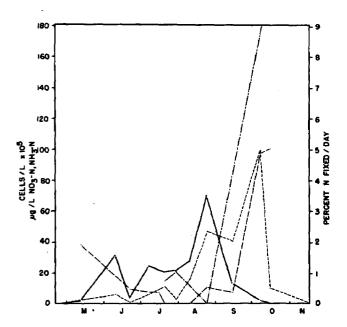


Figure 31. RELATIONS AMONG RATE OF NITROGEN FIXATION, CONCENTRATIONS OF AMMONIA AND NITRATE AND NUMBER OF CELLS OF ANABAENA AND DATE IN SANCTUARY LAKE.

Percent fixation rate --- NO₃ - N
---- Anabaena --- NH₃ - N

The period of rapid increase in the Anabaena population occurred when the levels of inorganic combined nitrogen had fallen to nearly undetectable quantities. The concurrent rise in fixation rate suggests that the expected advantage nitrogen fixers would have over non-fixers under these conditions is indeed realized, eventually resulting in what Professor Hutchinson calls "Anabaena soup".

The second noteworthy event is the startling decline and final disappearance of fixation at a time when the Anabaena population was still nearly at peak abundance. Although we have no experimental evidence, this decline can probably be ascribed to the simultaneous increase to very high values of ammonia concentration in view of the well-documented depressing effect of available combined nitrogen on nitrogen fixation (Fogg, 1954). The subsequent rise of nitrate and the first appearance of nitrite indicate the beginning of active nitrification.

Phosphorus concentrations (not shown) fell with the onset of the spring blue-green bloom and remained low for the rest of the summer, indicating a rapid rate of turnover or a high rate of exogenous supply. The total combined nitrogen in the lake increased several fold in late August as a result of the very high rates of fixation prevailing at that time. The process of accumulation of fixed nitrogen is self-accelerating wherever percentage rates of fixation remain consistently high.

It is, of course, possible that the observed fixation is attributable to organisms other than the algae. We have, however, shown a clear dependence of the process of fixation in lake water upon light by incubating samples in ordinary transparent flasks together with similar samples in darkened flasks, finding that fixation is reduced or obliterated in the latter. The process, as it occurs in lake water, seems clearly to be energized by photosynthesis directly or indirectly.

Some observations made in Lake Mendota (at Madison, Wisconsin) during the summer of 1959 suggest that a very similar series of events takes place there. In mid-September, daily percentage fixation rates were measured above the thermocline as follows: 0.019 (at 1 meter below the surface), 0.023 (2 meters) and 0.014 (4 meters). At this time the phytoplankton was not dense and among the blue-greens only Gleotrichia echinulata was present. Nitrate was at undetectably low concentrations in the entire epilimnion (0-12 meters), although there was a small maximum in the thermocline; ammonia concentrations were not measured. Nitrate began to appear in the upper water after October 13, immediately after complete turnover of the lake, and at the end of that month fixation rates had dropped to zero. By themselves, these data are not particularly useful, but they would be expected if one assumed an inverse relationship between nitrate concentration and fixation rate such as was found in Sanctuary Lake.

Fixation rates so low that they should probably be termed non-existent have been measured under the completely different conditions found in two lakes on Afognak Island, Alaska.

Conditions for Intense Nitrogen Fixation

A working hypothesis may be constructed at this point as a guide for further experimentation and observation. The following conditions are likely to be necessary for the support of intense fixation in a body of water:

- 1. The general physical and nutritional characteristics of the body of water must be such as to encourage the growth of blue-green algae.
- 2. Some factor(s) must operate to reduce the concentrations of the various forms of combined nitrogen to very low levels. This may be accomplished by the phytoplankton population itself, or by some other agency such as denitrification.
- 3. The work of Sawyer, et al. (1945) indicates that phosphorus is important. Although prevailing concentrations apparently need not be strikingly high, an adequate supply would appear to be a critical factor. The experiment of Buljan (1957) provides dramatic support for this point of view. In it, a clear, blue, unproductive lagoon of the Adriatic Sea, whose water contained very low concen-

trations of phosphorus and combined nitrogen, was fertilized with phosphorus alone, the assumption being that, with an adequate supply of phosphorus available, the nitrogen requisite for increased productivity could be supplied by fixation. The results of the experiment amply proved this to be the case, the blue water of the lagoon changing to an unappealing green. These results are similar to those obtained very much earlier by investigators studying fertilization of central European carp ponds, showing that increased yields of fish from ponds fertilized with combined nitrogen, phosphorus and the two in combination depended upon the quantities of phosphorus provided, whether this was used alone or with nitrogen, and not upon amounts of added nitrogen. The latter was presumably supplied by local fixation.

4. Certain elements in trace amounts are now known to be specifically necessary to permit nitrogen fixation by particular species of blue-green algae; e.g., Eyster (1959) has shown that Ca, B, and Mo are necessary for fixation by Nostoc muscorum, and has determined amounts necessary for optimum growth in the absence of an external source of combined nitrogen. A comparison of these amounts with those ordinarily available under natural conditions suggests that Ca is usually present in sufficient concentration in fresh waters, but that B and Mo are likely to be in short supply. Little is known about the distribution of the latter in fresh waters; the few analyses available indicate that it will seldom be found in concentrations greater than about 1 µg/1, about one-hundredth the concentration required for optimum fixation by algae in culture (Fogg, 1956). The success of Buljan's experiment may well be due to the more-thanadequate supply of B and the nearly-adequate supply of Mo (about 10 μ g/1) ordinarily present in sea-water. Hilliard (personal communication) has found in a series of brackish lagoons near Cape Thompson, Alaska, the same three species of Anabaena (flos-aquae, spiroides and circinalis) that have apparently been responsible for fixation in Sanctuary Lake. These findings are not inconsistent with the results of Allen (1956), who found that Anabaena cylindrica could tolerate NaC1 in concentrations as great as 1.5 percent. Allen (1959) has also isolated nitrogen-fixing species of Calothrix from coastal waters of California. All of these observations seem to indicate that dilute sea-water is a reasonably good medium for nitrogen-fixing blue-green algae, perhaps owing more than anything else to favorable quantities of the trace elements named above. It seems possible that some of these elements are concentrated in sewage, resulting under certain circumstances in the stimulation of nitrogen fixation by this material.

We wish to acknowledge the support of the National Institutes of Health for our studies on nitrogen fixation being carried out jointly at the Universities of Wisconsin and Pittsburgh. A very considerable portion of the work reported for

Sanctuary Lake and for lakes in Alaska has been done by Vera Dugdale; John Goering has likewise carried out a share of that reported for the Madison lakes.

REFERENCES

Allen, M. B. 1956. Photosynthetic nitrogen fixation by blue-green algae. Sci. Month., 83: 100-106.

Allen, M. B. 1959. Photosynthetic Nitrogen Fixation in the Marine Environment. International Oceanographic Preprints: 194.

Buljan, M. 1957. Report on the results obtained by a new method of fertilization experimented in the marine by "Mljetska Jezera", Acta Adriatica 6:6 1-54.

Burris, R. H., F. J. Eppling, H. B. Wahlin, and P. W. Wilson. 1943. Detection of nitrogen fixation with isotopic nitrogen. Jr. Biol. Chem., 148: 349-357.

Eyster, C. 1959. Mineral requirements of Nostoc muscorum for nitrogen fixation. Proc. IX Int. Bot. Congr., 2: 109.

Fogg, G. E. 1956. Nitrogen fixation by photosynthetic organisms. Ann. Rev. Plant Phys., 7: 51-70.

Fogg, G. E., and M. Wolfe. 1954. The nitrogen metabolism of the blue-green algae. Symp. Soc. Gen. Microbiol., 4: 99-125.

Hartman, R. T. and J. Herbert Graffius. 1960. Quantitative seasonal changes in the phytoplankton communities of Pymatuning reservoir. Ecology 44: 333-340.

Hutchinson, G. E. 1941. Limnological studies in Connecticut. IV. The mechanism of intermediary metabolism in stratified lakes. Ecology Mon., 11: 21-60.

Hutchinson, G. E. 1957. A Treatise on Limnology, Vol. I. New York, Wiley. 1015 pp.

Rittenberg, D., A. S. Keston, F. Rosebury, and R. Schoenhoomer. 1939. Studies in protein metabolism. II. The determination of nitrogen isotopes in organic compounds. Jr. Biol. Chem., 127: 291-299.

Rohlich, G. A., and W. L. Lea. 1949. The Origin of Plant Nutrients in Lake Mendota. Rept to Univ. of Wis. Lake Invest. Comm. (mimeo.) 7 pp.

Sawyer, C. N., J.B. Lackey, and R. T. Lenz. 1945. An Investigation of the Odor Nuisance Occurring in the Madison Lakes, Particularly Monona, Waubesa and Kegonsa, from July 1943 – July 1944. Rept. Gov. Comm., Madison, Wis. 92 pp., 25 fig., 27 tables.

Tryon, C. A. Jr., and D. F. Jackson. 1952. Summer plankton productivity of Pymatuning Lake, Pennsylvania. Ecol. 33: 342-350.

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Limitation of Nutrients as a Step in Ecological Control

THE MADISON LAKES BEFORE AND AFTER DIVERSION

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The Madison lakes are among the most intensively studied lakes in this part of the world. The early work of Birge and Juday is well known among limnologists. Domogalla (1926, 1935 and 1941) reported on studies of the lakes. Sawyer (1943 and 1944), acting as director of the Governor's Committee, published his investigations of the odor nuisances in the Madison lakes. Sawyer and Lackey (1945) reported on plankton productivity in Wisconsin lakes which included the Madison chain. Lawton and Bartsch (1949) reported on an investigation of Lake Mendota and its tributaries. Following 1947, twenty-five thesis studies were made by the Hydraulic and Sanitary Engineering Laboratories on the Madison lakes. Twelve of these theses (Darrow and Jackson, 1949; Haggerty, 1950; Levihn, 1951; and Teletzke, 1953) covered chemical and biological characteristics of the lakes and thirteen covered the hydrological characteristics. Flannery (1949) summarized the Madison lakes problem from a political science point of view in a Master of Science thesis. Other reports of investigations have appeared from time to time, including that of Bryson, et al. (1955). Presently a chemical and biological study of the lakes is in progress at the Sanitary Laboratory of the University of Wisconsin. Data for this discussion have been drawn from the above sources.

A map of the Madison lakes and their tributaries is shown in Figure 32. Pertinent physical characteristics of these lakes are given in Table 37.



Figure 32. MAP OF MADISON LAKES

The algae nuisance and odor problem has plagued Madison for many years as indicated by Professor Sarles earlier in this program. The odor nuisance over the years has been variously attributed to sewage, to industrial wastes, and to algae. The first really comprehensive study of the problem was made by the engineering firm of Alvord and Burdick (1920). They traced the foul odors to the decomposition of algae, and showed

Table 37. PHYSICAL CHARACTERISTICS OF THE MADISON LAKES*

Lake	Length, miles	Width, miles	Area, sq. miles	Max. depth, ft.	Mean depth, ft.	Volume, M.G.	Ave. drop between lakes, in.
Mendota	5.90	4.60	15.20	84.0	39.7	126, 385.35	36.0
Monona	4.16	2.40	5.44	74.0	27.6	31,409.95	3.4
Waubesa	4.20	1.40	3.18	36.6	16.1	10,634.58	18.0
Kegonsa	3.00	2.25	4.91	31.4	15.1	15,603.63	******
Wingra	1.00	0.37		14.1	8.9	1,497.52	*****

^{*} From Domogalla (1926)

that the nitrogen and phosphorus contained in domestic and industrial wastes were important contributing factors in the production of algal blooms.

A resume of the progress of waste treatment in Madison is given in Table 38.

The Governor's Committee, headed by Sawyer, sought to determine the quantity and quality of all fertilizing matter entering Lakes Monona, Waubesa and Kegonsa. A thorough two year study determined the sources of the nutrients, phosphorus and nitrogen, as shown in Figures 33 and 34. The data show clearly that the bulk of the nutrients in the lower lakes (Waubesa and Kegonsa) is derived from the liquid wastes of the Madison metropolitan area even though these wastes are subjected to complete treatment.

In 1943 the Lewis bill requiring effluent diversion was passed by the legislature and signed by the Governor. Following various legal battles the diversion sewers and channels were finally completed near the end of 1958 and diversion started in December of that year. The route of the diversion via Badfish Creek bypasses Lakes Waubesa and Kegonsa as shown in Figure 35 (Woodburn, 1959).

This route involves pumping the effluent over a divide 80 feet higher than the treatment works. The pumping is accomplished by means of four 24-inch single stage double volute centrifugal pumps, each with a rated capacity of 25,000 gpm at 110 feet of head. The present flow can be handled by one pump, but two will be necessary for peaks. The excess pumpage capacity was provided for emergency use and expansion. The effluent is pumped from two equalizing basins, each 130 feet in diameter, and discharged after chlorination through a 54-inch pipeline consisting of 5.1 miles of 100 psi reinforced concrete pipe. The effluent then flows by gravity through 3.8 miles of new open channel and into the Badfish Creek. To restore oxygen to the effluent two cascade aerators are provided in the new channel. One is located at the end of the pipeline, and the other 0.6 miles above the point of discharge into the creek. The creek channel was widened and deepened for 10.3 miles to the Dane-Rock County line. The remainder of the creek channel was considered to be adequate to carry the increased flow.

The average flow in the Yahara River, as computed from data contained in Sawyer's reports, is approximately 100 mgd. Domogalla's calculations of volumes for Lakes Waubesa and Kegonsa are

Table 38. WASTE TREATMENT AND DISPOSAL IN MADISON

Date	Development	Effluent disposal to
1886	Sanitary sewer construction started	Lake Monona Lake Mendota
1899-1902	Chemical treatment	Lake Monona
1902-1914	Septic tank and filter bed	Lake Monona
1914-1926	Burke plant-contact bed and trickling filters	Lake Monona
1926	First unit of Nine Springs plant-trickling filters	Lake Waubesa Lake Monona
1930	Metropolitan district formed - completely covered Lake Mendota and Lake Monona shoreline	
1936	Burke plant closed	Lake Waubesa
1942	U. S. Army leased Burke plant and reactivated it	Lake Waubesa. Lake Monona
1950	East Side interceptor placed in operation (all waste to Nine Springs plant)	Lake Waubesa
1952	Brewery ceased waste discharge to Lake Monona	
1958 (Dec.)	Diversion started	Yahara River (below Madison Lakes)

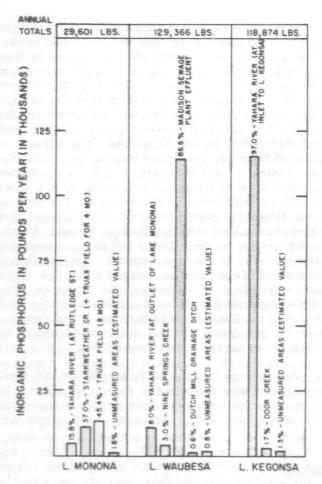


Figure 33. ANNUAL CONTRIBUTIONS OF INOR-GANIC PHOSPHORUS - 1943-1944. (From Sawyer and Lackey, 1944)

10,634 and 15,603 million gallons, respectively; thus the theoretical flow through times are about 3.5 and 5.2 months. Estimates of this type led many of the proponents of diversion to believe that the removal of the effluent from the lower lakes (Waubesa and Kegonsa) would quickly reduce the nitrogen and phosphorus to levels where algal blooms would occur only occasionally and of moderate intensity.

Sawyer's studies pointed out the fact that the unconsolidated bottom muds of these lakes release appreciable quantities of nitrogen and phosphorus to the overlying water. Reductions in the concentration of these elements in the lower lakes thus may be considerably less pronounced than anticipated.

In an attempt to evaluate the effects of diversion the present chemical and biological study was started in June, 1959. Data are being obtained at twelve stations located on Lakes Mendota, Monona, Wingra, Waubesa, and Kegonsa; on Badfish Creek just above the junction with the Yahara River; and on the Yahara River above and below the junction

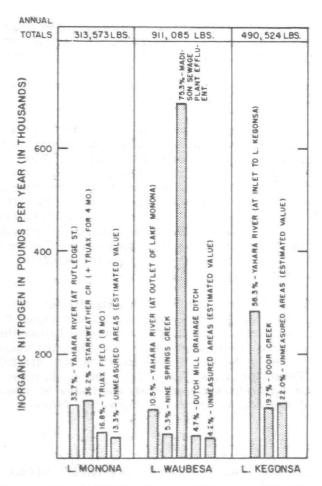


Figure 34. ANNUAL CONTRIBUTIONS OF INOR-GANIC NITROGEN - 1943-1944. (From Sawyer and Lackey, 1944)



Figure 35. NEW OUTFALL ROUTE via Badfish Creek bypasses Lakes Waubesa and Kegonsa. (From Woodburn, 1959)

with the Badfish Creek. Weekly samples are taken during the summer and bi-weekly samples during the winter. Determinations include temperature, pH, alkalinity, ammonia, nitrate and organic nitrogen, B.O.D., C.O.D., soluble phosphorus, volatile suspended solids, and estimates of amounts of predominant algal species.

Before attempting to draw conclusions from comparisons of data taken before and after diversion, it is important to consider other factors that enter into the production of algal blooms. Climatological factors that are of importance include variations in air temperature, water temperature, sunshine, rainfall, and velocity and direction of wind. Copper sulfate treatment of the Madison lakes, to control the growth of algae, was practiced with some regularity from 1925 to 1954. The effect of this treatment on the early data cannot be completely determined. A consideration of these factors emphasizes the fact that one must be extremely cautious when interpreting the available data.

CHEMICAL DATA

The data considered in this discussion include concentration of inorganic nitrogen, organic nitrogen, soluble phosphorus and volatile suspended each of the lakes are presented graphically for both the growing (June through October) and nongrowing (November through May) seasons. These values are based on samples taken at the outlet of each lake.

solids, all expressed in mg/l. Average values for

Summer average concentrations for Lake Mendota are shown in Figure 36. A considerable variation in concentration of inorganic and organic nitrogen over the years is apparent, but there appears no general trend. Soluble phosphorus is slowly but steadily increasing as might be expected with the increased use of land fertilizer.

Figure 37 presents average summer concentrations for Lake Monona. This lake received all of Madison's sewage treatment plant effluent until 1926 and smaller amounts until 1936. A decrease in both inorganic nitrogen and soluble phosphorus is noted following 1925. Burke plant effluent was again discharged to Monona between 1943 and 1950 and is reflected in the higher values of nitrogen and phosphorus in 1950, followed by decreased values in 1959. Organic nitrogen and volatile suspended solids content show no pronounced trends; however, they are appreciably higher than in Mendota, indicating a generally higher level of algae during the frowing season.

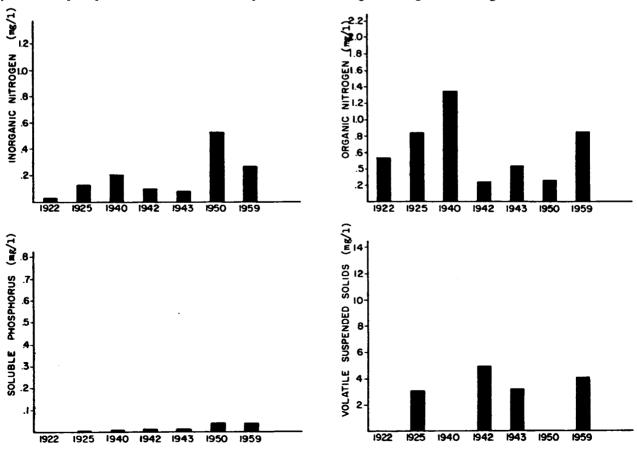
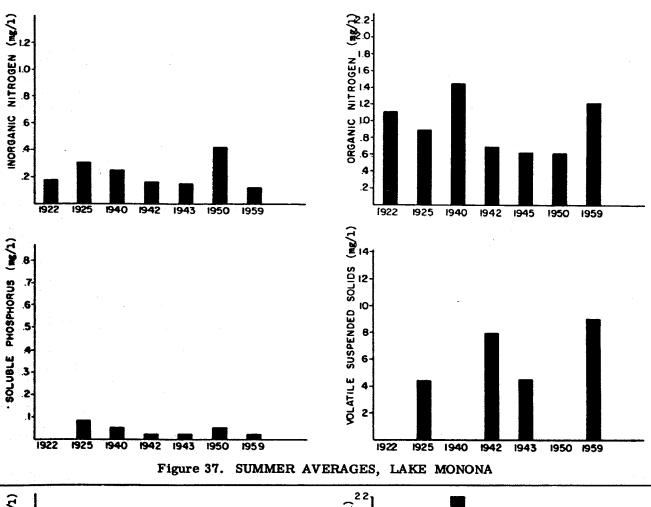


Figure 36. SUMMER AVERAGES, LAKE MENDOTA



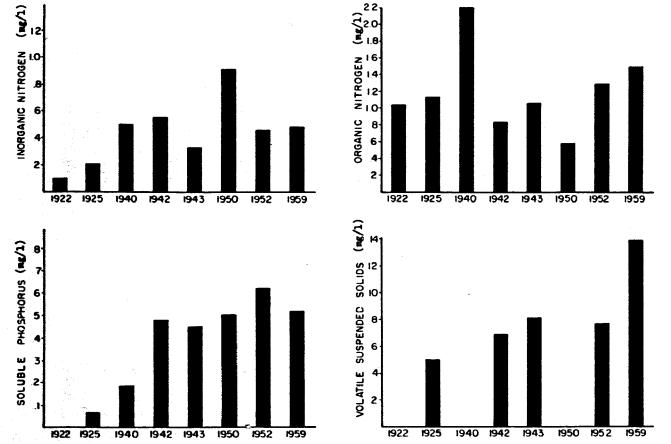


Figure 38. SUMMER AVERAGES, LAKE WAUBESA

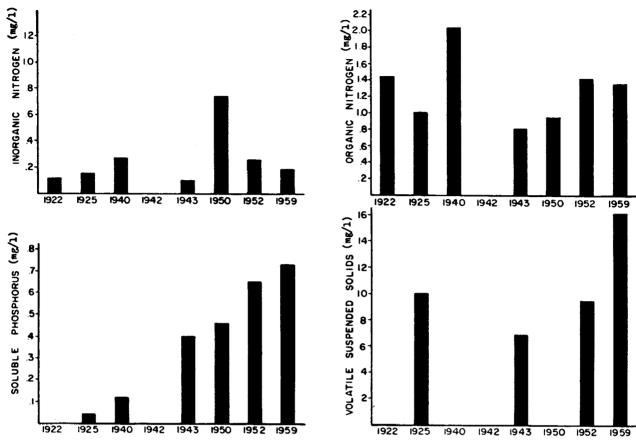


Figure 39. SUMMER AVERAGES, LAKE KEGONSA

Summer averages for Lake Waubesa, Figure 38, show no appreciable reduction in phosphorus or inorganic nitrogen following diversion. The increase in the concentration of both these elements following the opening of the Nine Springs plant in 1926 is very marked. The concentration of algae, as indicated by organic nitrogen and volatile suspended solids, was extremely high during the 1959 growing season.

Summer averages for Lake Kegonsa, Figure 39, indicate a slight reduction in inorganic nitrogen in 1959, but the phosphorus and volatile solids content are especially high.

In general, Lakes Waubesa and Kegonsa during the past growing season exhibited no clear cut reductions in nutrient materials. The concentration of algae in these lakes, as judged by organic nitrogen and volatile solids content, was generally higher than in most of the previous years of record. However, comments by residents living on the lower lakes indicated that they believed the conditions in the lakes were somewhat improved.

Winter average concentrations in Lake Mendota, Figure 40, show less fluctuation than the summer averages. The phosphorus content shows a gradual decrease, in contrast to the gradual in-

crease in summer values.

Winter averages for Lake Monona, Figure 41, show high phosphorus and inorganic nitrogen values for 1948 and 1949, reflecting the discharge of Burke plant effluent between 1943 and 1950. The organic nitrogen content exhibits little change over the years of study.

Figure 42 shows winter average concentrations for Lake Waubesa. The inorganic nitrogen and soluble phosphorus content for 1959 are sharply decreased, presumably due to diversion. The organic nitrogen content is also somewhat lower than normal. It appears from this figure that the removal of the effluent is beginning to affect the concentration of nutrient materials in Lake Waubesa.

Winter average concentrations in Lake Kegonsa, Figure 43, indicate no pronounced trends in any of the constituents considered. The slight reductions noted during the past winter are not comparable to those exhibited in Lake Waubesa. The calculated average retention times in Lakes Waubesa and Kegonsa are 3.5 and 5.2 months, respectively. On a straight displacement basis Lake Kegonsa would be free of effluent by now. Short circuiting and incomplete mixing are normal under the con-

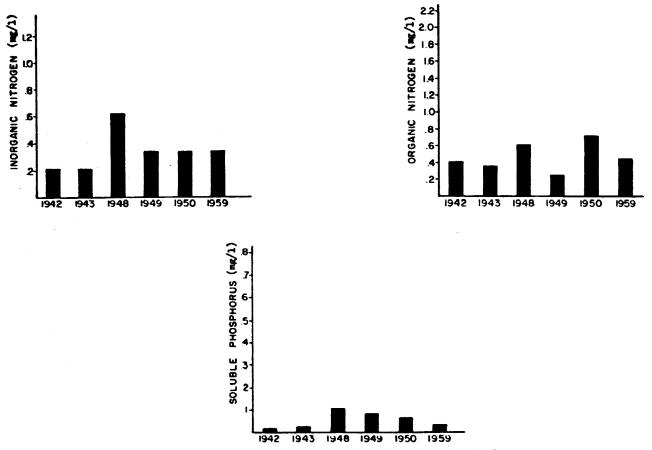


Figure 40. WINTER AVERAGES, LAKE MENDOTA

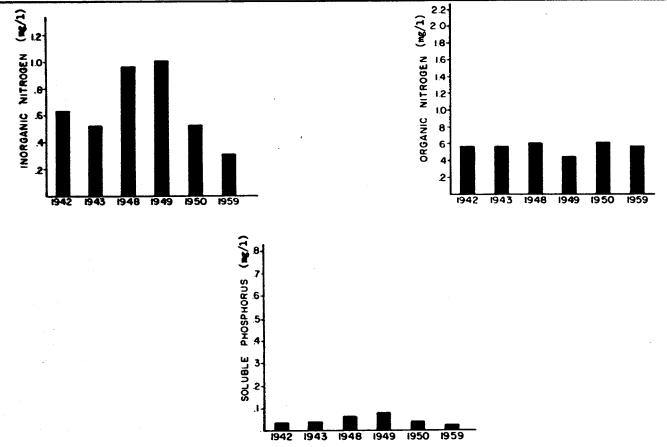
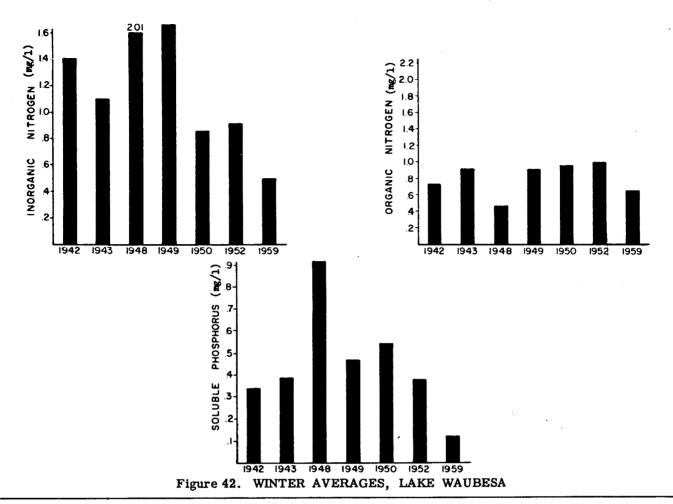
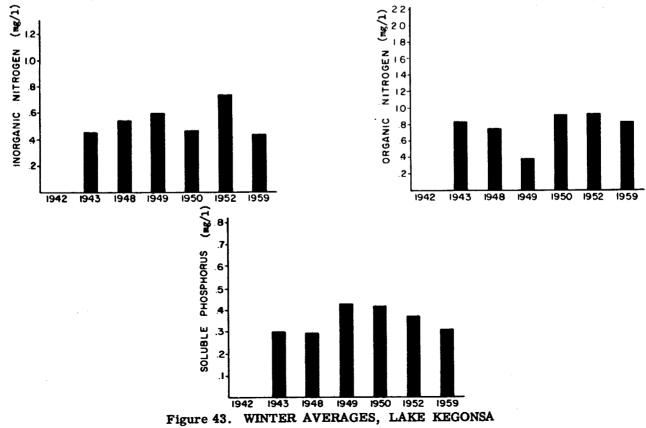


Figure 41. WINTER AVERAGES, LAKE MONONA





ditions found in these lakes; hence it is likely that a considerably longer period will be required to thoroughly flush them out.

PLANKTON DATA

Over the years of study of these lakes, the concentration of algae has been expressed in various terms-cell counts, volumetric standard units, mg/l, and per cent of various species. methods of concentrating the algae have included the following: filtration through plankton nets, filter paper, sand, and membrane filters; centrifuging; settling; and flotation. Samples have been taken at various depths with various types of sampling equipment during the many investigations. Methods of preserving the samples until examined also have varied widely. Any correlation of the plankton data obtained by these various methods was considered to be of such a low order as to be of little value. Consequently, only the plankton data obtained during the growing seasons of 1955, 1957, and 1959 are considered in this discussion. All samples during these years were examined by Dr. George Fitzgerald and are thus directly comparable.

In 1955 and 1957, the net plankton from 5 to 10 quarts of sample were further concentrated and preserved with the aid of formaldehyde and wetting agent. In 1959, the total plankton were concentrated 20-fold using Rohde's modification of Lugol's iodine-wetting agent method of concentration and preservation. The examination of the plankton consisted of identification of the predominant species and an estimation of the relative amounts of each species.

Table 39 summarizes the plankton data. During the period before diversion (1955 and 1957) there were many species of algae in Lakes Monona

Table 39. ALGAL SPECIES IN MADISON LAKES

	19	986 (T/23 to 10/5)
Lake	% Microcyetie	Other species in appreciable amounts
Mosses	1 - 75	Anabassa, Aphanisomenos, Oscillatoria and many others
Vanben	Over 96	Mone
Kegones	1 - 15	Melosira, Coelosphaerium, and many others
	10	157 (T/8 to 9/4)
Mosona	20 - 30	Anabassa, Oscillatoria, Melosira, and many others
Wantees	Over 90	None
Kegones	25 - 50	Melosira, Aphanisomenoa and many others
	- 11	99 (C/25 to 10/1)
Мочона	10 - 50	Anabasna, Melosira, Oscillatoria, Ceratium and many others
Wandoon	25 - 10	Melostra, Oscillatoria, Ceratium and others
Kegonan,	20 - 90	Melostra, Aplantmomenon, Oscillatoria and many others

and Kegonsa, with the dominant species changing from sample to sample. During the same period the algae of Lake Waubesa always consisted of 99 per cent or more Microcystis.

Samples taken after diversion indicated that again there were many species of algae in Lakes Monona and Kegonsa, but the importance of Microcystis in the plankton of Lake Waubesa decreased sharply to a point where it made up only 25 to 90 per cent of the total. Other algae of significance in Lake Waubesa during 1959 were Melosira, Oscillatoria, and Ceratium, plus others in lesser amounts.

It appears that, in general, the number of species and changes in dominant species in Lakes Monona and Kegonsa have not changed during the sampling periods, whereas the number of species in Lake Waubesa has been markedly increased since diversion.

SUMMARY

- 1. Variations in the amount of sewage treatment plant effluent added to Lake Monona over the years are reflected in the levels of nutrient concentration in that lake.
- 2. Diversion has completely stopped the addition of sewage treatment plant effluent to Lakes Waubesa and Kegonsa.
- 3. The first growing season following diversion showed no appreciable trend in the nutrient content of Lakes Waubesa and Kegonsa.
- 4. The first full winter season following diversion showed appreciable reductions in both the inorganic nitrogen and soluble phosphorus content of Lake Waubesa. Lake Kegonsa exhibited little change from previous years.
- 5. The number of predominant species of algae in Lake Waubesa has sharply increased since diversion. The number of predominant species in Lakes Monona and Kegonsa has shown no appreciable change during the same period.
- 6. It is realized that, due to the many factors affecting the growth of algae, one cannot arbitrarily conclude that the observed trends and changes are entirely due to diversion.
- 7. Data obtained over a longer time period will be necessary before the full impact of diversion can be determined.

REFERENCES

Alvord and Burdick Engineers. 1920. Report upon the cause of offensive odors from Lake Monona.

Bryson, R.A., G. P. Fitzgerald, H.M. Kaneshige, and G.A. Rohlich. 1955. The Madison Lakes Problem, Part I. Report on investigation of a project sponsored by Oscar Mayer & Co.

Darrow, R.A., and H.H. Jackson. 1949. A sanitary survey of the Yahara River. B. S. Thesis in Civil Engineering, University of Wisconsin.

Domogalla, B. 1926. Treatment of algae and weeds in lakes at Madison, Wis. Engineering New-Record 97: 950-54.

Domogalla, B. 1935. Eleven years of chemical treatment of the Madison lakes: Its effect on fish and fish foods. Transactions of the American Fisheries Society 65: 115.

Domogalla, B. 1941. Scientific studies and chemical treatment of the Madison lakes. A Symposium of Hydrobiology, University of Wisconsin Press: 303-09.

Flannery, J.J. 1949. The Madison lakes problem. M. A. Thesis in Political Science, University of Wisconsin.

Haggerty, J.R. 1950. A sanitary survey of the Yahara River. M.S. Thesis in Civil Engineering, University of Wisconsin.

Lawton, G.W. and A.F. Bartsch. 1949. Report on Lake Mendota studies. Wisconsin Committee on Water Pollution.

Levihn, Paul. 1951. A sanitary survey of the Yahara River and bottom muds of Lake Mendota. M.S. Thesis in Civil Engineering, University of Wisconsin.

Sawyer, C.N., and J.B. Lackey. 1943. Investigation of the odor nuisance occurring in the Madison lakes particularly Monona, Waubesa, Kegonsa from July, 1942 to July, 1943. Report of the Governor's Committee.

Sawyer, C. N., and J. B. Lackey. 1944. Investigation of the odor nuisance occurring in the Madison lakes particularly Monona, Waubesa, Kegonsa from July, 1943 to July 1944. Report of the Governor's Committee.

Sawyer, C.N., and J.B. Lackey. 1945. Plankton productivity of certain southern Wisconsin lakes as related to fertilization. Sewage Works Journal 17: 573-85.

Teletzke, G.H. 1953. A sanitary survey of the Yahara River and Badfish Creek. M.S. Thesis in Civil Engineering, University of Wisconsin.

Woodburn, J.G. 1959. Outfall around the Madison lakes. Water and Sewage Works 106: 497-500.

THE BADFISH RIVER BEFORE AND AFTER DIVERSION OF SEWAGE PLANT EFFLUENT

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INTRODUCTION

After Supreme Court decisions validated orders of the Committee on Water Pollution issued pursuant to a statute prohibiting discharge of effluent from treatment plants serving municipalities of specified size to lakes of specified area, it became necessary for the Madison Metropolitan Sewerage District to provide for diversion of its effluent in accordance with statutory provisions.

The Madison Metropolitan Sewerage District operates a complete treatment plant serving a population of about 135,000. The flow through the plant normally averages about 20 million gallons per day. Primary treatment consists of screening, grit collection, and sedimentation. About one-fourth of the sewage receives secondary treatment in a trickling filter, and the remainder receives secondary treatment in an activated sludge process. The combined final effluent from the secondary processes is chlorinated before discharge.

Engineers were retained to make preliminary studies of possible diversion routes and to make estimates of costs for diversion of effluent from the Madison lakes. Fourteen separate routes were considered and it was concluded that a route utilizing Badfish River was the most practicable for several reasons, including the probable use of the effluent for irrigation of lands adjoining a proposed ditch leading to the river.

Badfish River was a small meandering stream which flows through typical agricultrual lands in Dane and Rock Counties. Many years ago, the Dane County portion was straightened and widened to serve as a drainage ditch to lower the water table under adjoining lands. From the Dane-Rock County Line to its junction with the Yahara River, however, it has a substantially larger natural flow and a correspondingly greater channel capacity. Badfish River has an average slope of six feet per mile and portions of the stream have been considered as marginal trout water. Effluent from the trickling filter treatment plant of the Village of Oregon, Wisconsin, amounts to 65,000 gallons per day and is discharged to Badfish River near its headwaters.

The route chosen for the diversion necessitated construction of five miles of 54-inch pipeline, straightening and reconstruction of about four miles of the drainage ditch, and straightening and improvement of 10 miles of Badfish River to the Rock County line. The improved ditch is 16 feet wide and the improved river channel is 16 to 20 feet wide. From the Dane-Rock County line to its junction with the Yahara River, the natural channel has adequate capacity for handling the added effluent flow without serious change in level, and no reconstruction was necessary. The Yahara River, after six miles, discharges into the Rock River.

To restore dissolved oxygen to the effluent after its passage through the pipeline, two cascade aerators are provided in the new channel. One, located at the discharge end of the pipe, is 35.5 feet wide and consists of a seven-step cascade with a total fall of 6.5 feet; the second is 3,240 feet above the point of discharge into Badfish River and is 30 feet wide with a five-step cascade having a total fall of 5.5 feet. The new ditch channel has a slope of 2.6 feet per mile while the improved river channel has an average grade of 4.75 feet per mile for the first four miles and an average grade of 8.05 feet per mile for the remaining six miles.

ORGANIZATION OF STUDY

The Madison Metropolitan Sewerage Commission and the Wisconsin Committee on Water Pollution concluded that here an opportunity would be afforded for study of the effect of sewage plant effluent on a stream and proposed a joint study to determine conditions before and after diversion of effluent. Surveys were conducted at one-week intervals during 1956 to determine stream characteristics before diversion; and after diversion of effluent to the Badfish River in December, 1958, the surveys were repeated during 1959 at the identical stations. Arrangements were also made by the Madison Metropolitan Sewerage District for installation of a U.S. Geological Survey stream gauging station on Badfish Creek at a point 9.2 miles above its confluence with the Yahara River,

this point being about nine miles down from the point of discharge of the 54-inch pipeline. Thus, a continuous record of stream flow at this point on the river is available.

The data reviewed in this report are based only on the bi-weekly samples collected by the representatives of the Committee on Water Pollution and analyzed by them and by the State Laboratory of Hygiene and represent the results of determinations for a 26-sample period prior to diversion, and a similar period subsequent to diversion.

Badfish River is approximately 16-1/2 miles long, and, for the purpose of this study, three sampling stations were selected. Station 1 is approximately one mile below the confluence of the effluent ditch with the river; Station 4 is approximately four miles downstream from Station 1 and in the immediate vicinity of the gauging station; Station 8 is the farthest downstream station on Badfish River and is eight miles below Station 4 (Figure 44).

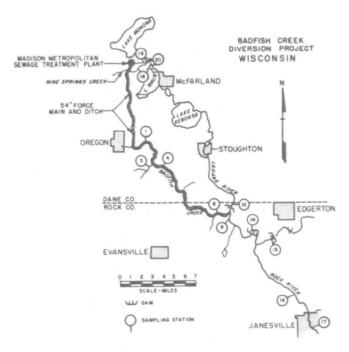


Figure 44. BADFISH CREEK DIVERSION PROJECT, WISCONSIN.

Three stations were chosen on the Yahara River, one above the confluence with Badfish Creek (Station 10) and two below this confluence (Stations 9 and 14). The distance of the Yahara River which is now affected by the effluent is 6.4 miles. On the Rock River, Station 15 is approximately two miles above the confluence of the Yahara and Rock Rivers, and Stations 16 and 17 are located six and ten miles, respectively, from this confluence.

Chemical determinations were made in accordance with procedures detailed in the Tenth Edition of Standard Methods for the Examination of Water, Sewage, and Industrial Wastes. Enumeration of phytoplankton was carried out according to the procedures outlined by Prescott.

The important chemical data and the phytoplankton were analyzed statistically (Table 40). The mean and the 95% confidence interval were calculated for 26 paired dates prior and subsequent to diversion for nitrogen, phosphorus, 5-day B. O. D. and phytoplankton. The coefficient of variation on these data normally do not appear excessive, especially when the climatic and seasonal influence is taken into consideration.

RESULTS OF STUDY

Physical Observations

As the effluent leaves the 54-inch pipeline, it passes over a seven-step reaeration cascade and enters a rather straight ditch with steep banks. The first half mile of this ditch often carries a blanket of detergent foam. For the next mile, the banks are less steep, and within a year after diversion was commenced, there was evidence of some vegetation encroachment, principally roundstemmed bulrush. Badfish River was dredged to a bottom width of 16 feet for about four miles and a width of 20 feet for six miles. This is a great change from the original meandering stream bed. Along with the changes created by physical disturbance, there is a change in flow produced by the introduction of slightly over 20 million gallons per day of effluent. Prior to diversion, Badfish River, at about its midpoint (Station 4) between its source and confluence with the Yahara River, had an average flow of 9.6 c.f.s. for the 2-1/2 years in which records were kept. Following the diversion, the flow averaged 43 c.f.s. for the summer portion of the study with 32.8 c.f.s. being contributed as effluent from the Madison Metropolitan Sewerage District plant. In the unimproved stretch of Badfish River, there was little gross physical change noted.

Badfish River originally contained many riffle areas with a bottom composed principally of small rock and gravel. The bottom was, of course, altered in the improved section, but remains a coarse gravel over much of the area.

The effluent normally contains about 25 p.p.m. of suspended solids and a substantial amount of these solids has settled to build a sludge deposit over most of the upstream portion of Badfish River. In some areas, especially in small pockets along the side of the stream, this deposit approaches six to ten inches in depth. In most of the upstream region, as well as the ditch itself, the sludge is of sufficient thickness to provide a suitable habitat for a bountiful population of midge larvae.

Chemical and Bacterial Determinations

The organic nitrogen (Table 40) shows a sizeable increase at all stations in Badfish River following diversion. At Station 4, where stream flow information was available, calculations show a mean of 30 pounds per day of organic nitrogen prior to diversion, and a mean of 286 pounds per day following diversion.

In the Yahara River, there appears to be no statistical difference between the samples collected before or after diversion. There is an indication, however, as shown at Station 10 for the 1959 samples, that the organic nitrogen is less in the Yahara River above the entrance of Badfish River than it is below this confluence. The lower value at this point can be attributed to diversion. as the effluent formerly discharged through Lakes Waubesa and Kegonsa and down the Yahara River. There was no significant change in the organic nitrogen concentrations in the Rock River. Calculations show that the effluent contributed 732 pounds per day of organic nitrogen to the stream, and that this was reduced to 286 pounds per day in the Badfish by the time the flow reached Station 4. The reduction was most likely due to settling of the suspended solids to create sludge deposits in the upstream region.

The inorganic nitrogen, as shown in Table 40, includes the total of ammonia, nitrite and nitrate nitrogen. The 1959 samples indicate a 5-fold increase in concentration. Again at Station 4, determinations showed a mean of 110 pounds per day before diversion and 3,153 pounds per day after diversion, a more than 30-fold increase. The effluent contributed 3,192 pounds per day or inorganic nitrogen during the test period. Most of the increase in the stream is attributed to the ammonia nitrogen contributed in the effluent although there was a slight increase in nitrite and nitrate nitrogen. The inorganic nitrogen persists in solution in the Badfish River, and the effect of its presence is demonstrated in the Yahara River and in the Rock River by the increase in concentration in these rivers after diversion.

Soluble phosphorus is characteristically high in sewage effluent as compared to natural drainage. Following diversion, Badfish River displayed a great increase in the soluble phosphorus content. Consideration of soluble phosphorus passing Station 4 reveals 9 pounds per day in 1956 and 1,351 pounds per day in 1959. The effluent discharged to Badfish River contributed 1,458 pounds of soluble phosphorus per day. The Yahara River samples were all quite high in 1956 reflecting the discharge from Lakes Waubesa and Kegonsa which received the effluent that year. In 1956, there was no significant difference between the determinations at any of the three stations on the Yahara River. In 1959, after diversion, stations

below the confluence of the Badfish River showed a 100% increase in soluble phosphorus as compared to the station on the Yahara River above the confluence. The Rock River samples indicated a similar differential between the station above the confluence with the Yahara and the stations below.

High B. O. D. (biochemical oxygen demand) values and low D. O. (dissolved oxygen) values were encountered in Badfish River during the post-diversion survey. Considering the summer period (June 1 to October 1), there were 75 pounds of B.O.D. per day and 475 pounds of D.O. per day prior to diversion at Station 4. After diversion for this same period in 1959, the water at Station 4 was carrying 1,602 pounds per day of B.O.D. and 900 pounds of D.O. per day, definitely a D. O. deficit. The effluent during this period contributed 3,724 pounds of B. O. D. per day. It should be noted that in the four-mile stretch between Station 1 and 4, a substantial amount of the contributed B.O.D. was removed. The Yahara and Rock Rivers did not appear to be appreciably affected by this B.O.D.-D.O. relationship.

The most probable number of coliform organisms per 100 ml. was quite variable throughout the course of the surveys (Table 40). The effluent from the Oregon sewage treatment plant was not chlorinated and did have an effect on Badfish River prior to diversion, with readings of above normal concentrations of coliform organisms. Following diversion, the MPN determinations for Badfish River were higher than those recorded for 1956. The influence of the effluent was also noticeable in the Yahara River. On the Rock River, MPN determinations were higher at all stations than the determinations made in 1956 and can be attributed to factors other than diversion.

Biological Determinations

The phytoplankton volume throughout the course of the study displayed considerable variability as would be expected (Table 40). However, the mean volume, although showing an increase for the larger rivers, showed no statistical difference either between the three stations on a given river or between the two periods of study for the same station. It thus appears that a sizeable increase in nutrients in a flow water situation has no substantial effect upon a volumetric production of phytoplankton.

The phytoplankton volume of Badfish River was generally lower than that of the Yahara River or Rock River. Although there was little change in phytoplankton volume evidenced as a result of diversion, the change was most pronounced at Station 1 on Badfish River. There was an indication of a volumetric reduction following diversion which suggested inhibited growth. The blooms of Euglena which were present before diversion at

Table 40. SUMMARY OF BIOLOGICAL AND CHEMICAL DATA BEFORE AND AFTER DIVERSION ON BADFISH CREEK, YAHARA RIVER, AND ROCK RIVER - BASED UPON 26 BI-WEEKLY PAIRED DATES E

			nge	±95% Confid	lean lence Interval	Stan Devi	ation	Coeff of Variat	ion (%)		Pounds Per	M	ean
Phytoplankton ²	<u>Sta.</u> 1 4	1956 0.12-40.93 1.09-15.43	1959 0.37-10.16 0.55-12.45	3.98±2.58		1956 10.03 3.20	1959 2.28 2.70	1956 153 80	1959 83 83	1956 56-791	1959 247-1,435	1956 259	1959 622
	10 9	1.35-16.74 0.04-37.43 0.33-24.38	0.22-13.99 0.11-56.87 0.39-30.71	7.59±2.55	12.70±6.34 9.09±3.10	3.85 10.17 6.73	4.06 14.35 7.51	60 95 83	83 112 82				
	14 15 16	0.16-37.38 0.58-62.19 0.55-56.51	0.15-52.53 0.53-57.55 1.03-62.20	25.59±8.59	11.63±5.49 18.37±6.15 21.36±6.96	9.41 20.38 17.15	13.32 14.91 16.89	98 82 67	114 81 79				
Organic N. ²	17 1 4	1.06-62.24 0.33-2.20 0.27-2.00	0.23-53.67 1.33-11.74 0.93-9.50	24.64±7.61 0.73±16 0.61±.17	19.50±6.56 4.13±1.14 3.22±.89	0.40 0.41	15.93 2.69 2.17	73 55 67	81 65 67	13-71	206-447	30	286
	8 10 9	0.21-2.30 0.60-4.34 0.64-2.32	1.03-7.10 0.00-2.44 0.94-3.34	0.54±.18 1.61±.41 1.30±.19	2.59±.66 1.29±.25 1.49±.23	0.42 0.96 0.48	1.59 0.63 0.56	77 60 37	61 49 38				
	14 15	0.60 -2. 70 0.90 -3. 71	0.60-2.65 0.94-3.54	1.54±.27 1.99±.25	1.58±.23 1.77±.27	0.66 0.60 0.71	0.54 0.65	43 30	34 37 39				
Inorganic N. ²	16 17	0.88-3.51 0.94-3.31 1.98-5.53	0.74-3.34 0.84-3.24 13.4-21.1	1.86±.29 1.86±.25 3.73±.35	1.78±.29 2.14±.56 17.98±2.07	0.60	0.69 1.42 2.03	38 32 23	66 11				
	4 8 10	1.73-3.64 1.38-4.49 0.09-1.24	10.0-18.7 7.2-17.6 0.09-2.82	2.65±.08 2.34±.31 0.47±.14	15.17a.91 11.42a.97	0.21 0.77	2.20 2.35	33 72	15 21	89-143	2,171-4,246	110	3,153
	9 14	0.10 -2.18 0.10-1.51	1.09±11.31 0.80-4.64	0.66±.21 0.55±.16	0.62±.25 3.53±1.11 2.00±.37	0.34 0.52 0.40	0.61 2.73 0.93	72 79 73	98 77 46				
	15 16 17	0.09-0.68 0.09-0.91 0.05-1.13	0.10-3.32 0.15-3.10 0.19-3.09	0.27±.07 0.29±.08 0.30±.10	1.34±.47 1.42±.41 1.43±.39	0.17 0.21 0.26	1.18 1.01 0.97	63 72 86	88 71 68				
Soluble P. ²	1 4 8	0.30-1.56 0.10-0.30 0.01-0.12	5.5-12.0 4.4-7.3 3.0-8.4	1.07±.12 0.19±.02 0.08±.01	8.22±.64 5.96±.37 5.22±.64	0. 33 0.05 0.0 3	1.51 0.90 1.56	30 25 37	18 15 30	7-12	996-1,701	9	1,351
	10 9 14	0.46-1.30 0.16-1.30 0.40-1.28	0.23-1.5 0.56-6.4 0.58-2.6	0.94±.10 0.83±.12 0.81±.10	0.88±.12 1.86±.52 1.22±.49	0.26 0.33 0.28	0.33 1.28 0.41	28 40 35	38 69 34				
	15 16 17	0.01-0.19 0.01-0.41 0.02-0.44	0.01-0.68 0.15-0.94 0.13-1.40	0.05±.02 0.18±.04 0.17±.04	0.22±.01 0.46±0 0.48±.02	0.05 0.11 0.10	0.02 0.01 0.03	95 61 63	8 3 6				
B.O.D. ²	1 4 8	1.8-7.7 0.8-5.4 0.6-8.8	4.1-39.4 3.1-55.8 3.3-38.4	2.11±2.30	21.01±8.13 17.25±5.59 13.99±4.44	1.46 5.29 1.56	18.80 13.56 10.75	40 250 74	89 79 77	39-113	755-2,333	75	1,602
	10 9 14	1.3-14.5 1.8-15.7 1.5-14.3	1.5-7.70 2.5-19.7 2.4-15.4	6.14±1.75 4.90±1.24 5.90±1.50	5.86±1.44	4.23 3.08 3.70	2.17 3.55 2.79	69 63 63	56 61 55				
	15 16 17	3.5-17.1 2.7-14.3 3.0-15.4	2.2-15.3 2.1-12.9 2.4-10.2	7.18±1.26	5.23±1.17 5.46±1.19 4.95±.89	3.46 3.09 3.51	2.91 2.94 2.21	45 43 50	56 52 45				
D.O. ²	1 4 8	3.1-13.4 7.8-15.9 6.6-16.7	0.1-8.9 1.7-10.7 2.2-11.1							368-636	413-1,749	475	904
	10 9 14	4.2-21.6 8.9-19.3 5.6-15.4	5.9-17.5 3.9-16.7 2.9-15.7										
	15 16 17	4.4-20.9 6.6-18.3 5.3-18.1	3.0-25.8 6.4-22.0 6.1-20.5										
рH	1 4 8	7.5-8.2 7.7-8.6 7.7-8.8	7.4-8.1 7.5-8.2 7.7-8.1		Flow in c.	f.s. in 19	56 ranged	on 9 bi-we l from 8.0- a mean of	-10.0 wit	ed dates (Jur h a mean of t	ne 1 – October 1 1.7; in 1959 the	l) for St flow in	ation 4. c.f.s.
	10 9 14	8.0-9.9 8.2-9.8 8.1-9.6	7.7-9.2 7.0-8.9 7.7-8.9		2. Parts per								
	15 16	8.3-9.2 7.8-9.7	7.7-9.1 7.6-9.3										
M.P.N. (x 10 ³)	17 1 4 8	7.9- 9 .4 7-540 0.5-160 0.4-240	7.8-9.2 3.3-790 4.9-350 0.8-1,200										
	8	U. 9-44U	0.8-1,200		*								

15 16 17

0.2-35 0.2-17 0.2-54

0.5-210 0.3-170 0.3-160

Station 1 did not appear in the 1959 samples. Oscillatoria sp. did appear in the samples following diversion and quite possibly came from the rather extensive growth of this genus over the bottom deposits. The principal diatoms occurring in the 1956 samples consisted of Navicula, Nitzschia, Gomphonema, and Synedra. In the 1959 samples, populations were dominated by species of Navicula and Nitzschia with other genera appearing only occasionally and in very small numbers. On the occasions when green algae appeared, these consisted of Chlamydomonas and Closterium, both in the 1956 and 1959 samples. In general, the 1959 samples, especially at Station 1, appeared more heterogeneous to class and more homogeneous to genera than those collected in 1956.

The tendency toward inhibited growths was apparent although much reduced at Station 4, following diversion. In 1956, the greatest diatom volume appeared in late summer and consisted principally of Navicula with several other genera represented in varying numbers. The 1959 samples did not reveal as great a volume nor as great a variety of species, but did indicate a more equal representation between the diatoms, blue-green algae, and green algae in the phytoplankton.

At Station 8, the principal constituents of the diatom population prior to diversion were Navicula and Nitzschia with Synedra, Cyclotella, Gomphonema, and Cocconeis contributing to the total volume regularly. In 1959, Navicula and Nitzschia were the principal constituents of the diatom population, with the other genera appearing only occasionally and contributing less to the total volume. Green algae and blue-green algae appeared occasionally in the 1959 samples and not in the 1956 samples, although the total plankton volume was rarely affected by these occurrences. The Euglena group appeared more often in the 1959 samples, but they, too, seldom affected the total phytoplankton volume.

The phytoplankton volumes on the Yahara River stations reveal little change subsequent to diversion. The diatom population at Station 10 above the confluence with Badfish River prior to diversion was dominated by Melosira with species of Navicula, Nitzschia and Cyclotella appearing regularly but in lesser numbers. After diversion, the same genera of diatoms were encountered, but the volume became more equally proportioned among those present and no particular genus predominated. Blue-green algae appeared in both the 1956 and 1959 samples with Anacystis and Aphani-The most commonly zomenon predominating. recorded genera of green algae were Scenedesmus and Ankistrodesmus, Chlamydomonas, and Coelastrum in both the 1956 and 1959 samples. On only two occasions in the spring and early summer of 1959 did the green algae volume exceed the diatom volume.

Stations on the Yahara River below its confluence with Badfish River generally revealed a greater proportionate volume of green algae following diversion. Blue-green algae at these stations were noted only occasionally and contributed little to the total volume. The diatom population, especially in the summer months, appeared similar both before and after diversion. In mid-winter of 1959, a bloom of Cyclotella approached a population of 7,000 organisms per ml. and extended over a period of six weeks. The species were very small and contributed little to the total volume.

The phytoplankton in the Rock River revealed no detectable difference between stations in a given year. The prominent genera in both 1956 and 1959 were <u>Stephanodiscus</u>, <u>Melosira</u>, and <u>Cyclotella</u>. <u>Navicula</u> and <u>Nitzschia</u> appeared consistently scattered but rarely exceeded one p.p.m. in volume. <u>Cyclotella</u> was a major constituent of the population during the entire year. During December, 1958 and January, 1959, it was the principal genus found, and populations at this time approached 30,000 organisms per ml.

All stations on the Rock River revealed a substantial volume of blue-green algae during all except the winter months. This consisted principally of Anacystis and Aphanizomenon. Green algae appeared more prominent in the 1959 samples, particularly in the spring and summer months. Volumes of green algae exceeded 10 p.p.m. only rarely. The principal constituents were Closterium and Coelastrum in 1956 and Coelastrum in 1959. Scenedesmus appeared regularly but the volume seldom exceeded 2 p.p.m. in any particular sample.

The organisms which dwell upon and within the bottom deposits were studied at seven separate stations on four different dates in Badfish River. Pre-diversion surveys were conducted on August 1. 1956 and March 1, 1957, whereas post-diversion surveys were conducted on September 17, 1959 and December 1, 1959. Prior to diversion at Station 1, the stream was 3 to 6 feet wide and approximately 6 inches deep. It gradually increased in width downstream until a width of around 30 feet was attained before the confluence with the Yahara River. The depth at this point, however, was still relatively shallow, varying between 6 and 18 inches. The bottom material at the sampling stations consisted principally of rock and coarse gravel and, at some points, gravel mixed with sand. Submerged aquatic vegetation was abundant prior to diversion and, at some points, streamers

of filamentous algae were attached to the submerged vegetation. In September, 1959, following diversion, the improved portion of Badfish River still maintained a coarse gravel bottom and, in the Station 1 area, the stream was already choked with submerged vegetation. In the downstream areas, this vegetation appeared to be less dense than in 1956. Long streamers of filamentous green algae (Stigeoclonium and Rhizoclonium), some of which were estimated to be 50 feet in length, were attached to bottom materials at numerous locations. In the upper areas of the stream, there was a green blanket of Oscillatoria covering the bottom. Sludge had deposited along the edge of the stream and covered portions of the vegetation. A definite sewage odor was present in the Station 1 area in September, and this odor extended the full length of Badfish River in December, 1959. Much of the stream bottom was covered with a slimy mat of the blue-green algae, Oscillatoria, and, especially in the December survey, much of the vegetation was covered with a prolific growth of a stalked protozoa belonging to the family, Epistylidae. These formed a gray mass not unlike a dense growth of fungus.

The degradation of the stream following diversion is apparent when one examines the community of biological life living upon and within the bottom materials. Prior to diversion, between 10 and 14 different invertebrate species were recovered from each of the samples collected. Following diversion, the number of species was reduced to about five.

Prior to diversion, also, a balanced community of intolerant and tolerant organisms was observed. At nearly every station, caddis fly larvae (Cheumatopsyche and Hydropsche), mayfly nymphs (Baetis and Caenis), and riffle beetle larvae were found in association with cranefly larvae, horsefly larvae, scuds, and miscellaneous midges. Very tolerant forms such as sludge worms (Tubificidae) were also found, but occurred in very low numbers. In some locations, the intolerant caddis fly larvae formed the bulk of the total population.

Following diversion, all stations in the ditch and in the improved portion of Badfish River supported a bottom-dwelling population comprised of sludge worms (Tubificidae) and at least three species of very tolerant midge larvae (Tendipes plumosus, T. tendipediformis, and T. decorus). These are all considered to be very tolerant organisms and were found to be living in the sludge deposits on the bottom and along the sides of the stream. Near the lower end of Badfish River in the unimproved portion, tolerant and very tolerant bottom-dwelling organisms predominated. Occasionally, an intolerant form was observed, but this was only one among many of the more tolerant forms.

SUMMARY

- 1. Studies have been conducted on the biological and chemical effects resulting from the diversion of approximately 20 million gallons a day of effluent from the Madison, Wisconsin, Metropolitan Sewerage Commission Treatment Plant to a small stream which originally had a flow of 9.6 cubic feet per second. This stream, Badfish River, discharges into the Yahara River, and the Yahara River into the Rock River. The effects upon all three river systems were investigated.
- 2. In addition to physical and biological observations, and bottom fauna studies made at intervals, 26 bi-weekly samples were collected and analyzed from selected stations before and after diversion for chemical and phytoplankton determinations.
- 3. Considering that 10 of the 14.5 miles of stream were improved to a bottom width of 16 and 20 feet, that the flow was increased nearly five-fold, and that a deposition of solid materials created substantial sludge deposits in some areas, a tremendous physical change, especially in the upper regions, was exerted upon Badfish River as a result of diversion.
- 4. The water chemistry of Badfish River especially responded to diversion with substantial increases in organic nitrogen, inorganic nitrogen (influenced principally by ammonia nitrogen), phosphorus, and B. O. D. The dissolved oxygen was reduced to a critical level many times throughout the summer, and a D. O. deficit of 700 pounds per day existed at Station 4 during this period.
- 5. Phytoplankton populations were of substantially the same concentration between the three stations on a given stream and between the two periods of study for similar stations on the same stream, but were greater in the Yahara River than in Badfish River, and greater in the Rock River than in the Yahara River. There was an indication of a population depression following diversion at the upper stations on Badfish River and a difference in genera encountered between the pre-and post-diversion samples.
- 6. Submerged aquatic vegetation was abundant prior to diversion and, already in 1959, had become abundant in the dredged portion of the River. Perhaps it is yet too early to judge, but the submerged plants do not now present a problem. Long streamers of filamentous algae were attached to plants and bottom materials at numerous loca-

tions. A blanket of <u>Oscillatoria</u> covered much of the bottom of the upper Badfish River.

7. A study of bottom organisms indicated severe stream degradation following diversion. Stream biota changed from a balanced population containing several species and many intolerant organisms, prior to diversion, to a population

containing few species and only very tolerant sludge worms and midges following diversion.

8. The benthos in Badfish River exhibited a much greater response than the phytoplankton to the addition of nutrients, suspended solids, and B. O. D. contained in the effluent of the Madison Metropolitan Sewerage District Treatment Plant.

REFERENCES

Mackenthun, K. M., L. A. Lueschow, and C. D. McNabb. 1960. A study of the effects of diverting the effluent from sewage treatment upon the receiving stream. To be published in Volume 49 of the Trans. Wis. Acad. of Sci., Arts and Letters.

Standard methods for the examination of water, sewage and industrial wastes (1955). Amer. Publ. Health Assoc., Inc., 1790 Broadway, New York 19, N.Y., 10th Ed.

Woodburn, James G. 1959. Outfall around the Madison lakes. Water and Sewage Works 106 (11): 497-500.

SPRAY IRRIGATION FOR THE REMOVAL OF NUTRIENTS IN SEWAGE TREATMENT PLANT EFFLUENT AS PRACTICED AT DETROIT LAKES, MINNESOTA

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Detroit Lakes is a resort city located in a recreational area of 412 lakes within a radius of 25 miles.

The first sewage treatment plant at Detroit Lakes was erected in 1929, though as early as 1909 there was in existence a septic tank for the treatment of waste from a limited sewage system. The 1909 septic tank drained to the same water course as the 1929 plant addition. The 1929 plant, by reason of the growth of the city, was enlarged and improved by additional construction in 1941. The 1950 census gives Detroit Lakes a population of 5,734. The plant now treats on an average of 750,000 gallons of waste daily. The present sewage treatment plant consists of a coarse screen, a fine screen, a primary high-rate trickling filter, an intermediate settling tank, a secondary low-rate trickling filter, a final settling tank, and a chlorination tank. Sludge is digested and removed to sludge drying beds and sludge lagoons.

The effluent from the plant was originally discharged into a ditch which runs through what was formerly the easterly arm of St. Clair Lake, then into Lake St. Clair proper, and from there into St. Clair Ditch, which empties into the Pelican The Pelican River, after its confluence with St. Clair Ditch, empties into Muskrat Lake which empties into Lake Sally. Lake Sally is a navigable lake and, as the water flows, lies between 4 and 5 miles from the sewage treatment plant. It is one of the recreational lakes in the vicinity of Detroit Lakes in the so-called Pelican River chain. The recreational uses of the lake are swimming, boating, and fishing; and, with its wooded shores, it has attracted many residents for summer living. It appears that until the year 1947, there was no particular complaint about the algal bloom or weed growth in Lake Sally, though there were statements made that there had been a gradual increase in the growth of lake vegetation since 1940. The bloom is due to a large increase in the number of algae in the upper part of the

The summer of 1947 was unseasonably warm with a low amount of precipitation, and the amount

of algae in the lake was particularly extensive, and the odor, at times, extremely offensive. On August 18, 1947, there was a fish kill in the lake. A large number of dead fish were lodged on the shores, and that, together with the great amount of algae in the lake, some of which were washed on the shore, caused a great deal of disturbance and alarm among the summer residents. Bathing in the lake was unpleasant on account of the algae and recreational use of the lake except for fishing was greatly reduced.

Complaints were made by the residents to the City of Detroit Lakes, alleging that the source of the trouble was the effluent from the sewage treatment plant; while there was no dissatisfaction with the effluent from a sanitary viewpoint, it was alleged to be rich in nutrients, particularly phosphorus and nitrogen, which were conducive to the excessive growth of algae and weeds in Lake Sally.

STUDY BY THE DEPARTMENT OF HEALTH AND CONSERVATION

As this problem was an entirely new one for the city officials, it was referred to the Minnesota Department of Health and Conservation for investigation and study. Sampling stations were set up at various points between the waste treatment plant and Lake Sally and at other points at water courses in the area which were not affected by sewage treatment plant effluent. Reports issued in 1948 and again in 1951 indicated that some artificial fertilization was due to the effluent from the sewage treatment plant, although test data indicated a reduction of 90 per cent in phosphorus between the sewage treatment plant and Lake Sally. In the summary and conclusion of the 1951 report, the following was stated:

"To obtain a proportionate evaluation of the effect of the sewage treatment plant effluent on the present rate of enrichment of Lake Sally, the following program is offered:

"(a) A continuous study should be carried out, consisting of at least weekly sampling and flow estimation at all significant stations for at least a year.

- "(b) A sanitary survey of all lakeshore and watershed watershed property should be made.
- "(c) The nutrient content of ground waters and their effect on Lake Sally should be adequately studied.
- "(d) The relative algal productivities of Lakes Sally, Monson, Mellissa, and Detroit Lakes should be scientifically determined over at least one year's time.
- "(e) The kinds and amount of nutrients from all sources should be evaluated according to the nutrient requirements of plant life in general and of blue-green algae in particular."

OTHER STUDIES

During the period from 1948 to 1951, investigations were made of studies and reports on similar conditions throughout the nation. Information and data were very limited. We found that Madison, Wisconsin, had a similar condition and that the University of Wisconsin was carrying on various studies on the nutrition of algae, methods of control, removal of nutrients from sewage plant effluent, etc. At that time, no practical solution was available.

EXPERIMENT AT THE UNIVERSITY OF WIS-CONSIN

On February 13, 1950, W. L. Lea and G. A. Rohlich issued a paper on "The Removal of Phosphates from Sewage Treatment Plant Effluent." Their data showed that approximately 96 per cent of the soluble phosphate phosphorus was removed from effluent employing alum as a coagulant in the amount of 200 parts per million. Sixty-two per cent of the nitrogen containing organic compounds was also removed from the effluent. The data showed that addition to the treatment plant effluent of 200 parts per million of alum produced no significant change in the concentration of free ammonia, nitrites or nitrates.

The data presented in that study showed that, in order to remove 95-99 per cent of the soluble phosphates from a sewage plant effluent containing 5.0 parts per million of phosphates, approximately 6-10 times as much coagulant is required as is ordinarily used in the clarification of surface water supplies employed by a community as a source of potable water. Obviously then, the cost of chemicals required to remove the phosphates from one million gallons of effluent would be 6-10 times the chemical cost ordinarily incurred in treating one million gallons of a surface water supply.

REPORT OF THE MINNESOTA DEPARTMENT OF HEALTH

The Minnesota Department of Health, Division of Water Pollution Control, issued a "Report on the Experimental Removal of Phosphorus from Sewage Plant Effluents with Lime" in April, 1950. Experiments were conducted at the Detroit Lakes Treatment Plant.

The summary and conclusions stated that:

- 1. Approximately 700 ppm slaked lime or 530 ppm of unslaked lime were required to remove 80 per cent of the total phosphorus from the effluent of the low rate filter at Detroit Lakes.
- 2. The existing facilities did not lend themselves readily to full scale trial of the efficiency of the lime method, and it is estimated that the removal of phosphorus could be increased and the amount of chemical used decreased with proper mixing and efficient flocculation and settling of the treated waste.
- 3. The volume of sludge produced would be approximately three times the volume without the use of lime.
- 4. Under the conditions existing during the survey, the chemical costs only for the year-around treatment with unslaked lime would be approximately \$7,600. Assuming a sewage flow of 0.5 mgd, changes and additions would have to be made to the treatment plant units and equipment in order to provide chemical treatment and additional operating personnel would be needed.
- 5. The use of this method of phosphorus removal would not be recommended for this plant on the basis of this study.

SUGGESTED SOLUTIONS

Another suggested solution to the problem was to pond the effluent in large gravel pits to the north of the city. This possibility was ruled out as it is believed that the outwash gravel in the pits was directly connected with aquifers that supplied wells within the city.

A non-recreational lake to the north of the city was also studied with the possibilities of using it as a ponding reservoir. This lake had no outlet and its level is evidently maintained by surface run-off. However, studies indicated that the introduction of effluent would raise the water level approximately 6 inches per year. This would cause flooding of additional land and consequently lead to trouble and possible litigation.

LITIGATION

All actions of the city council and studies and reports by public agencies were open for public review and comments. What originally was the theory of a few, under proper influence, became the conviction of many. It was finally concluded by certain of the cottagers and their representatives that fantastic sums of money could be collected. Civil action was served on the City of Detroit Lakes on or about August 22, 1951, and additional ones shortly thereafter. In these actions the plaintiffs asked for a permanent injunction against the City of Detroit Lakes, together with money damages. A total of 26 claims were eventually filed totaling \$258, 500.

In the decision handed down by Gunnar H. Nordbye of the United States District Court, District of Minnesota, Sixth Division, he stated that he could not assume to speculate or conjecture the causes of any excessive growth of algae or weeds in Lake Sally, when the weight of the evidence establishes that the present data were insufficient to form any definite conclusions thereon. The evidence did not reflect any other practical method which would reduce the nutrients in the effluent more effectively than the present system.

The case was dismissed, without prejudice to the plaintiffs' right to apply again for equitable relief and damages in the event sufficient data and evidence exist to justify the granting of such relief.

IRRIGATION FOR THE DISPOSAL OF EFFLUENT

As the citizens of Detroit Lakes were interested in protecting the recreational lakes in their area from possible damage, the City Council instructed our firm to investigate further possible means of preventing the effluent from reaching these waters.

An investigation was made of a 55-acre wooded knoll lying directly west of the treatment plant. Borings indicated that gravel lay to a depth of 12 feet. A contour map was prepared of the area. On the west and east side of this knoll, there exist drainage ditches which converge to the south of the knoll and enter St. Clair Lake. We were quite confident that the porosity of the soils and transpiration by the trees would dispose of an enormous amount of water. Laboratory tests indicated that phosphorus would precipitate in filtering through the soils. Growth of vegetation would aid in the removal of nitrates. Oxidation of organic wastes would take place.

Factors which were unknown and had to be found out by experimentation were:

1. Soil permeability and irrigation rates

- 2. Effect on vegetation
- 3. Odors
- 4. Operating conditions during the winter months
- Effect on ground water

As there were a number of factors which were unknown and could cause failure of the project, we proposed to proceed slowly. The initial installation included a turbine pump with a capacity of 600 gallons per minute against a total pumping head of 110 feet and driven by a 20 horsepower motor. This unit was installed in the chlorination tank and made automatic with a float switch. A weir was installed in the outlet to the chlorination tank and set at an elevation approximately six inches above the high water level of the float switch. This would permit an automatic by-pass of effluent in the event of a surge greater than the pump capacity or in the event of pump failure and still provide for practically 100 per cent of the effluent going to the irrigation system during normal operation. The pump was equipped with a check valve, gate valve and a propeller type main line meter.

The initial piping system consisted of galvanized steel irrigation pipe with 29 sprinkler heads. Sizes and lengths of piping were as follows: 1312' of 8", 288' of 4", and 2304' of 3".

Sprinkler heads were those manufactured by the Skinner Irrigation Company: 1" utility with 7/32" main nozzle and 3/16" secondary nozzle. These were to have a sprinkling radius of 45 feet at 30 pounds of pressure and discharge 12.3 gallons per minute each.

Pipes were laid on the surface of the ground and at a gradient so as to be self-draining. The eight-inch main was provided with a two-inch valved tap at the low point which would be left open as desired during cold weather so as to provide drainage when the pump stopped. The 1,300 feet of eight-inch pipe were covered with hay and weighed down to help retain the water temperature. End caps of the laterals were tapped to provide drainage and prevent freeze-upduring periods of pump stoppage. Pipes were set on blocks where necessary to provide the gradient. Trees were removed only where necessary to provide for reasonable pipe alignment.

Equipment and materials for the initial installation, in place, cost approximately \$6,500. Cost of land was \$3,000.

A test well was installed in the irrigation area and a sample of the ground water was taken before operation began. The static level from the top of the test well was measured and found to be 11' - 10-1/2". Operations began on October 14th, 1955. Ground water analysis showed the following in ppm:

Total hardness	300
Alkalinity (M.C.)	
Chlorides	9
Nitrate nitrogen	1
Nitrite nitrogen	
Ammonia nitrogen	0.19
Organic nitrogen	1.4
Total phosphorus	0.6
Total nitrogen	2.6

The average of samples of sewage treatment plant effluent showed a pH of 7.4 and the following analysis in ppm:

Suspended solids	3	34
B.O.D. (5 day)	3	33
Soluble phosphorus		7.8
Total phosphorus		
Nitrate nitrogen		

During the month of March, 1956, effluent was applied to a 4-acre tract at the following rates:

Effluent pumped	21,547,500 gals.
	(695, 080 G. P. D.)
Pumping time in 31 days	651 hours
Gals/Acre/Day	173,470
Gallons per acre foot	325, 829
Total gallons per foot,	•
4 acres	1,303,400
Total feet of irrigation .	16.5 per month
Total inches of	· · ·
irrigation	198 per month
Sprinkling rate	
Sprinkling period daily .	20 hours

The flow was 360 gallons per minute through the sprinkler heads and 190 gallons per minute through drains during this period of the year. At this high rate of application, we found that during the summer months, vegetation would not grow in the sprinkled areas and a number of trees died. Some washing and ponding occurred but no objectionable odors were present.

In the fall of 1956, the irrigation area was doubled in size and a 6-inch main installed to feed the 4-inch and 3-inch laterals. Valves were installed on each lateral so that one branch could be shut off at a time for maintenance of nozzles and providing rest periods for each area served by the lateral.

In a four year period, 994,426,300 gallons of effluent have been pumped through the irrigation system during a total pumping time of 26,871 hours for an average rate of 617 gallons per minute. The pump operates approximately 20 hours per day.

The static water level in the test well on November 21, 1958, was 8' - 9-1/2" having raised 3'-1" during the four year period. Analysis of the sample of ground water taken November 21, 1958, was as follows in ppm:

Total hardness	420
Alkalinity (M.O.)	320
Chlorides	130
Nitrate nitrogen	31.0
Nitrite nitrogen	NF
$ Kjeldahl N_2 \begin{cases} ammonia \ nitrogen \\ organic \ nitrogen \end{cases} $	2.2
Total phosphorus	
Total nitrogen	

COST OF OPERATION

Cost of operation of the system is largely power costs. At its designed condition, the pump motor will require 0.47 kilowatt per 1,000 gallons pumped. This would be an increase in power consumption of 11,000 to 12,000 K.W. per month for an average of 24,000,000 gallons. Power meter readings during the years 1954 through 1959 confirm this figure. At two cents per kilowatt, the cost of power for irrigation by this method is slightly less than one cent per 1,000 gallons.

CONCLUSIONS

Limited personnel, equipment, and finances do not permit the gathering of sufficient data to thoroughly weigh the effects of the system insofar as nutrient removal is concerned. It is our opinion that results are being obtained chiefly through visual observance of the water course and limited test data. No doubt a large amount of the organic wastes are being oxidized in the soil.

The system has definite value as an additional unit to the treatment works.

If normal vegetation were to be grown, five feet of irrigation water a year would perhaps be the maximum allowable, considering the limited growing season of our area. If this rate were used to dispose of 287,000,000 gallons of effluent per year, approximately 200 acres would be required. This would require frequent moving of irrigation pipe and, consequently, additional personnel. Such an extensive system would be very difficult to operate during the winter months. If the higher rates can be maintained in a smaller space without

ill effects and without regard to the growth of vegetation, forced irrigation appears to be the most economical method to waste effluent in this area, where rainfall is quite adequate.

Experience has shown that with proper provisions, an irrigation system can be operated during the winter months without difficult maintenance problems, if the object is chiefly to dispose of water.

If the topography and soil characteristics are satisfactory, we believe that a "ridge and furrow" system could be used quite well as a method for inducing the effluent into the soils. The operating costs could be reduced considerably over spray irrigation as it would not be necessary to pump against a high head; however, the benefits gained by aeration and evaporation in the overhead spray irrigation system would be lost. Again, more research and investigation is necessary.

CHEMICAL METHODS FOR THE REMOVAL OF NITROGEN AND PHOSPHORUS FROM SEWAGE PLANT EFFLUENTS

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As part of the program of lakes and streams investigations at the University of Wisconsin, studies have been carried out on the removal of plant nutrients from sewage and sewage effluents. In particular, attention has been given to methods for the removal of nitrogen and phosphorus, essential elements which are usually considered of principal concern in supporting algal blooms. Inasmuch as other contributors to this seminar will discuss removal of nutrients by biological growths, this paper will be confined to a discussion of chemical methods for the removal of nitrogen and phosphorus from sewage and sewage effluents.

Rudolfs (1947) has reported on the quantities of phosphates in sewage and sewage effluents. Obviously the amount of phosphate will vary depending on the quantity and composition of industrial wastes present. In a report on the removal of phosphorus from sewage plant effluent, Owen's (1953) analyses of samples of domestic sewage from communities in Minnesota, with populations varying from 1200 to 940,000, showed that the raw sewage of those communities contained 1.5 to 3.7 grams of phosphorus per capita per day, with a median of 2.3 grams. His study showed also that the amount of phosphorus removed in conventional sewage treatment processes varied from 2 to 46 per cent. The higher removals generally were found in plants with secondary treatment and in those which showed the highest 5-day B.O.D. reductions.

Following laboratory studies, Owen (1953) carried out an investigation at a municipal plant consisting of fine screens, high-rate trickling filter, secondary clarifier, low-rate trickling filter, and final clarifier. Slaked lime was added to the influent of the final settling tank in a concentration of 545 ppm CaO, and the results indicate that the total phosphorus was reduced from a concentration of 7.4 ppm to 1.7 ppm indicating a removal efficiency of 77 per cent. He estimated that the chemical costs for treatment with unslaked lime would be approximately \$7,600 when treating a sewage flow of 0.5 mgd. His laboratory studies indicate that with the same dosage, a pH of 10.9 resulted, and the phosphorus concentration

was reduced from 6 ppm to 0.3 ppm, a reduction of 95 per cent. In the laboratory studies, a settling period of one hour was used. He further showed that when the settling period was increased to 18 hours, the phosphorus was reduced to .015 ppm, a reduction in excess of 99 per cent. Owen concluded that the plant scale tests would have more closely approached the laboratory results if adequate mixing and efficient flocculation could have been obtained. He found that the sludge produced was approximately three times the volume ordinarily handled at the plant.

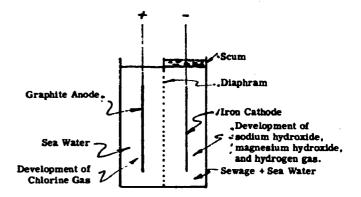


Figure 45. PRINCIPLE OF DR. E.FØYN'S DIA-PHRAGM CELL.

Dr. Ernst Føyn at Oslo, Norway, developed a process referred to as the Electrolitic Sewage Purification Method. The principle involved in Dr. Føyn's method is shown in Figure 45. A divided container is equipped with electrodes connected to the negative and positive poles of a battery. One portion of the chamber contains sea water and the other sewage mixed with approximately 10 to 15 per cent sea water. Chlorine is developed at the graphite anode and hydrogen and the alkali in the chamber containing the iron cathode. In this way, the chemical conditions necessary for precipitation of the phosphorus are established. The phosphate is adsorbed on the magnesium hydroxide floc and is floated to the surface by the hydrogen bubbles that are formed.

Following Føyn's laboratory study, a pilot plant investigation was carried out under the direction of Mr. L. R. Hougen of the Institute for Industrial and Technical Research at the Technological University in Trondheim. In Hougen's scheme, the diaphragm in the electrolitic cell is eliminated. The electrodes are arranged horizontally and the difference in the density between the sea water and sewage is assumedly sufficient to separate the two liquids. Using a retention time of about 1/2hour, the results indicated a removal of phosphate of between 90 and 95 per cent. The pilot plant test showed power requirements of 1 kilowatt hour per cubic meter of sewage flow. The patents to Dr. Føyn's invention have been assigned to Elektrokemisk. In addition to the phosphate reduction, it is reported that the Kjeldahl nitrogen was reduced from 18.8 to 5.4 mg/l.

In our laboratories, and in a pilot plant constructed adjacent to the outfall sewer of the Madison Nine Springs Sewage Treatment Plant, studies were conducted on phosphate removal using ferrous sulfate, ferric sulfate, cupric sulfate, diatomaceous earth, and aluminum sulfate. The most intensive study of phosphate removal was made using aluminum sulfate in the form of filtered alum as the coagulant. Initial studies were made to determine the effects of concentration of coagulant and pH in the coagulating system. In addition, studies were made on the influence of mixing time. Details of these studies have been presented elsewhere (Lea, et al., 1954).

In summary, the data showed that an alum dosage of about 200 mg/l was required to effectively remove from 95 to 99 per cent of the soluble phosphates from a sewage treatment plant effluent.

Approximately 6 to 10 times as much coagulant is required as is ordinarily used in the clarification of surface water supplies employed by a community as a source of potable water. Obviously, the cost of the chemicals required to remove the phosphates from 1 mg of effluent will be 6 to 10 times the chemical cost ordinarily incurred in treating 1 mg of a surface water supply. As an approach to the problem of reducing the costs for the removal of soluble phosphates from sewage treatment plant effluents, a study was made of coagulant recovery and purification.

After preliminary studies of alum recovery at low pH values involving acidification and the use of an ion exchange resin, this approach was abandoned in favor of an alkaline or high pH recovery process. The chemical reactions involved in the alkaline recovery and purification of the aluminum hydroxide floc are quite simple, as shown by the following:

Al(OH)₃ · [PO₄]
$$\longrightarrow$$
 + 4NaOH \longrightarrow NaAlO₂ + Na₃PO₄ + 2H₂O + 3OH - NaAlO₂ + 2Na₃PO₄ + 3CaCl₂ \longrightarrow NaAlO₂ + Ca₃(PO₄)₂ + 6NaCl

The mechanics of the process also are simple. The aluminum hydroxide floc with its adsorbed phosphate is pumped from the bottom of a sedimentation basin to a recovery tank. The concentration of aluminum hydroxide in the settled floc normally is equivalent to a 10,000 ppm solution of aluminum sulfate. Sodium hydroxide is added to the floc suspension until the pH of the solution is raised to approximately 11.9. At pH 11.9, the insoluble aluminum hydroxide is converted to soluble sodium aluminate and the phosphate to a soluble sodium phosphate. Addition of calcium chloride to the solution at this point results in the formation of insoluble tricalcium phosphate. The calcium phosphate is readily separated from the sodium aluminate solution by sedimentation and is a by-product of the process. The comparatively phosphate-free sodium aluminate solution is then adjusted in strength and pumped back to the flocculation process to be re-used as a coagulant for removing more phosphate from sewage treatment plant effluent.

The chemical aspects of alum recovery and reuse are shown by the following equations and explanatory remarks:

$$Al_2(SO_4)_3 \cdot 14H_2O + H_2O \longrightarrow 2Al(OH)_3 + 3H_2SO_4$$
 (1)

200 ppm $Al_2(SO_4)_3$ 14H₂O yields 52.5 ppm $Al(OH)_3$ and 99 ppm H_2SO_4

$$Al(OH)_3 + NaOH \longrightarrow NaAlO_2 + 2H_2O$$
 (2)

 $52.5 \text{ ppm Al(OH)}_3 = 55.2 \text{ ppm NaAlO}_2$

$$NaAlO_2 + H_2CO_3 + H_2O \longrightarrow Al(OH)_3 + NaHCO_3$$
 (3)

 $55.2 \text{ ppm NaAlO}_2 \text{ requires } 29.6 \text{ ppm of CO}_2$ (Neutralization)

The requirement of 29.6 ppm of CO₂ is close to the average concentration of carbon dioxide present in the Nine Springs effluent. Consequently, the addition of excess sodium hydroxide over the amount required to form sodium aluminate must be avoided. Excess sodium hydroxide will neutralize or consume a part of the carbon dioxide naturally present in the effluent and thereby result in incomplete hydrolysis of the recovered coagulant, sodium aluminate. If the coagulant loss in the over-all process is 10 per cent, or 20 ppm of alum, and this loss is made up through

the addition of 20 ppm of new alum just previous to the addition of the recovered sodium aluminate, advantage may be taken of the acidity of the added alum. The amount of acidity, as carbon dioxide, made available in this manner is shown by the following: 200 ppm of alum upon hydrolysis produce 99 ppm of sulfuric acid (see Eq. 1).

$$Ca(HCO_3)_2 + H_2SO_4 \longrightarrow CaSO_4 + 2H_2CO_3 \longrightarrow 98$$

$$2H_2O + 2CO_2$$
 (4)
 $2 \times 44 = 88$

Therefore, hydrolysis of 200 ppm of alum will produce, through reaction with the bicarbonates present in the effluent, 88 ppm of carbon dioxide. Ten per cent of this amount of alum will produce a proportionate amount of carbon dioxide. This proportionate amount, 8.8 ppm, is 29.6 per cent of the amount of carbon dioxide required to neutralize an amount of sodium aluminate equivalent to 200 ppm of alum.

PILOT-PLANT STUDIES

With the laboratory studies as a background, a pilot plant for continuous operation was constructed adjacent to the outfall sewer of the Madison Nine Springs sewage treatment plant.

With the cooperation of the Madison Metropolitan Sewerage District, a concrete blister was constructed on the effluent outfall structure. Installed in the concrete blister was a 4-inch gate valve. This construction permitted the withdrawal of sewage effluent from the outfall structure.

As the effluent was pumped from the blister into a constant-head tank, an automatic sampler was used to collect a composite sample of the effluent. From the constant-head tank, the effluent flowed into a baffled mixing tank, to which the alum solution was added. Mixing was accomplished by the use of air.

The effluent flowed over the mixing tank weir, through a trough into the settling tank, in which the theoretical detention time was about 2 hours at a flow rate of 10 gpm. Another sampler was used at the effluent end of the settling tank, from which the treated supernatant flowed into a ditch.

A manifold was provided for drawing off the settled floc. At the drawoff points, valves were provided to control the flow of sludge, which was drawn off by gravity into a thickening tank, where the supernatant was removed and from which a sump pump lifted the sludge into the reaction tank. The reaction tank was 4 ft. in diameter and 5 ft. high, and was constructed with a conical bottom to facilitate removal of the calcium phosphate pre-

cipitate. Sodium hydroxide was added to the reaction tank for pH adjustment and calcium chloride to precipitate the calcium phosphate. From the reaction tank, the precipitate was drawn off to a drying bed. The supernatant of the reaction tank was then discharged to a storage tank, from which it was pumped to the alum feed tank. With the exception of the conical bottom, the storage tank was of similar dimensions to the reaction tank. Both tanks were designed to hold about 400 gallons.

As a result of the studies, the following conclusions may be made:

- 1. Laboratory studies show it is possible to remove approximately 96 to 99 per cent of the soluble phosphates from the effluent of a sewage treatment plant. This removal can be accomplished in a coagulation process employing any of the following coagulants: (a) aluminum sulfate, (b) ferrous sulfate, (c) ferric sulfate, or (d) copper sulfate.
- 2. The use of copper sulfate as a coagulant is not advisable because the floc formed by hydrolysis of this salt in the sewage treatment plant effluent has very poor settling characteristics.
- 3. Filter alum appears to be the most suitable coagulant for removal of soluble phosphates from sewage treatment plant effluent because: (a) The residual phosphate concentration of the effluent following coagulation with 200 ppm of alum is, on the average, 0.06 ppm, expressed as P. (b) The optimum pH range for the removal of phosphates through coagulation with alum is 7.1 to 7.7. The average pH value of Nine Springs Treatment Plant effluent lies in this range; therefore, no adjustment of pH would be required at this plant. (c) The concentration of aluminum hydroxide in the effluent of the coagulation process is only approximately 1.0 to 1.5 ppm and represents a loss of only 0.75 per cent of the coagulant. It is assumed that 1.0 to 1.5 ppm of aluminum hydroxide in the effluent is not objectionable. (d) The aluminum hydroxide floc resulting from the hydrolysis of alum may be recovered, purified by removing the adsorbed phosphates in the form of tricalcium phosphate, and re-used for further phosphorus removal in the form of sodium aluminate. This recovery and purification reduces by 80 per cent the cost of chemicals required to remove phosphates from sewage treatment plant effluent.
- 4. A study was made of the mechanism by which phosphates are removed through coagulation with alum, cupric sulfate, ferric sulfate, or ferrous sulfate. This study revealed that for the first three coagulants the phosphates are removed through adsorption upon the hydroxide flocs formed. The data obtained on phosphate removal through coagulation with ferrous sulfate indicated

that adsorption is not the sole means of removal, but that precipitation may also enter into the reaction during the transition from ferrous to ferric hydroxide.

- 5. The results of this study showed that approximately 85 per cent of the biologically oxidizable organic compounds and 68 per cent of the organic nitrogen compounds are removed along with the soluble phosphates in the coagulation process employing 200 ppm of alum.
- 6. The study showed that there was no removal of inorganic nitrogen compounds by the use of alum coagulation.
- 7. Pilot-plant studies show that with the use of the alum recovery process, from 77 to 89 per cent of the soluble phosphates can be removed. Filtering of the effluent showed that from 93 to 97 per cent of the soluble phosphate can be removed. Improved settling facilities should give phosphorus removals that lie between the unfiltered and filtered values obtained in the pilot-plant study.

NITROGEN REMOVAL

Much less attention has been given to the removal of nitrogen from sewage and sewage effluent by chemical means obviously because soluble nitrogen compounds are least affected by precipitation processes.

It is of interest, however, to note that Gleason and Loonam (1933), in reporting their work on the development of the Guggenheim process, used the removal of nitrogen as a criterion for the effectiveness of the process.

The Guggenheim process, as described by Gleason and Loonam (1934), consisted of the following steps:

- 1. Removal of suspended solids by coagulation with iron compounds and lime, and settling of the coagulated solids.
- 2. Disposal of the sludge by filtration and circulation and regeneration of the iron as ferric sulphate from the incinerated ash.

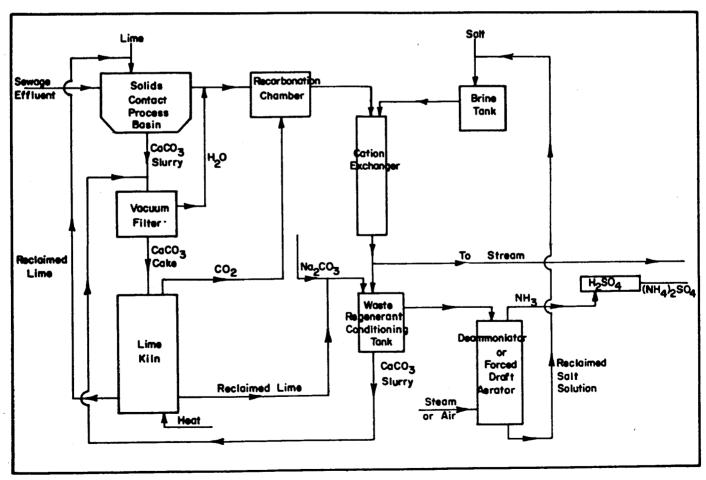


Figure 46. PROCESS DESIGN—NITROGEN AND PHOSPHORUS REMOVAL FROM SEWAGE EFFLUENT. Ammonia by Cation Exchange.

- 3. Removal of the basic nitrogen compounds by an exchange reaction using a zeolite.
- 4. Regeneration of the zeolite and concentration of the basic nitrogen compounds in a salt solution, and subsequent recovery of ammonia from this solution. Results reported by these workers indicated that the total nitrogen in the raw sewage was 24 28 ppm and in the effluent from the process, 2 3 ppm. The ammonia nitrogen was reduced from a concentration of 12 14 ppm in the raw to 0.5 to 1.0 ppm in the effluent.

Subsequent studies by these workers (Gleason and Loonam, 1934) carried out at the North-side Sewage Treatment Works at Chicago over a sevenmenth period showed organic nitrogen removals of about 79.3% and ammonia nitrogen removals of about 67.4%.

From studies in our laboratories carried out by Nesselson (1954) using ion exchange, the following conclusions were made:

1. Strong base anion exchangers, regenerated with common salt, perform satisfactorily for the

removal of nitrate. A number of evaluations established that Amberlite IRA-410, in the treatment of trickling filter effluent, has an exchange capacity of 8.7 to 11.7 kilograms/cu. ft. as CaCO₃. It operates under an efficiency of 4.0 to 6.8 lbs. of NaCl/kilograin of anions removed. Nalcite SAR had respective values of 6.5 to 7.7 kgrs/cu. ft. as CaCO₃ and 6.0 to 9.9 lbs. NaCl/kgr of anions removed. Both media were regenerated to an end point of 1 ppm of nitrogen in the waste regenerant. A minimum volume of about 7 per cent of the influent feed was required for this operation.

2. The removal of ammonia nitrogen by nuclear sulfonic cation exchangers was investigated. Nalcite HCR has an exchange capacity of 16.0 to 22.0 kgrs/cu. ft. as CaCO₃ in the treatment of activated sludge effluent. It operates with an efficiency of 1.4 to 2.5 lbs. NaCl/kgr cations removed. Amberlite IR-120 has respective values of 13.0 to 17.0 kgrs/cu. ft. as CaCO₃ and 1.3 to 2.6 lbs. NaCl/kgr cations removed. A minimum volume of about 6 per cent of the influent feed was required to perform regeneration.

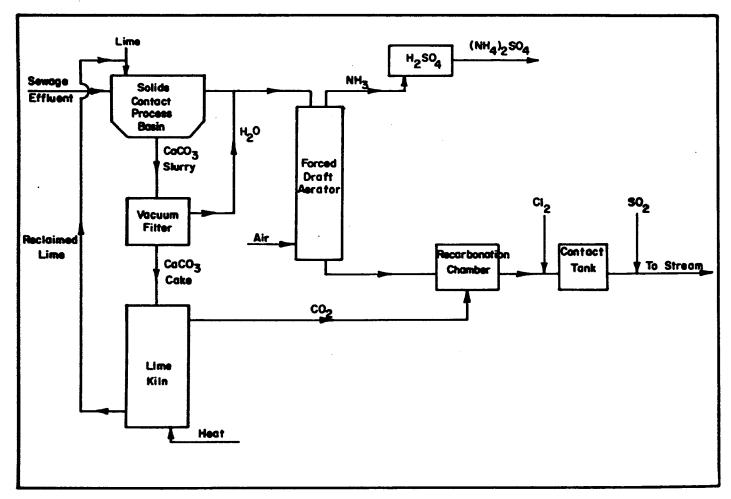


Figure 47. PROCESS DESIGN—NITROGEN AND PHOSPHORUS REMOVAL FROM SEWAGE EFFLUENT. Ammonia by Forced Draft Aeration.

Kuhn (1956) in further work at Wisconsin carried out studies on air stripping in packed towers to remove ammonia nitrogen. As a result of these studies the following conclusions were made.

It is possible to remove ammonium nitrogen from sewage effluent by stripping with air.

Desorption may be accomplished in a tower packed with Raschig rings.

In the studies conducted, the optimum pH for stripping was 11.0. The optimum pH value was determined from a study of ammonia nitrogen removals through the range pH 8.0 to pH 12.0. Increased removals were obtained by increasing pH values. No significant difference existed between removals at pH 11.0 and pH 12.0.

The effect of air/liquid loading, expressed as cfm of air per gpm of liquid, was studied at ratios of: 40, 59, 85, 230, and 447 cfm/gpm. Respective removals obtained at these ratios were: 15.1, 28.5, 37.8, 67.0, and 78.7 per cent, respectively. These results are consistent with theory which predicts best removals at an air/liquid ratio of 453 cfm/gpm.

Ammonium nitrogen removals were studied at depths of 1/2" Raschig rings of: 2.5, 4.0, 5.0, 5.5, and 7.0 feet in an 8" diameter column. At the optimum pH value, pH 11.0, removals of ammonia nitrogen from sewage effluent of 53.9, 71.1, 78.5, 82.2, and 92.3 per cent, respectively, were obtained at the depths above. Loadings for these removals were 52 to 55 cfm of air and 0.10 gpm of effluent. These results are consistent with the theory of design of desorption columns.

The studies on phosphorus and nitrogen removal have suggested again two possible process designs originally proposed by Nesselson (1954).

The units described next are suggested primarily for removal of nutrients from the effluent of an activated sludge or high-rate filtration plant. The extent of nitrification in these two types of plants can be minimized by proper operational control.

The essential features of the treatment scheme shown in Figure 46 are as follows: Sewage effluent enters a softening unit for hardness reduction. The effluent stream from this unit is then recarbonated to convert NH4OH to NH4+. The liquid then passes through the cation exchanger and is discharged to the watercourse. Reclamation of waste regenerant is effected by precipitating the calcium and magnesium and diverting the slurry to the lime recovery units. Supernatant liquid containing NaCl and NH4OH is passed through either a forced draft aerator or a deammoniator. The liquid effluent from this unit is recirculated to the brine tank and the stripped ammonia is recovered in a sulfuric acid solution.

Treatment of sewage effluent in the process design shown in Figure 47 is as follows: Effluent from the pH adjustment tank flows to a forced draft aerator. Since air requirements for complete ammonia removal are quite high, only sufficient air is supplied to reduce the ammonia concentration to a lower level amenable to economical treatment by chlorine. Following recarbonation and chlorination, it may be necessary to dechlorinate.

REFERENCES

Rudolfs, W. 1947. Phosphates in sewage and sludge treatment. I. quantities of phosphates. Sewage Works Journal 19: 1, 43.

Owen, R. 1953. Removal of phosphorus from sewage effluent with lime. Sewage and Industrial Wastes Journal 25: 5, 548.

Lea, W. L., G. A. Rohlich, and W. J. Katz. 1954. Removal of phosphates from treated sewage. Sewage and Industrial Wastes Journal 26: 3, 261.

Gleason, G.H. and A.C. Loonam. 1933. The development of a chemical process for treatment of sewage. Sewage Works Journal 5: 1, 61.

Gleason, G.H. and A.C. Loonam. 1934. Results of six months operation of a chemical sewage purification plant. Sewage Works Journal $\underline{6}$: 3, 450.

Nesselson, E. J. 1954. Removal of inorganic nitrogen from sewage effluent. Unpublished Ph.D. Thesis on File, University of Wisconsin Library.

Kuhn, P.A. 1956. Removal of ammonia nitrogen from sewage effluent. Unpublished M.S. Thesis on File, University of Wisconsin Library.

STRIPPING EFFLUENTS OF NUTRIENTS BY BIOLOGICAL MEANS

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Studies have been made over a two year period on the factors influencing the absorption of algal nutrients from treated and untreated domestic sewages by algae, both in controlled laboratory experiments and in field experiments with a halfacre pond. This work was supported by the Oscar Mayer Company and the Rockefeller Foundation and was carried out by the author and Dr. Harry Kaneshige.

Laboratory experiments were carried out to clarify or substantiate some of the data obtained in the pond studies. These studies have shown that (1) there is little influence of different lake waters

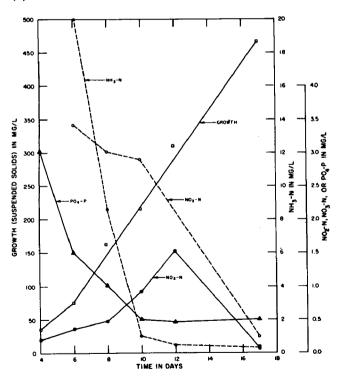


Figure 48. GROWTH AND NUTRIENT UTILIZATION BY CHLORELLA IN SEWAGE EFFLUENT.

as a source of inocula (1 ml/100 ml) on the subsequent growth of algae in secondary sewage effluent, (2) the amount of growth of algae in treated effluents is influenced by temperature and the level of nutrients available for the algae, (3) growth and nutrient utilization by Chlorella pyrenoidosa is similar in both primary and secondary effluents (see Figures 48 and 49), and (4) the CO2 content of effluents will influence the pH, solubility of nutrients, and the growth and nutrient utilization of Chlorella (see Figure 50).

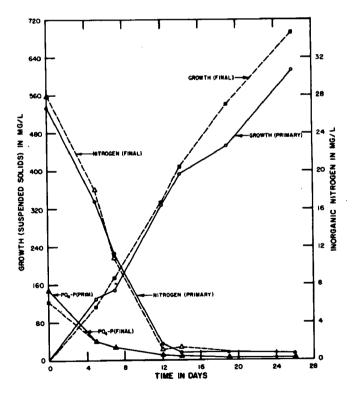


Figure 49. ALGAL GROWTH AND NUTRIENT UTILIZATION IN PRIMARY AND FINAL EFFLUENT.

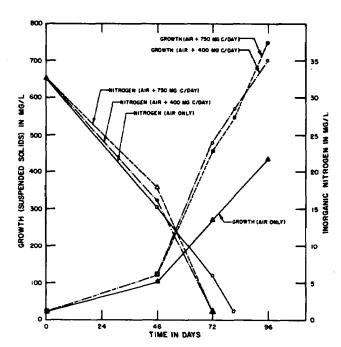


Figure 50. GROWTH AND NITROGEN UTILIZATION BY CHLORELLA IN SEWAGE EFFLUENT WITH DIFFERENTIAL LEVELS OF CO₂.

In general, laboratory experiments have indicated that the growth of an alga, such as <u>Chlorella</u>, in domestic sewage effluents, should efficiently remove algal nutrients from solution during that portion of the year when the temperature is conducive to the growth of algae.

In contrast to the results of controlled laboratory experiments, data from the operation of the half-acre pond have indicated that there were considerable periods of time, during what might be considered the optimum algae growing period of the year, when there was very little algal growth or changes in nutrient levels.

The data obtained in 1956 with secondary effluent as feed are summarized in Figure 51 as the amount of suspended solids in the outlet of the pond, the percentage removal of the inorganic nitrogen as the waters passed through the pond, and the water detention time in the pond.

An early bloom of <u>Euglena</u> in April had little effect on nitrogen removal. The peak and valley of nitrogen removal at this time is due to a slug of ammonia from a fertilizer plant and its subsequent passage through the pond.

During the latter part of May, a bloom of Chlamydomonas brought about a removal of 50% of the inorganic nitrogen.

A period in the latter part of July, with 10 days or more detention time, brought about nitrogen removals in excess of 80%, despite only minor amounts of algae (<u>Closteridium</u> and <u>Eudorina</u>).

The feed to the pond was changed from secondary effluent about the first of September. The early nitrogen removals recorded during the first part of September are due to dilution of the sewage with the pond waters and the fact that the flow to the pond was not continuous due to mechanical failures.

Despite heavy blooms of <u>Euglena</u> and <u>Chlamy-domonas</u> during October and November, the nitrogen removal was only about 20% with detention times of about 5 days.

During 1957, the feed to pond was secondary effluent. Since the data of 1956 indicated that 9 or 10 days detention time appeared best, this flow was used for most of 1957. The data obtained are summarized in Figure 52.

The peaks in inorganic nitrogen removed appear to coincide with the peaks in algal content of the pond.

The data do not indicate the reason for the low algal content of the pond waters during June, despite periods of very low flow. During this time, the pond was inhabited by a quite dense bloom of Daphnia. However, observations did not indicate whether the Daphnia brought about the decline in the algal population or if the Daphnia came into dominance after the algae had decreased.

There was a period of high inorganic nitrogen removal during July and August brought about by a dense bloom of <u>Euglena</u>. This was followed by another peak in nitrogen removal due to the growth of a mixture of <u>Chlorella</u>, <u>Chlamydomonas</u>, and <u>Ankistrodesmus</u>.

The algal population declined during the middle of September and was replaced with <u>Daphnia</u>.

The pond operation is summarized as follows:

- 1. The impoundment of final effluent without artificial aeration causes the D.O. content to decrease considerably below the 6-8/mg/1 D.O. of the influent. Lower levels are obtained during the summer than in winter. With the growth of algae in the water, the D.O. rises to levels considerably higher than that of the influent.
- 2. Variations in the depth of the pond (2 feet vs. 3.5 feet) had little effect on D. O. during periods of low algal activity.
- 3. Vertical mixing by means of an air compressor and 1-1/2 inch plastic pipe with 1/8 inch holes every two feet was found to be very effec-

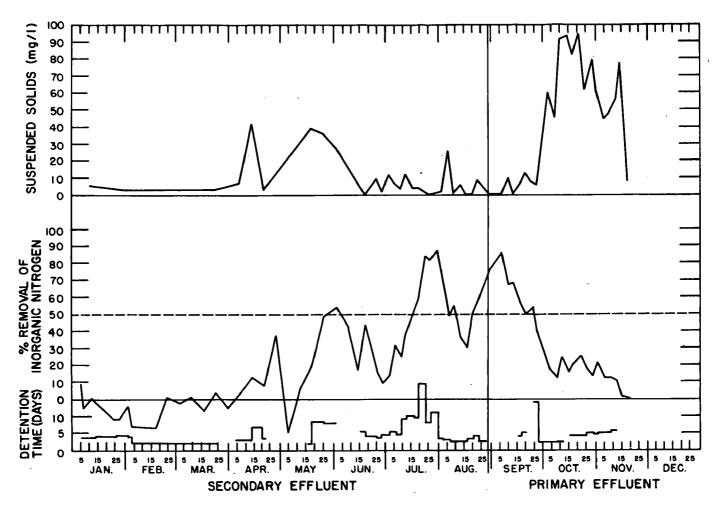


Figure 51. NUTRIENT UTILIZATION BY ALGAE IN A HALF-ACRE POND RECEIVING SEWAGE EF-FLUENT, 1956.

tive (as measured by suspended solids (algae) and D.O. concentrations). No apparent effect on B.O. D. removals was noted.

- 4. Recirculation inside each cell or from the effluent end to influent end of the pond had no effect on B.O.D. removals.
- 5. Detention time variations between 2 to 5 days during the winter did not cause B. O. D. removal to vary. During summer periods, 10 days or more detention time appears to be required for substantial inorganic nitrogen removal.
- 6. Suspended solids removal and B.O.D. reduction during periods of low algal activity took place in Cell 1 of the pond, with little change taking place in the other cells. During two such periods in 1957, the suspended solids and B.O.D. were decreased 70-80% in Cell 1.

- 7. The pond appears capable of removing 60% of the influent B. O. D. when either primary or secondary effluents are the feed.
- 8. The average nitrogen removal throughout the year is about 30%, with removals in the summer reaching about 70%. It should be pointed out, however, that there were only 33 days in 1956 and 76 days in 1957 when the inorganic nitrogen removal exceeded 50% with secondary effluent as feed.
- 9. Phosphorus removal during periods of high algal activity coincides with high pH values in the pond and is probably due to precipitation. This is borne out by the fact that during winter periods the effluent phosphate concentrations frequently surpassed influent levels—probably due to the dissolution of phosphorus previously precipitated (similar results were obtained in laboratory experiments when the pH of solutions were adjusted to 9.5 and then back to 8.0).

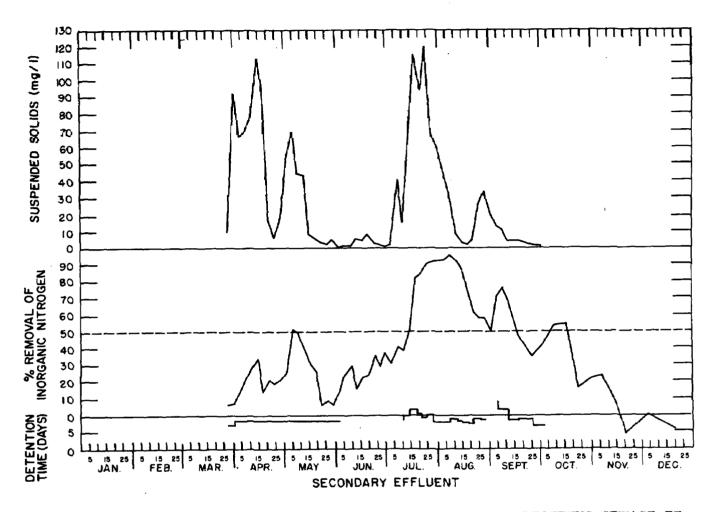


Figure 52. NUTRIENT UTILIZATION BY ALGAE IN A HALF-ACRE POND RECEIVING SEWAGE EFFLUENT, 1957.

THE USE OF ALGAE IN REMOVING NUTRIENTS FROM DOMESTIC SEWAGE

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INTRODUCTION

Excessive enrichment of receiving waters by nutrient rich wastes appears to be emerging as a major water pollution problem in many areas.

Limnological investigations indicate that, where domestic sewage serves as the primary source of eutrophication, treatment for the removal of phosphorus would provide a practical and effective means of control (Sawyer, 1953). Treatment, unlike the use of algicides, would be permanent and, as in the case of diversion, would not suffer the possible disadvantage of degrading other drainage basins. In spite of these obvious advantages, a suitable method of treatment has yet to be placed in practice.

Phosphorus may be removed from sewage by biological and by chemical means. Either approach is aimed at converting soluble inorganic phosphorus into recoverable insoluble matter. Of the two, chemical coagulation has received the greatest attention. Several promising but costly chemical treatment methods have been proposed. Little has been done to date with biological treatment.

The purpose of this paper is to explore the concept of employing algae as a means of removing nutrients from domestic sewage. The concept of employing algae for this purpose does not appear to have received much attention, hence much of what follows is based on research carried out at the University of Washington during the past three years. Wherever possible, pertinent data reported by others has been drawn upon.

THEORETICAL CONCEPTS

Phosphorus Metabolism

The concept of removing nutrients by biological means can hardly be considered as new or unique. In any actively growing biological system, nutrient materials are constantly being extracted from the environment through conversion to cell tissue, the

rate and degree of removal being dependent upon the biological system employed and upon the environmental conditions provided.

Rate of removal, other things being equal, is a function of the rate of cell tissue synthesis. Growth rate varies considerably with type of organism, as shown in Table 41. The mixed microbial culture

Table 41. COMPARISON OF COMMONLY RE-PORTED GROWTH RATES.

Type of organism	k ^a (day ⁻¹)		
Algae	0.20 to 2.0		
Protozoa	1.0 to 4.0		
Bacteria	2.0 to 60		

a Based on $N_t = N_0 e^{kt}$

provided by the activated sludge process would appear to be the most effective biological system in terms of removal rate. Theoretical daily phosphate removal rates are shown in Figure 53 for several concentrations of cell tissue having growth rates comparable to those commonly reported for algae.

Cell tissue composition together with the mineral content of a sewage will determine the amount of phorphorus which can be extracted. Examination of the data presented in Table 42 indicates that assimilation of 1 mg/1 of phosphorus.

Table 42. COMPOSITION OF ACTIVATED SLUDGE AND COMMON FRESH WATER ALGAE.

Biological system	C	N	P
	% dry wt.	%dry wt.	Hdry wt.
Activated sludge ^{a, b,} Algae ^C	41 - 53	8 - 12	0.7 - 2.2
	49 - 60	1.4 - 11	0.9 - 2.0

a. Hoover, S. R., and N. Porges, 1952.

b. Helmers, E. N. et. al., (1952).

c. Burlew, 1953. See R. W. Krauss, Chapter 8.

would be accompanied by the metabolism of 25 to 50~mg/l or more of carbon and 2 to 12~mg/l of nitrogen. It is interesting to note the similarity in the C, N, and P content of algae and of activated sludge.

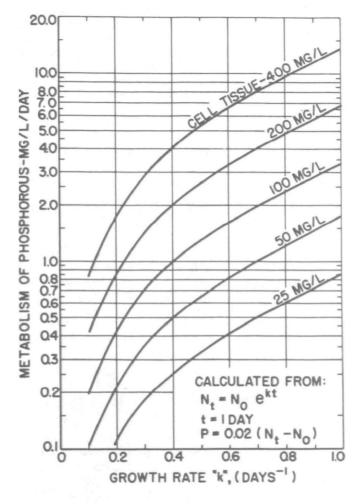


Figure 53. THEORETICAL RELATIONSHIP BETWEEN CELL TISSUE CONCENTRATION, GROWTH RATE AND METABOLIC CONVERSION OF PHOSPHORUS BY ALGAE. Light intensity will determine cell tissue concentration. Temperature, diet and species will regulate "k". In the Seattle area, algal concentration ranged from 25 to 50 mg/-1 in 4 ft. deep lagoons. The mean "k" was 0.30 days.

Nutritional Limitations

Ordinary domestic sewage does not provide a balanced diet for activated sludge, being deficient in carbon and nitrogen with respect to the amount of phosphorus normally present. Organic carbon is usually limiting. Algae do not suffer from the same dietary restrictions. Adequate amounts of carbon are normally available in the form of alka-

linity. The inorganic nitrogen content of domestic sewage, however, still may be in short supply. There is evidence that atmospheric nitrogen fixation might be made to serve as a significant source of nitrogen (Sawyer, 1953). Agricultural grade NH₄-N could also be employed as a relatively inexpensive source of nitrogen.

Viewed in terms of nutritional requirements, algae appear to offer the most effective biological system for extracting phosphorus from domestic sewage.

Observed Phosphorus Reductions

Based on a carbon to phosphorus ratio of 100 to 1 (Helmers, et al., 1953) and a settled raw sewage B.O.D. lying in the range of 100 to 200 mg/l, it becomes obvious that phosphorus reductions during the course of biological sewage treatment would on the average be limited to about 1 to 2 mg/1. Owen (1953), in an investigation of sewage treatment plant performance in Minnesota, found phosphorus removals ranged from an average 2 per cent for promary treatment plants to an average 23 percent for plants employing biological treatment; this was equivalent to approximately 1 to 2 mg/l of P. Analysis of biological sewage treatment plant effluents in the Seattle area disclosed a reduction ranging from 15 to 40 per cent which was equivalent to 0.80 to 2.0 mg/l of P.

A number of excellent research reports have appeared in the literature dealing with algal behavior in oxidation ponds and in raw sewage lagoons (Burlew, 1953; Oswald, et al., 1953; Oswald and Gotaas, 1955; and Anon., 1957). Attention has been centered mainly upon the use of photosynthetic oxygen production. Very little data has been reported regarding nutrient reductions realized in such processes. Available information indicates phosphorus reductions ranging from 10 to 90 per cent or better. Performance appears to be erratic and unpredictable. Considerable difficulty has been experienced in harvesting algal cell tissue; this coupled with slow growth rate would account for some of the wide fluctuations noted in the mineral composition of lagoon effluents.

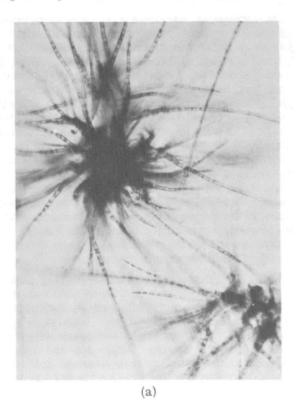
The Use of Algae

The most direct method of employing algae as a means of recovering nutrients would appear to be an oxidation pond followed by a separation operation for harvesting algae. With such an arrangement sewage would be continually mixed with actively growing algae and the nutrients gradually converted to cell tissue; cell tissue would be recovered for reuse or wasted according to need. Experience indicates that the principal problem would be one of harvesting the algae.

Several unit operations have been investigated as a means of harvesting algal cell tissue (Burlew, 1953). Those most frequently referred to in the literature are screening, settling, centrifuging and chemical coagulation. All have been found wanting in some aspect, generally in terms of efficiency and often in terms of cost. From the standpoint of performance and economy, some type of screening device appears to be the most promising.

Screen performance obviously would be related to the nature of the algal culture. It was reasoned, however, that if some readily recovered alga could be established, then through the simple mechanism of recovery and reuse, it could in turn be made to predominate. Other things being equal, the process would naturally tend to contain the most readily utilized population. The ideal organism would appear to be a large, rapidly growing, filamentous alga.

There is little evidence that filamentous species of the type desired normally grow in oxidation ponds or sewage lagoons. This does not preclude the possibility of their being cultivated under such conditions. Failure of filamentous species to constitute a significant part of the population of most oxidation ponds may be due to the fact that they are relatively slow growers and simply tend to be overgrown by Chlorella and Scenedesmus.



EXPERIMENTAL RESULTS

The use of algae in removing phosphorus from sewage was studied in both the laboratory and the field. Several fundamental concepts relative to using algae for such a purpose came under investigation. Space does not permit a detailed accounting of all major aspects of this research. There follows a brief summary of some of the more significant findings dealing with the topics of available algae, mechanism of phosphorus removal, physical chemical behavior of orthophosphate, photosynthetic pH adjustment, and cell tissue recovery.

Available Algae

Several common fresh water algae were grown in the laboratory on mixtures of lake water and raw and treated sewages. However, except for Chlorella and Scenedesmus, most species gradually died out after a brief period of active growth. A large filamentous alga, subsequently identified as Stigleoclonium stagnatile, was recovered from the rock of a biological filter in the area and successfully cultured. This alga, when grown under aeration, developed into settleable floc particles resembling activated sludge. Photomicrographs of Stigleoclonium stagnatile are shown in Figure 54. Its growth characteristics and nitrogen and phosphorus content are shown in Tables 43 and 44.

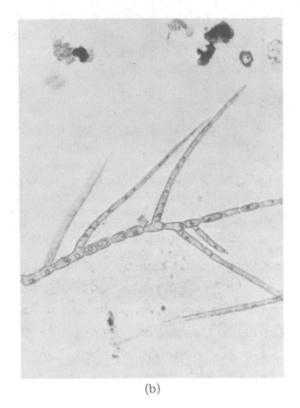


Figure 54. PHOTOMICROGRAPHS OF STIGLEOCLONIUM STAGNATILE. (a) Floc-like colonies which developed in aerated cultures (220x). (b) View of individual organism (520x).

Table 43. EFFECT OF TEMPERATURE AND CULTURE MEDIA ON GROWTH RATE, STIGLEO-CLONIUM STAGNATILE.

pH variation 8.3 - 9.5. Illumination 400 ft. candles

	k days -1*	
Synthetic sewage employing NO ₃ - N	Synthetic sewage employing NH ₃ - N	Secondary STP effluent
0.165	0.140	0.170
15 0.188		0.215
0.252	0.131	0.131**
	sewage employing NO ₃ - N 0.165 0.188	Synthetic Synthetic Synthetic Sewage employing NO ₃ - N NH ₃ - N

^{*} k computed from $N_t = N_0 e^{kt}$

phosphate reductions comparable to those observed in laboratory batch and pilot plant studies.

PO4 Behavior

Under suitable conditions, orthophosphate may combine with a number of substances commonly present in sewage to form relatively insoluble complexes. Calcium ion concentration and pH were found to be the principal controlling factors in determining $PO_{\overline{4}}$ solubility; their relationship is shown in Figure 57. Ammonia, iron and magnesium in the amounts generally encountered in domestic sewage were not found to exercise any discernible effect on phosphate solubility.

Constituent Synthe		sewage	Secondary STP	1/2 STP Eff
	$NH_3 - N$	effluent	$1/2 \text{ NO}_3$ - N Syn Sew	
N	5.71	6.59	6.52	6.00
P	2.16	1.81	1.89	2.07
N/P	2.64	3.63	3.44	2.89

a Expressed as percent dry cell weight. Values reported represent an average of several determinations.

Table 44. PHOSPHORUS AND NITROGEN CONTENT OF STIGLEOCLONIUM STAGNATILE HARVESTED FROM VARIOUS CULTURE MEDIA².

Mechanism of PO4 Removal

It was expected that phosphorus removal would follow a pattern predicted by cell tissue composition and growth rate (see Figure 53). Contrary to expectations, orthophosphate residuals in batch fed cultures were found to decrease at a rate considerably in excess of that predicted by biological uptake. Response to repeated heavy phosphate doses was most interesting; in general, some 80 to 90 per cent of the phosphate added was removed from solution within two hours as shown in Figure 55. Obviously, more than metabolic uptake was involved. Examination of culture characteristics disclosed that phosphorus removal was related to pH. It thus appeared that coagulation and adsorption may have played a significant role.

At moderately high pH levels, generally in the range of 9.5 to 10.0, large amounts of phosphorus were extracted rapidly from solution without the use or need of auxiliary chemicals. At lower pH levels, the rate of phosphorus removal should, of course, be determined largely by algal growth rate. This hypothesis was subsequently tested in the laboratory and in the field. Laboratory pilot plant performance is shown in Figure 56. At pH levels of 9.5 and above, field cultures exhibited

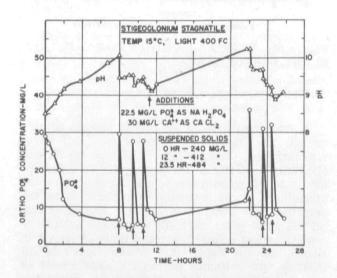


Figure 55. RESPONSE OF BATCH FED ALGAL CULTURES TO REPEATED DOSES OF PO Acrated Stigeoclonium cultures exhibited a remarkable capacity for coagulating and adsorbing orthophosphate. Photosynthetic response was not visibly impaired by repeated use of cell tissue.

^{**} Scenedesmus appeared and began to predominate after approximately 15 days.

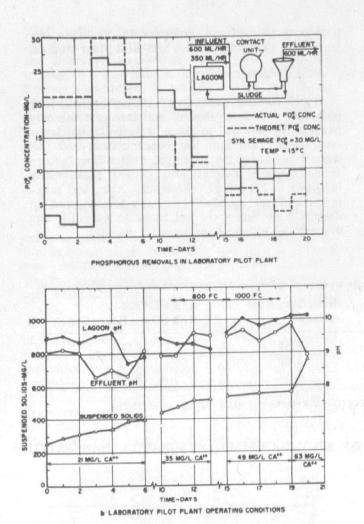


Figure 56. TYPICAL LABORATORY PILOT PLANT PERFORMANCE.

Photosynthethetic pH Adjustment

A study was made of the role of algae in adjusting pH. Such factors as temperature, cell tissue concentration, composition of culture media and alkalinity naturally would be expected to influence the rate of photosynthetic pH adjustment. Interestingly, light intensity was found to be the principal rate controlling factor. Photosynthetic pH response under carefully controlled laboratory conditions and under field conditions is shown in Figure 58 and Table 45. Where light was not limiting, pH response followed a pattern such as that shown in Figure 58. Under field conditions light intensity became limiting and the rate of change of pH was consequently markedly reduced.

Judged solely on the basis of rate of pH adjustment, minimum light intensity requirements appear to lie in the vicinity of 100 to 200 f.c. Where such light intensities are possible, the pH of raw and treated sewages may be photosynthetically increased to 9.0 and above within 4 to 12 hours.

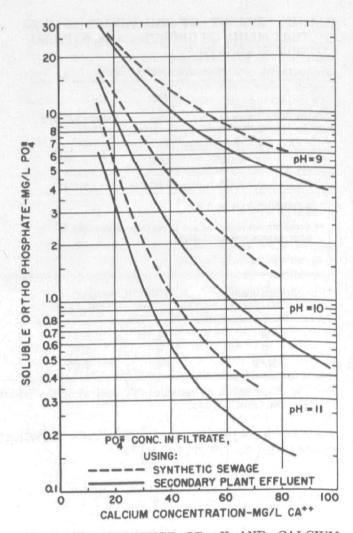


Figure 57. INFLUENCE OF pH AND CALCIUM CONCENTRATION ON ORTHOPHOSPHATE SOLUBILITY. Calcium ion concentration and pH adjusted by means of CaCl₂, Ca(OH)₂ and NaOH. Samples of synthetic sewage and sewage treatment based on filtered supernatant.

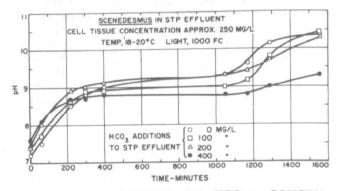


Figure 58. INFLUENCE OF HCO3⁻ CONCENTRATION ON PHOTOSYNTHETIC pH SHIFT. At light intensities above 200 f.c., Scenedesmus, Chlorella and Stigleoclonium caused a rapid increase in the pH of raw and treated sewages. Alkalinity in amounts generally encountered in most sewages had little effect on pH response.

Lagoon cell	Day								
1	1	2	3	5	7	10	12	15	Remarks
1	7.7	7.6	8.4	8.7	8.5	9.4	9.5	10.1	Intermittent aeration plus artificial illumi- nation (400 F.C.) PM
2	8.0	7.7	8.4	8.8	8.9	9.2	9.2	9.6	Int. aeration: no
3	8.1	8.0	8.5	9.1	9.3	9.7	9.7	10.4	artificial illum. No. aeration; no artificial illum.
4	8.1	7.5	8.2	10.4				_	Int. aeration. Air off after day 6
Time of sampling	1:30 PM	11: AM	11: AM	11:30 AM	10:30 AM	3 PM	11:30 AM	12N	All lagoon cells filled with secondary
Weather	Cloudy	Cldy	Pt. Cldy.	Rain	Sunny	Pt. Cldy.	Sunny	Sunny	treatment plant ef-
Temperature ^O C			14		12	11	10	11	fluent and seeded with equal volume of algal culture.
Susp. solids mg/l		28	33	36	36	62	62	70	aigai cuiture.

Table 45. PHOTOSYNTHETIC pH CHANGES IN LAGOON CELLS - Autumn, 1958.

Cell Tissue Recovery

When it was found that Stigleoclonium stagnatile could be effectively recovered by sedimentation, the concept of screening as a recovery device was abandoned. Repeated use of a Stigleoclonium culture, such as during successive batch culture feedings or during cell tissue recycling in pilot plant operations, resulted in a marked improvement in settling characteristics. It thus appeared that adsorption of insoluble calcium phosphate salts by the algae caused coagulation of cell tissue, thereby increasing its rate of subsidence.

During pilot plant studies, the Stigleoclonium culture became contaminated with Chlorella and Scenedesmus. At temperatures above 20°C, Chlorella and Scenedesmus tended to predominate; Stigleoclonium was simply overgrown. This gave rise to some serious misgivings until it was observed that culture settling characteristics varied little with species owing to the coagulation effect of the insoluble phosphate salts produced at high pH levels. This is most interesting for it implies that the algae in most oxidation ponds might be effectively recovered by sedimentation if they are first permitted to increase pH levels above approximately 9.5 before recovery is attempted.

DISCUSSION

Algae are capable of removing phosphorus from solution both by metabolic uptake and by chemical coagulation and adsorption. Adsorption and coagulation appear to play the major role where rapid removal of large concentrations of phosphorus is involved. The relative significance of biological uptake depends, of course, on algal growth rate, environmental conditions, and upon time available for growth. In either case, it is the photosynthetic activity of the algae which governs the rate of removal.

High Rate Process

Laboratory pilot plant studies employing an illuminated contact unit followed by sedimentation have demonstrated that a high rate continuous flow process is functionally feasible when light is not limiting. Orthophosphate concentrations can be reduced to less than 1 mg/l within 6 to 12 hours. This is equivalent to a 90 to 95 per cent reduction in the phosphorus content of most sewages. Residual phosphate concentrations of about 3 to 5 mg/l were realized with contact times as brief as 2 to 4 hours.

While a high rate algal treatment process appears to be functionally sound, economic considerations restrict the use of algae to the more leisurely conditions prevailing in oxidation ponds and sewage lagoons. Process costs are related to the cost of pH adjustment, which in turn is a function of light requirements and holdup time. Where artificial illumination is employed, power requirements and hence, cost can be computed from a knowledge of lamp performance and the rate of light attenuation in the algal culture. Cost of pH adjustment employing artificial and natural illumination is compared in Figure 59 together with the cost of pH adjustment by lime alone. Lime requirements were computed from titration curves of a number of treated sewages in the Seattle area. Economical considerations appear to preclude the use of artificial illumination.

Lighting Limitations

Owing to a high rate of light attenuation, it is exceedingly difficult to maintain adequate illumination in large scale cultures. Theoretical considerations based on the Beer-Lambert Law indicate that it ordinarily would not be possible to maintain light intensities above 100 f. c. by natural or artificial means in depths much greater than

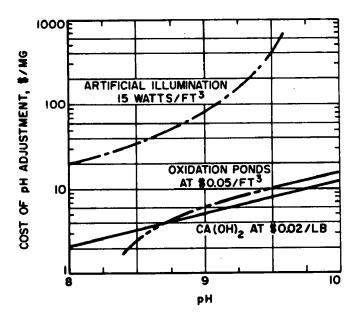


Figure 59. ECONOMIC COMPARISON OF VARIOUS METHODS OF ADJUSTING pH. Cost of electrical energy was taken at 0.01/kw-hr. Power requirements were based on high voltage fluorescent elements and a culture extinction coefficient of $2 \times 10^{-3} \text{ cm}^2/\text{mg}$. Oxidation pond costs were based on a construction cost of $0.05/\text{ft}^3$, a useful life of 20 years, and a liquid depth of 4 feet. Lime requirements were based on titration curves for sewages in the Seattle area; mechanical equipment costs were neglected.

one foot. Current practice is to construct oxidation ponds and lagoons with depths ranging from 3 to 5 feet.

As a practical matter where thorough mixing occurs, the effect of employing depths greater than that involved in photosynthesis is roughly equivalent to illuminating the entire culture at a proportionately lesser intensity. The net photosynthetic response is thus reduced and a longer contact time must be provided to achieve a given degree of pH adjustment. For example, it was found that 12 hours contact time at 100 f.c. was approximately equivalent to 10 to 12 days lagoon retention time under field conditions prevailing in the Seattle area during the fall of the year.

Detention Time Requirements

Theoretically, an oxidation pond should be capable of very high efficiencies of phosphorus removal. In order to realize this potential, active

photosynthesis and/or high pH conditions must prevail for some time prior to discharge. Where biological fixation is the principal mode of phosphorus removal, efficiency will be a function of detention time, growth rate, and cell tissue concentration.

During field pilot plant studies, algal cell tissue concentrations in lagoon units having a depth of 3 to 4 feet varied from 25 to 50 mg/l. Average growth rate was equivalent to a k of 0.30 day-1. Thus, in order to biologically extract 5 mg/l of P (equivalent to 80 to 90 per cent reduction for most sewages), it appears that lagoon retention times on the order of 14 to 28 days would be required. Theoretical retention time requirements for any other set of circumstances can be calculated from the data presented in Figure 53. These considerations suggest that pond volume in excess of that employed in most oxidation ponds may be necessary where a high degree of phosphorus removal is a treatment objective.

SUMMARY AND CONCLUSIONS

The concept of employing algae as a means of removing phosphorus from sewage was studied in the laboratory and in the field on a pilot plant scale. A high rate process was developed in the laboratory whereby soluble phosphate reductions equivalent to 90 per cent or better were achieved with contact times as brief as 6 to 12 hours. The process subsequently was studied in the field along with the behavior of conventional sewage lagoons. Certain aspects of this work were discussed in this paper and are briefly summarized below.

- 1. In the presence of adequate amounts of light, it is possible to realize rapid biological extraction of phosphate. Minimum light requirements appear to be in the vicinity of 100 to 200 f.c.
- 2. Under normal field conditions, adequate light intensities seldom prevail in algal cultures at depths much in excess of one foot. The use of deeper ponds, in common practice today, serves in effect to decrease net illumination roughly in proportion to the ratio of light and dark volumes. Photosynthetic reaction times are markedly increased.
- 3. Adsorption and coagulation appear to play the major role where rapid removal of large amounts of phosphate is involved. Metabolic conversion is the principal removal mechanism under the more leisurely conditions prevailing in oxidation ponds and sewage lagoons. In the latter case, efficiency of phosphate removal is proportional to detention time, other things being equal.
- 4. Three algae, <u>Chlorella</u>, <u>Scenedesmus</u>, and Stigleoclonium, were grown in raw and treated

sewages. Repeated reuse of these algae generally improved subsidence properties without noticeably impairing photosynthetic response. Settleability appeared to be influenced by the formation of insoluble phosphates at elevated pH. It appears that the photosynthetic pH shift may be employed as a means of enhancing the recovery of algae by sedimentation.

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REFERENCES

Anon. 1957. Development of design criteria for waste stabilization ponds. University of Texas, Civil Engineering Department, Final Report to the AEC, March 1, 1957.

Burlew, John S., Editor. 1953. Algal culture from laboratory to pilot plant. Carnegie Inst. of Washington, Publication 600, Washington, D.C.

Helmers, E.N., et al. 1952. Nutritional requirements in the biological stabilization of industrial wastes. Sewage and Industrial Wastes 24: 496.

Hoover, S.R., and N. Porges. 1952. Assimilation of dairy wastes by activated sludge - II. Sewage and Industrial Wastes 24: 306.

Oswald, W.J., et al. 1953. Algal symbiosis in oxidation ponds - \overline{II} Growth characteristics of Chlorella pyrenoidosa cultured in sewage. Sewage and Industrial Wastes 25: 26.

Oswald, W.J., and H.B. Gotaas. 1955. Photosynthesis in sewage treatment. ASCE Proceedings, <u>81</u>, Sep. No. 636.

Owen, R. 1953. Removal of phosphorus from sewage plant effluent with lime. Sewage and Industrial Wastes 25: 548.

Sawyer, C. N. 1953. Some new aspects of phosphates in relation to lake fertilization. Sewage and Industrial Wastes 24: 708.

Use of Algicides

THE PRACTICAL USE OF PRESENT ALGICIDES AND MODERN TRENDS TOWARD NEW ONES

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Prolific and extensive algal growths give rise to many damaging effects and hinder man in his full utilization of this great natural resource, water. These effects have been well documented (Bartsch, 1946; Bartsch, 1954; Mackenthun, 1952; and Mackenthun, 1958). Algae can cause tastes and odors in water supplies, clog filters in industrial and municipal treatment operations (Matheson, 1952) and interfere with the manufacture of a product in industry, such as paper. They may decrease the fishability of water, destroy a fish population through the elimination of the dissolved oxygen resulting from decomposition (Mackenthun, Herman and Bartsch, 1948), and decrease such recreational uses as swimming, skiing, speed boating, skin diving, etc. Some species have been found to produce toxic blooms which upon accidental or purposeful ingestion have been known to cause abdominal discomfort to man, and kill fish, pets, all types of wildlife, and domestic animals (Davidson, 1959; Ingram and Prescott, 1954; Mackenthun, Herman and Bartsch, 1948; Prescott, 1948; Rose, 1954; and Senior, 1960). Some wildlife kills, especially, have been most extensive. The decomposition of a large algal mass may liberate sufficient hydrogen sulfide to react with the lead compounds used as paint pigments, and discolor paint on lakeshore residences. The staining is especially noticeable on weathered white paint, forming brown specks or blotches. odors arising from the decomposition of this mass of algae and dead fish are so vile that cottages remain unrented, vacations are cut short, business is hampered, and property values are lowered.

Today our waters are subjected to the onrush of civilization, population pressures, year-around shoreline dwellings, increased pollution, and the concept of multiple use. Because of these increasing demands placed upon our recreational waters, adequate control of an algal nuisance is of utmost importance, especially to those who live in close association with highly productive lakes.

An ideal algicide for adequate control should possess certain attributes (Mackenthun, 1959). It must kill the specific nuisance plant or plants, be

non-toxic to fish and most fish-food organisms at the plant-killing concentration, not prove seriously harmful to the ecology of the general aquatic area, and be of reasonable cost. Since 1904 (Moore and Kellerman, 1904) and up to the present time, the chemical which has most nearly met these specifications for the control of algae has been copper sulphate (blue vitriol). Despite this extensive usage, copper sulphate does have shortcomings in that it may poison fish and other aquatic life in excessive concentrations, it may accumulate in bottom muds as an insoluble compound following extensive usage, and it is corrosive to paint and equipment (Bartsch, 1954).

Initial attempts at prescribing definite dosage rates for the control of various types of algae with copper sulphate were first undertaken by Moore and Kellerman in 1905 (Moore and Kellerman, 1905) and have been extensively reprinted in tabular form giving specific dosages for some 70 organisms (Hale, 1954). The practical application of such a table is difficult, however, because of the many variables which one encounters in nature. Because solubility of copper in water is influenced by pH and alkalinity as well as temperature, the dosage required for control depends not only upon the chemistry of the water itself, but also upon the species or genus variation of the organisms and their resistance to copper sulphate. Thus, rather arbitrary dosage rates have been successfully used, especially in the midwestern states, for a number of years (Mackenthun, 1958; and Bartsch, 1954). Since a total alkalinity of 40.0 ppm seems to be a natural separation point between soft and hard waters (Moyle, 1946), those lakes which have a total methyl orange alkalinity of 40 ppm or greater are treated at a rate of 1 ppm blue vitriol for the upper 2 feet of water regardless of actual depth. On an acreage basis, this concentration would amount to 5.4 pounds of commercial copper sulphate per surface acre. The 2-foot depth has been determined to be about the maximum effective range of a surface application of copper sulphate in such water since algae will be killed with increasing depth only if the rate of downward diffusion exceeds the rate of copper precipitation. The algae killed by such a treat-

ment are those which are suspended near the water surface and which commonly occur with blooms in calm weather. In lakes with a total methyl orange alkalinity below 40 ppm, a concentration of 0.3 ppm commercial copper sulphate for the total volume of water has been selected. This is roughly comparable to 0.9 pounds of copper sulphate per acre foot of water. It is obvious that if a low alkalinity lake has an average depth of about 6 feet, the dosage would be about the same as in a high alkalinity lake of the same area. When lesser average depths are involved, a greater concentration of chemical results in the high alkalinity lakes. This apparent paradox would be even more striking were it not for the fact that in low alkalinity lakes, the algae frequently are of the filamentous types that may lie at the bottom and, therefore, the entire volume of water must be calculated to insure that a sufficient concentration of the chemical will reach the algae to effect a kill. In high alkalinity lakes, algae frequently are planktonic and tend to concentrate near the surface which is the only stratum at which appreciable concentrations of soluble copper can be expected. Certainly, when copper sulphate is used as the algicide, the best and most lasting control will result if the lake water has a total alkalinity around 50 ppm or less.

Algal control treatments may be marginal or complete and the type applied to a given body of water must be determined by the size, shape, and relative fertility of the water, and the estimated cost of the project. Complete treatment in which the calculated amount of copper sulphate is systematically applied over the entire surface area is the most satisfactory. It insures that a major portion of the total algal population is eliminated at one time so that a long algal recovery period is required before a bloom condition may again occur. The interval of time between necessary treatments will be directly correlated with climatalogical conditions and the available nutrients utilizable by the remaining algal cells which are not killed as a result of chemical application. One to three complete treatments per season may be sufficient to give reasonable control. Marginal treatment, on the other hand, is a method designed to obtain temporary relief in a restricted area where more extensive activity is not practicable or financially possible. It is a procedure in which a lane 200 to 400 feet wide, lying parallel with the shore, and all protected bays are sprayed in the same manner as for complete treatment. All other parts of the area are not affected even though much algae may be present.

As a result of treatment, the algal population and the intensity of odors along the periphery of the lake are reduced. The duration of freedom from the algal nuisance following this type of treatment is dependent upon the density of the algal population in the center of the lake and its ability to infiltrate the treated area through the action of wind, waves, and currents, in addition to those repopulation factors presented for a complete treatment operation. Any marginal control operation should definitely be considered on a periodic repeat basis. Providing fertility is not excessive, large bodies of water might gain enough relief from marginal treatment to warrant this type of control. However, it would probably be a waste of money to consider this type of control for a large, fertile lake which is long and narrow and subject to considerable wind and wave action. Complete treatment in this case might be the only present answer, and the cost of complete treatment might be prohibitive.

Copper sulphate may be applied in a variety of ways: bag-dragging, dry feeding (Monie, 1956), liquid spray (Mackenthun, 1958), and airplane application of either dry or wet material (Anon., 1959). Regardless of the method, rapid and uniform distribution of the algicide is essential. Therefore, the size and scope of the problem would determine to some extent the method employed. In general, liquid spraying systems operated from a boat or barge have thus far been most widely used.

Copper sulphate is a highly corrosive chemical. Therefore, materials which are used in the construction of spraying equipment should be resistant to its corrosive nature. These include red brass, containing 85% copper and 15% zinc; commercial bronze, containing 90% copper and 10% zinc; stainless steel; and a good grade of copper. Cast iron is unsuitable. A more complete list of materials may be found in the Chemical Engineer's Handbook (Anon., 1950).

The life of chemical spraying equipment depends to a great extent upon the care given the equipment. Equipment which is used in spraying a corrosive chemical should be thoroughly cleaned in clear water following the spraying operation. This may be accomplished by spraying clear water at the end of treatment before the equipment is taken apart. It is most important that a residue of copper sulphate not be left in the equipment at the end of the operation, thus producing corrosion over a long period of time.

Essential equipment for the distribution of a solution of copper sulphate is illustrated in Figure 60. The equipment, as shown, can be easily fabricated for about \$200. The copper sulphate, crystals or powder, is placed in the chemical solution tank to which water is constantly added as the saturated copper solution is withdrawn. The saturated copper solution is bled into the suction line containing clear lake water producing a 2% to 5% solution. This is drawn through the pump and discharged through a smooth fire hose nozzle which is continuously swept back and forth over

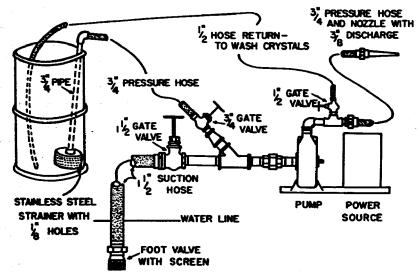


Figure 60. BASIC EQUIPMENT DESIGN

the area receiving treatment so that good distribution is obtained. A small portion of the discharge solution is returned to the chemical solution tank to furnish dilution water for the crystals. This will naturally reduce the nozzle pressure and decrease the diameter of the spray pattern. To compensate for this, the equipment may include a small auxiliary pump whose sole function is to maintain a constant level of dilution water in the chemical solution tank. Other modifications may include the substitution of a California redwood baffled wooden box for the chemical solution drum, the substitution of a burlap bag of chemical for the stainless steel strainer in a chemical solution tank, and the addition of mechanically oscillating spray nozzles. A wooden barrel may be used as a chemical solution tank since it will not corrode and lasts well; however, an ordinary steel drum will usually suffice for one season providing the treatment is not too extensive. A good quality of pressure hose or steam hose, with similar fittings and clamps, should be used for all hose connections in assembling the equipment.

The selection of the pump which will exert the right pressure on a particular size of nozzle to produce the desired volume of discharge and spray combination should be given consideration. Early experiments (Fleming, 1918) on fire hose and small nozzles supplied basic information on the distance of throw when the nozzles are operated under varying pressures (Table 46). It is indicated that the tip of the nozzle should be reamed out for a distance of at least one-half inch in order to obtain a good fire stream.

The boat, barge, or launch which is used to transport the spraying equipment is equally as important as the equipment itself—and generally, more costly. It must be adequate to carry the equipment, required personnel, and additional chemical. Speed and ease of operation are essentail for good chemical distribution.

The amount of commercial copper sulphate to apply to a given area can be readily calculated either by arithmetic or through the use of a dosage

Table 46. DISCHARGE FROM SMALL NOZZLES AT VARYING PRESSURES.

Results of Experiments at University of Illinois with Small-size Nozzles attached to 1-1/2-inch hose.

		\$/16" Nozzle			1/2" Nozzle			
	Discharge (g.p.m.)	Good; hori- sontal fire stream jet (ft.)	Extreme hori- zontal drops (feet)	Discharge (g.p.m.)	Good hori- zontal fire stream jet (ft.)	Extreme hori- zontal drops (feet)		
20	12	15	53	33	15	63		
30 40	15	18	63	40	20	79		
40	17	21	71	46	25	91		
50	19	23	78	52	30	102		
- 60	21	26	84	57	33	111		
70	23	28	90	61	37	120		
80	-24	29	96	65	40	127		
90	26	30	102	69	43	. 134		
100	28	31	107	73	46	140		

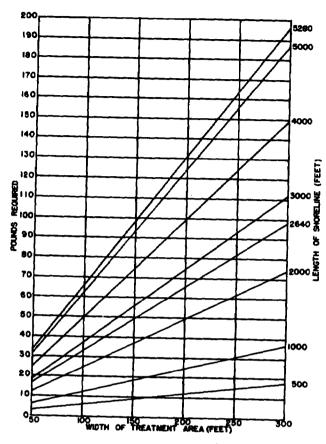


Figure 61. COMMERCIAL COPPER SULPHATE DOSAGE CHART FOR LAKES WITH A METHYL ORANGE ALKALINITY GREATER THAN 40 PPM. Treating a mile of shoreline for a width of 200 feet requires 130 pounds of blue vitriol.

chart (Figure 61). The area of the spray pattern and the concentration of chemical solution as it leaves the nozzle determine the concentration in the receiving water. It thus remains for the operator to effect an even distribution of the calculated amount of chemical within a given specified area. This may be quite easily accomplished by adjusting the percentage of the solution sprayed with the diameter of the spray pattern and the speed of the boat. Acidified copper sulphate standards containing a 1%, 2%, 3%, 5%, and 8% solution of commercial copper sulphate should always be available for testing purposes. The test is made by collecting a small sample from the pump discharge, adding sufficient acid (powdered citric acid has been found easiest to handle in the field) to clear up the precipitate and matching the test color with the standard colors to determine the percentage of solution being sprayed. Other methods include the use of the known pump capacity to determine that a given percentage solution will involve the distribution of a given number of pounds of copper sulphate within a given number of minutes. In this method, the speed of the boat must be regulated so that the entire area is covered in the specified time. Calculations can also be made using the average speed of the boat or barge coupled with the diameter of the spray pattern to determine the pounds of chemical which must be used in a given amount of time.

The relative cost for the control of algae through the use of copper sulphate on high alkalinity lakes is in the neighborhood of \$1 per acre for chemicals, plus the cost of distribution for each treatment undertaken. The cost of distribution may range anywhere from 25¢ to \$1 per acre, depending upon the methods used in control and the circumstances surrounding the particular control project. In a liquid spray operation from a boat or barge, it is possible to distribute about 300 to 350 pounds of commercial copper sulphate per hour of spraying time, thus covering about 60 acres per hour.

The effect of copper sulphate treatment on an algal population can be noted soon after the treatment is applied. Within a few minutes, the color of the water will change from dark green to grayish-white. Although at no time are all the algae in the lake entirely eliminated (Domogalla, 1926), the water should be visibly free of cells two or three days following a complete application. Treatment has proven beneficial, both when applied to large bodies of water (Domogalla, 1935; and Domogalla, 1941) and also to small fishrearing ponds (O'Donnell, 1945). Both in Wisconsin and Minnesota (Moyle, 1949), there is an indication that certain species of algae, particularly Aphanizomenon, seem to have acquired an increased tolerance to copper as a result of many years of treatment. Two to five times as much copper sulphate must now be used as was necessary some 26 years ago to achieve similar control.

Much has been recorded on the toxicity of copper sulphate to various forms of aquatic life (Doudoroff and Katz, 1953; Ellis, 1937; Marsh and Robinson, 1910; Prescott, 1948; Rushton, 1924; Schaut, 1939; and Anon., 1939). Extensive field and laboratory studies have clearly shown that fish are not killed by copper sulphate at concentrations normally used for algal control and that fishing and fish yields of lakes which have been treated over a long period of time have not deteriorated (Moyle, 1949). It is well known that copper will accumulate upon the lake bottom following repeated treatments and that the greatest amount of accumulation is found in the profundal region (Nichols, Henkel and McNall, 1946). Attention has also been directed to the possible deleterious effect of this accumulation on lake ecology (Hasler, 1947; Schoenfeld, 1947; and Schoenfeld, 1950). However, it has been shown

experimentally that the accumulation of copper in bottom muds resulting from the use of nearly two million pounds of copper sulphate to control algae in a hard-water lake over a period of 26 years was considerably lower in concentration than the amounts determined to have a deleterious effect on the profundal bottom-dwelling organisms (Mackenthun and Cooley, 1952). More recent work (Antonie and Osness, 1959) on this same lake (Lake Monona, Madison, Wisconsin) has shown that the copper content is significantly less in the upper 1" of bottom mud than in the stratum 6" lower. Since this lake has received reduced treatment with copper sulphate since 1947 and practically no control since 1953, this would suggest that the constant seepage of silt into the deeper areas of the lake will in time cover up the heavy concentrations of copper that were deposited during the years of continuous and heavy treatment.

In recent years, there has been a constant search to find a chemical replacement for copper sulphate which would be more effective, nonaccumulative, and perhaps even less costly over an extended period of time (Maloney and Palmer, 1956; and Palmer and Maloney, 1955). Work has been done on Phygon XL (Fitzgerald, Gerloff, and Skoog, 1952; and Fitzgerald and Skoog, 1954), which is rather selective for blue-green algae, and more recently on related naphthoquinone compounds which are believed to be less toxic to fish. Delrad (dehydroabietylamine acetate) has proven successful under specific situations (Johnson, 1955; and Lawrence, 1954), but care must be exercised in its use because of its toxicity to fish in some waters. Some newer algicides are just now entering the field-testing phases following preliminary work. These will require many tests and observations under the varying conditions found in nature before their future potential can be determined. In all cases, where a newer algicide is involved, the cost of treatment of a large area has thus far exceeded the cost based upon the use of copper sulphate (Hooper and Fukano, 1955) and none have made up the difference in the amount of control offered. As a result, present day usage of some of the newer algicides has been confined to the smaller operations where a more costly chemical is feasible. Copper sulphate remains as the most effective, least harmful, and least expensive algicide for large-scale control at the present time.

Occasionally the need arises for an algicide to control a specific type of growth. By and large, however, the problem is one of a general algicide to control a general algal nuisance under the majority of circumstances encountered in field operations. This, then, is the basic challenge which must be met by a striving "new" chemical—it must control a general algal nuisance at a concentration not seriously harmful to lake ecology and it must achieve control at a competitive cost

to existing standard procedures. If the cost is greater, it must have more desirable qualities to offer, and, generally speaking, it must either do a better or a more lasting job of control.

Any algal control measures should be undertaken at the right time in the development of the algal bloom and this is especially true in treating confined areas. If, for some reason, treatment is not applied to a given area until the algal cells have become dense, one must use good judgment in determining the extent of the area which should receive treatment at one time. Usually, it is a good practive to sub-divide the area into sections in an attempt to control the nuisance in one section at a given time. Other sections may be treated later to insure that sufficient dissolved oxygen is present to satisfy the demand of the decomposing algae.

Recently, a questionnaire was directed to each state to obtain information on aquatic nuisance control activities. The problems of algal nuisances vary considerably from state to state. Many states do receive requests and many do carry out some type of control program. Some states report that no problem exists and some report a much reduced problem because of high water turbidity. In many states, it is necessary to receive authorization prior to the treatment of public waters for algal control. About 40 per cent of the states regulate the introduction of chemicals for the control of aquatic nuisances by statute or executive order and require a permit for the introduction of chemicals into public waters. About 57 per cent of the states report complete supervision of field application of chemicals and an additional 21 per cent spot check on field applications.

Wisconsin is the only state with a long-standing special statute pertaining specifically to aquatic nuisance control and the only state which assesses an established fee for permits and a definite rate of charge for supervisory service. To set up a project, an application for permit and a \$10 application fee is filed with the Wisconsin Committee on Water Pollution. The Committee determines, through the law enforcement division of the Conservation Department and the appropriate county clerk, if there are any objections on the part of citizens to the proposed chemical application. If objections are forthcoming within a specified period of time, a meeting or possibly a hearing is held and as a result the permit is granted, rejected, or modified. Upon receipt of a permit, the sponsoring party may hire one of the commercial operators who will carry out treatment in accordance with a specified plan which the operator submits to and is approved by the Committee, or the sponsoring party may provide suitable equipment and all necessary materials and labor to complete the project. Under either method, the distribution of chemicals into public waters for purposes of aquatic nuisance control must be supervised by a representative of the Committee on Water Pollution. There is a charge of \$8 per hour or fraction thereof for this supervisory service, or a minimum seasonal charge of \$20 if the seasonal supervision requirements do not exceed two hours of supervisory service.

Although 21 states report the use of copper sulphate for the control of algae, the greatest

large-scale use is apparently confined to the midwestern lake states. Minnesota, in 1959, reports the use of 221,745 pounds of copper sulphate in the treatment of 17,574 acres of water. In Wisconsin, 6,270 acres on 29 lakes received 54,765 pounds of copper sulphate for algal control. Other states report the use of lesser amounts. The majority of this chemical, at the present time, is distributed through the use of pumps powered by gasoline engines and transported by boats or barges.

REFERENCES

Anonymous. 1939. Chemical treatment of lakes and streams. Wisconsin Committee on Water Pollution, Wis. State Board of Health.

Anonymous. 1950. Chemical Engineers' Handbook. Textbook Edition, McGraw Hill Book Company, p. 1426.

Anonymous. 1959. Great Lakes Newsletter. Great Lakes Commission, Rackham Bldg., Ann Arbor, Michigan 3(6): 1-10, July 1959.

Antonie, C. J. and W. H. Osness. 1959. Toxicityand accumulation of copper as used in the copper sulfate treatment of lakes. West High School, Madison, Wis. (Mimeo)

Bartsch, Alfred F. 1946. Aquatic nuisance control in Wisconsin. Wisconsin Committee on Water Pollution, Madison, Wis. 35 pp.

Bartsch, Alfred F. 1954. Practical methods for control of algae and water weeds. Public Health Reports 69(8): 749-757.

Davidson, Floyd F. 1959. Poisoning of wild and domestic animals by a toxic waterbloom of Nostoc rivulare Kuetz. Jour. Amer. Water Works Assoc. 51(10): 1277-1287.

Domogalla, Bernard. 1926. Treatment of algae and weeds in lakes at Madison, Wisconsin. Engineering News-Record, pp. 3-7, Dec. 9, 1926.

Domogalla, Bernard. 1935. Eleven years of chemical treatment of the Madison lakes - its effects on fish and fish foods. Trans. Amer. Fish. Soc. 65: 115-120.

Domogalla, Bernard. 1941. Scientific studies and chemical treatment of the Madison lakes. A Symposium on Hydrobiology, University of Wisconsin Press, Madison, Wis., pp. 303-310.

Doudoroff, Peter and Max Katz. 1953. Critical review of literature on the toxicity of industrial wastes and their components to fish. II. The metals, as salts. Sewage and Ind. Wastes <u>25</u>(7): 802-839.

Ellis, M.M. 1937. Detection and measurement of stream pollution. Bulletin No. 22, U.S. Bur. of Fisheries; Bull. Bur. Fisheries 48: 365-437.

Fitzgerald, George P., Gerald C. Gerloff, and Folke Skoog. 1952. Studies on chemicals with selective toxicity to blue-green algae. Sewage and Ind. Wastes 24(7): 888-896.

Fitzgerald, George P. and Folke Skoog. 1954. Control of blue-green algae blooms with 2,3-Di-chloronaphthoquinone. Sewage and Ind. Wastes, 26(9): 1136-1140.

Fleming, Virgil R. 1918. Fire streams from small hose and nozzles. University of Illinois, Bulletin No. 105 XV(37): 43-60.

Hale, F. E. 1954. Use of copper sulphate in control of microscopic organisms. Phelps Dodge Refining Corp., New York, 30 pp., 6 plates.

Hasler, Arthur D. 1947. Antibiotic aspects of copper treatment of lakes. Wis. Acad. Sci., Arts and Lett., 39: 97-103.

Hooper, F. F. and K. G. Fukano. 1955. Laboratory and field experiments with new algaecides, 1944-1955. Report No. 1461 of the Institute for Fisheries Research, Michigan Dept. of Cons., Ann Arbor, Mich.

Ingram, W. M. and G. W. Prescott. 1954. Toxic fresh-water algae: Summary article of literature. Amer. Mid. Nat. 52(1): 75-87.

Johnson, Leon D. 1955. Control of <u>Ulothrix zonata</u> in circular ponds. Prog. Fish Culturist <u>17(3)</u>:126-128.

Lawrence, J. M. 1954. Control of branded alga, Pithophora, in farm fish ponds. Fish Culturist 14 (2): 83-88.

Mackenthun, Kenneth M. 1952. Cleaner lakes can be a reality. Wis. Cons. Bulletin 17(1): 1-4.

Mackenthun, Kenneth M. 1958. The chemical control of aquatic nuisances. Wis. Committee on Water Pollution, Madison, Wis., 64 pp.

Mackenthun, Kenneth M. 1959. Summary of aquatic weed and algae control research and related activities in the United States. Wis. Committee on Water Pollution, Madison, Wis., pp. 1-14. (Mimeo)

Mackenthun, Kenneth M., Elmer F. Herman, and Alfred F. Bartsch. 1948. A heavy mortality of fishes resulting from the decomposition of algae in the Yahara River, Wisconsin. Trans. Amer. Fish. Soc., 75: 175-180, (1945).

Mackenthun, Kenneth M. and Harold L. Cooley. 1952. The biological effect of copper sulphate treatment on lake ecology. Trans. Wis. Acad. Sci., Arts and Lett. 41: 177-187.

Maloney, Thomas E. and C. Mervin Palmer. 1956. Toxicity of six chemical compounds to thirty cultures of algae. Water and Sewage Works 103(10): 509-513.

Marsh, M. C., and R. K. Robinson. 1910. The treatment of fish-cultural waters for the removal of algae. Bull. Bur. Fisheries 28 (Part 2), 871-890, (1908).

Matheson, D. H. 1952. The effects of algae in water supplies. General report prepared for the 1952 Congress for the International Water Supply Assoc., Paris, 82 pp.

Monie, W.D. 1956. Algae control with copper sulfate. Water and Sewage Works 103(9): 392-397.

Moore, George T. and Karl F. Kellerman. 1904. A method of destroying or preventing the growth of algae and certain pathogenic bacteria in water supplies. Bulletin 64, Bureau of Plant Industry, U. S. Dept. of Agriculture.

Moore, George T. and Karl F. Kellerman. 1905. Copper as an algicide and disinfectant in water supplies. Bull. Bureau of Plant Industry, U.S. D.A. 76: 19-55.

Moyle, John B. 1946. Some indices of take productivity. Trans. Amer. Fisheries Soc. <u>76</u>: 322-334.

Moyle, John B. 1949. The use of copper sulphate for algae control and its biological implications. Limnological Aspects of Water Supply and Waste Disposal. Pub. of the Amer. Assoc. for the Adv. of Sci., Washington, D.C., 79-87.

Nichols, M. Starr, Theresa Henkel and Dorothy McNall. 1946. Copper in lake muds from lakes of the Madison area. Trans. Wis. Acad. Sci. 38: 333-350.

O'Donnell, D. John. 1945. Control of Hydrodictyon reticulatum in small ponds. Trans. Amer. Fisheries Soc., 73: 59-62, 1943.

Palmer, C. Mervin and Thomas E. Maloney. 1955. Preliminary screening for potential algicides. Ohio Jour. of Sci. 55(1): 1-8.

Prescott, G.W. 1948. Objectionable algae with reference to the killing of fish and other animals. Hydrobiological, 1-13.

Rose, Earl T. 1954. Blue-green algae control at Storm Lake. Proceedings of Iowa Acad. of Sci., 61: 604-614.

Rushton, W. 1924. Biological notes. Salmon and Trout Magazine, No. 37, 112-125.

Sawyer, C. N., J. B. Lackey, and A. T. Lenz. 1945. An investigation of the odor nuisance occurring in the Madison lakes, particularly Monona, Waubesa, and Kegonsa from July, 1943 - July, 1944. Rept. Governor's Committee, 92 pp., 25 fig., 27 tables.

Schaut, G. G. 1939. Fish catastrophes during droughts. Jour. Amer. Water Works Assoc., 31(5): 771-882.

Shoenfeld, C. 1947. Don't let 'em spray. Field and Stream, No. 4: 46, 79, 80, 81, August 1947.

Shoenfeld, C. 1950. The case against copper. Hunting and Fishing, 11, 12, July 1950.

Senior, V. E. 1960. Algal poisoning in Saskatchewan. Canadian J. of Comparative Medicine and Veterinary Science 24(1): 26-30, Jan. 1960.



RESEARCH NEEDS IN WATER QUALITY CONSERVATION

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I should like to thank the Program Committee for approving the subject of this talk. Such assignments force us to contemplate our position with respect to the adequacy of the tools and techniques of the profession. This is a hard and sometimes thankless task—but it is an essential function of the Robert A. Taft Sanitary Engineering Center.

The SEC is in an advantageous position for securing such information because of the many requests we receive for assistance on difficult and unusual problems. Although our relatively small resource permits us to respond to only a few of these requests, knowledge of important new problems is literally forced on us. This is desirable from our point of view. Not only do we learn of emerging problems, we obtain guidance as to the direction our research should take. I propose to base my discussion largely on the information so obtained.

We find that our uncertainty touches on every aspect of a waste's origin, behavior and ultimate fate. Proceeding in logical fashion from the origins of waste to ultimate water reuse, our broad areas of ignorance are encompassed by the following questions:

Do we account for all pollution reaching streams?

Having a new waste in hand, can we predict its potential pollutional effects?

Assuming we can determine tolerance levels for this waste, can we really treat it effectively?

In disposal of waste effluents, do we make effective use of the receiving water?

Having disposed of the waste effluent, can we monitor effectively the quality of waters which have received it?

Can we effectively treat polluted water for reuse?

Each of these questions offers its major challenge to research.

ORIGIN OF WASTES

Do we account for all pollution reaching streams?

When we think of pollution, we normally consider sewage and industrial wastes. This is not surprising. One expects an industrial plant to produce an industrial waste just as a community produces sewage. But only recently have we begun to consider the growing pollutional effect of land drainage, particularly that from agricultural lands. Even more recently have we become aware of pollution resulting from chemical poisons deliberately applied to the water course to kill weeds, insect larvae, fish, and other forms of We thus have at least four major aquatic life. avenues through which man-made pollution may reach streams: sewage, industrial wastes, land drainage, and direct chemical applications. Each warrants close study by the sanitary engineer.

- 1. Sewage which is assuming new, important characteristics. For example, synthetic organic chemicals are often undetected and unsuspected components of sewage. It is probable that the presence of alkyl benzene sulfonate, the basic ingredient of household synthetic detergent, would be unsuspected if it were not associated with foam. One could speculate on the presence of other substances, equally resistant to treatment and equally persistent in the stream, which give no physical or chemical clues to their identity.
- 2. It is not necessary to belabor the point that the "traditional" industrial wastes, the dinosaurs of our experience, are still major problems. However, new and perhaps even more troublesome wastes are being encountered. Increasing interest is being directed to radioactive wastes from uranium ore refining plants and nuclear power reactors, thermal pollution from power plants and industrial cooling operations, and the vast complex of waste substances discharged by the chemical industries. Synthetic organics are a particular problem because of the very rapid growth of the petrochemical industries. Many of these organic compounds resist destruction by the communities of microorganisms on which we depend for or-

ganic waste stabilization in the treatment plant and in the stream. They may persist almost indefinitely in the stream and reach locations of water use many miles below the point of waste discharge. An interesting example is a benzene derivative we followed 1000 miles down the Mississippi River.

- The increasing use of agricultural poisons, particularly insecticides, is reflected in the appearance of these chemicals in bodies of water receiving farm land drainage. DDT has frequently been recovered from streams, and even from treated water supplies. One of the new chlorinated hydrocarbon insecticides is the most toxic material yet encountered for fish. It has been used in Pennsylvania to destroy rodents in orchards. Like other such substances, it is persistent in water solution and is not easily removed by conventional water treatment processes. As materials of increased potency come on the market, public concern with their impact on water use must inevitably grow. One aspect of this problem is to determine how to control a waste whose source is general land drainage rather than a specific outfall sewer.
- 4. The growing use of synthetic organic poisons to destroy nuisance water vegetation, mosquito larvae, and undesirable fish has introduced new water quality control problems. Dickinson, North Dakota, provided us with a typical case. To destroy worthless coarse fish prior to restocking with game fish, it was proposed that a fish poison be applied to the municipal raw water impoundment in use. As one would expect, the health authorities were concerned with the ability of the water treatment plant to prevent this material from reaching the treated water. With the increasing recreational appeal of stored waters, it is clear that problems of this kind will be experienced widely and frequently.

Is our concern with these synthetic organics exaggerated? Of the many thousands of compounds of industrial origin reaching waterways daily, we have information on only a few. However, we can't help being impressed by the low concentrations in which these materials may exert their pollutional effects. For example, I have mentioned a certain chlorinated hydrocarbon insecticide which will kill fish in a concentration of less than 1 ppb. Phenols in concentration of just a few ppb have been implicated in taste and odor problems in drinking water. Alkyl benzene sulfonate has been associated with frothing in concentrations of about 1 ppm. Ignorance of the effects of other compounds does not certify their harmlessness.

The possibility of toxicity for humans is a dominating concern. However, we know of no case of human illness in which synthetic organic substances in a public water supply have been involved. Yet, we cannot escape the fact that as the number and size of plants engaged in synthetic organic chemical manufacture increase, their wastes will increase in flow and complexity. No matter how slowly the concentrations build up in receiving waters, this situation may have ultimate public health implications. Here I should like to emphasize that it is not the acute episode we fear—this would be readily identified. It is the insidious effects that could be associated with repeated exposure over long periods of time to low concentrations of the waste.

POLLUTIONAL CHARACTERIZATION OF A NEW WASTE

Having a new waste in hand, can we predict its potential pollutional effects?

Can we confidently predict the effect of the waste on the dissolved oxygen of the stream, its toxic effect on fish and other aquatic life, its tainting effect on fish flesh, its possible damage to water treatment processes, its effect on water palatability, and its harmful effect on persons who drink that water?

I think you will agree that few agencies are presently equipped to do more than estimate the waste's probable effect on the dissolved oxygen of the stream, and to determine, within rough limits, its toxic effect on fish in the receiving water.

Several years ago, the Center undertook to evaluate its ability to make such predictions. We selected a group of six organic cyanides as the wastes of challenge. Chemists, engineers, biologists, and toxicologists were assigned to determine the pollutional character of these com-They were instructed to exercise pounds. ingenuity wherever standard methods and equipment could not be used. Improvisation was necessary. For example, it was found that conventional organic analysis could not detect nor measure the low concentrations of compounds found to be significant. Quantitative analysis based on fish bioassay solved this particular problem.

Our experience indicates that a well-equipped and well-staffed laboratory can obtain the information needed to predict with confidence a new waste's probable impact on certain important downstream water uses. However, it is an expensive, time-consuming project—not one to be undertaken as a routine task. The toxicological phase of the study is perhaps its most perplexing aspect. The specialized services and cost necessary for determining the effect of repeated exposure to low concentrations of the waste for long periods of time would inevitably place this job out of reach of most public agencies. Equally discouraging, perhaps, is the probability that the toxicological study may take as long as two years.

WASTE TREATMENT

Assuming we can determine tolerance levels for this waste, can we really treat it effectively?

Many yardsticks of waste strength and charac-Biochemical Oxygen Demand ter are in use: (BOD) is unquestionably the most common. With the best treatment processes available, a BOD reduction of approximately 95% may be obtained. A reduction in BOD does not mean that the objectionable mineral salts—nitrates and phosphates have been removed. Just the opposite effect is produced in sewage stabilization by secondary treatment. Likewise, the soluble inorganic constituents of wastes are not removed by conventional secondary waste treatment processes. And it has already been noted that many persistent organics discharged in sewage and in chemical industry wastes do not have a BOD. They are either non-oxidizable or they exert their oxygen demand so slowly as to be considered nonoxidizable. Obviously, a reduction in a waste's BOD gives no assurance that such compounds have been removed or destroyed.

The possibility that so-called complete treatment of wastes may not always be sufficient, was pointed out by G. E. McCallum, Chief, Division of Water Supply and Pollution Control, Public Health Service, at the 1959 Purdue Industrial Wastes Conference. A new interest has been stimulated in the future's need for tertiary treatment processes and, beyond that, for ultracleansing of wastes. You may be interested in a few of the investigations under consideration at the Center.

- systems important in waste treatment. The potential of biological communities for breaking down organic compounds should be fully realized. This naturalistic treatment must, in our opinion, be exploited to the maximum because it costs less than other methods, and because it is a common sense preliminary to any necessary treatment of organic substances by non-biological methods. Research may yield valuable information on how to break down certain kinds of chemical structures now invulnerable to microbial attack and how to stimulate development of more adaptable microbial enzyme systems.
- 2. Fundamental studies on particle separation by filtration. The ultimate potential of filters for particle removal should be established. The relationship of filter design to the size of particles to be removed has not hitherto been adequately investigated. Because of the obvious usefulness of filters at points of waste production, and their very wide employment at points of water use, we should know the ultimate value of this unit process. As part of this project, methods could be explored for producing chemical flocs of controlled size, den-

sity, and toughness. Such flocs deposited on filters of various kinds will permit the realization of maximum filter potential.

- 3. Application of adsorption principle to waste treatment. Effective treatment of complex organic wastes may require, in many cases, the intelligent use of the principle of adsorption. For a number of years, the Center has studied intensively the adsorption of soluble organics from sewage and industrial waste streams and receiving waters. Many materials have been evaluated for their adsorption properties, with special attention directed to activated carbon which has been found, so far, to be the adsorbant of choice. In the summer of 1958, a special evaluation was made of the factors influencing adsorption of organic contaminants. The problem of regeneration of adsorbants is of paramount importance.
- 4. Destruction of organics by strong chemical oxidants. The feasibility of destroying the organic content of wastes by strong chemical oxidants such as ozone and hydrogen peroxide should be investigated. In this way, complex organic compounds may be converted to relatively simple inorganic structures which might be removed by some appropriate procedure.

Other physical-chemical phenomena may be useful in waste treatment. Advanced methods of freezing, ion exchange, solvent extractions, and foam fractionation should also offer interesting research opportunities in water cleansing.

WASTE DISPOSAL

In disposal of waste effluents, do we make effective use of the receiving water?

Presumably, available knowledge and experience on lateral mixing and dispersion of wastes in streams should permit full use of the stream's dilution ability. However, streaks of waste may often be traced many miles along a water course. Obviously, the full dilution effect is not being used. This often poses stream sampling problems because of variation in waste concentration in a cross-section of a stream. It also raises pertinent questions regarding the adequacy of outfall design techniques.

Many of you have personal knowledge of the relatively poor state of the art of waste disposal into tidal waters. A profound question is, "What is the fate of a particle of waste discharged into a mass of tidal water?" The intelligent use of coastal waters and estuaries for waste disposal was the subject of an International Conference held last year at the University of California in Berkeley. Every one of the 24 coastal and Gulf States is seeking, or will inevitably seek, effective techniques to answer this question. The use of models to supplement field studies is a recent interesting development.

WATER QUALITY MONITORING

Having disposed of the waste effluent, can we monitor effectively the quality of waters which have received it?

Until recently, few programs of water quality intelligence could present a realistic picture of changing quality conditions in a stream, and those were usually developed at great expense in special problem situations. You may recall that in December, 1955, the unsatisfactory condition regarding knowledge of the quality of our water impelled the President's Advisory Committee on Water Resources to note that improvement in basic data on water quality was urgent.

The monitoring problem has three important phases: to define what constitutes meaningful data for a given stream; to apply appropriate sampling and analytical procedures; and to fit these data into a scheme of known water uses, waste discharges, and stream flow to permit correct interpretation of data. Obviously, mere collection of data is not helpful if they do not truly reflect changes in water quality.

We are not badly off with respect to detection of certain types of pollution. Recent improvements in sampling and analytical techniques for coliform organisms and enterococci permit us to discern fecal contamination more readily than before. The successful development of equipment for continuous recording of dissolved oxygen represents a significant advance in the monitoring of streams for oxygen demanding wastes. The current emphasis on radioactivity monitoring is providing steadily improved instrumentation for this program. However, many important shortcomings remain.

Perhaps the most significant defect in data collection programs is the inadequacy of procedures for detecting pollution by highly stable organic compounds of industrial origin. A good example is the compound described earlier which could be recovered 1000 miles below its point of introduction into the stream. Its presence was suggested by an unusual peak in the infrared absorption spectrum. Only by using the most advanced techniques of sampling and analysis may we hope to detect such substances. An additional shortcoming is the failure to take full advantage of stream biota as useful indicators of pollution. Although biologists have repeatedly stressed the sensitivity of aquatic life to water quality changes, our approach remains relatively crude and expensive.

It must be re-emphasized that collection of data is not important if they do not relate to water quality. The time is ripe for close, critical scrutiny of the kind of information now being recorded in water quality basic data programs.

WATER TREATMENT

Can we effectively treat polluted water for re-use?

The main function of the modern water treatment plant is to remove particles suspended in the water and to destroy microorganisms that manage to survive flocculation and filtration procedures. Except for removal of hardness, little attempt is made to remove soluble chemicals in drinking water. Where taste and odor are highly objectionable, carbon is usually applied. But carbon is selective in its action and sometimes it is ineffective. We actually know little about the principle of adsorption as it applies to the use of carbon. Moreover, there is no assurance that harmful substances in solution will signify their presence by producing taste and odor. Here, as in waste treatment, interesting research opportunities exist.

CONCLUSION

My assignment has been to consider major needs of water quality research. I have addressed my remarks to the following questions:

- 1. Do we account for all pollution reaching streams?
- 2. Having a new waste in hand, can we predict its potential pollutional effects?
- 3. Assuming we can determine tolerance levels for this waste, can we really treat it effectively?
- 4. In disposal of waste effluents, do we make effective use of the receiving water?
- 5. Having disposed of the waste effluent, can we monitor effectively the quality of waters which have received it?
- 6. Can we effectively treat polluted water for reuse?

These, I believe, encompass the important areas of concern to agencies responsible for water quality conservation.

Perhaps in stressing areas of ignorance, I have been guilty of painting an unduly gloomy picture of the state of our art. We need not apologize. The success of sanitary engineers is controlling filth in water and, by so doing, eliminating the epidemic diseases associated with filth—one of the great triumphs of public health. We were able to solve the problems of the past. We may look forward with confidence to solving the problems now facing us.

PROGRAM

Wednesday, April 27, 1960

8:15 - 8:55

Registration and Assembly

8:55 - 9:00

Opening Remarks:

A. F. Bartsch, Assistant Chief, Research Branch, Division of Water Supply and Pollution Control

9:00 - 9:10

Welcome:

H. G. Hanson, Director, Robert A. Taft Sanitary Engineering Center

9:10 - 12:30 STATEMENT OF THE PROBLEM:

Chairman: A. F. Bartsch

Introduction

Induced Eutrophication -- A Growing Water Resource Problem:
A. F. Bartsch

Specific Problems In Lakes

Madison's Lakes: Must Urbanization Destroy Their Beauty and Productivity?
William B. Sarles, Department of Bacteriology,
University of Wisconsin, Madison, Wisconsin

Phosphate from Treated Sewage and Algae Blooms in Lake Zoar, Connecticut:
Richard J. Benoit, Research & Development,
General Dynamics Corporation, Groton, Connecticut and
John J. Curry, Water Resources Commission,
Hartford, Connecticut

Klamath Lake, an Instance of Natural Enrichment:

Harry K. Phinney, Department of Botany & Plant Pathology, Oregon State College, Corvallis, Oregon

Recent Changes in the Trophic Nature of Lake Washington:
G. C. Anderson, Research Department of Oceanography,
University of Washington, Seattle, Washington

Specific Problems In Rivers

Algae in Rivers of the United States:

C. M. Palmer, In Charge, Interference Organisms Studies, Research Branch, Division of Water Supply and Pollution Control

1:30 - 4:30 GROWTH CHARACTERISTICS OF ALGAE:

Chairman: Jack Myers, Department of Zoology, University of Texas, Austin, Texas

Nutrition

Fundamental Characteristics of Algal Physiology:

Robert W. Krauss, Department of Botany, University of Maryland, College Park, Maryland

Micro-Nutrients and Heterotrophic Requirements of Algae as Possible Factors in Bloom Production:

Luigi Provasoli, Haskins Laboratory, 305 E. 43rd St. New York, New York

Algal Density as Related to Nutritional Thresholds:

James B. Lackey, Department of Civil Engineering,
University of Florida, Gainesville, Florida

Productivity And How To Measure It

Methods of Measurement of Primary Production in Natural Waters:

Lawrence R. Pomeroy, Marine Institute,
University of Georgia, Athens, Georgia

Factors Which Regulate Primary Productivity and Heterotrophic Utilization in the Ecosystem:

Eugene P. Odum, Department of Zoology, University of Georgia, Athens, Georgia

Thursday, April 28, 1960

9:00 - 12:30 SOURCES OF NUTRIENTS:

Chairman: Harold B. Gotaas, Northwestern Technological Institute, Northwestern University, Evanston, Illinois

Land Drainage

Land Drainage as a Source of Phosphorus in Illinois Surface Waters:

R. S. Engelbrecht & James J. Morgan, Department of Civil Engineering, University of Illinois, Urbana, Illinois

Urban, Agricultural and Forested Areas:

R. O. Sylvester, Department of Civil Engineering, University of Washington, Seattle, Washington

Wastes

Metropolitan Wastes and Algal Nutrition:

William J. Oswald, School of Public Health, University of California, Berkeley, California

Limnological Relationships

The Role of Limnological Factors in the Availability of Algal Nutrients:

George H. Lauff, Department of Zoology,
University of Michigan, Ann Arbor, Michigan

Nitrogen Fixation in Natural Waters under Controlled Laboratory Conditions: C. N. Sawyer & Alfred F. Ferullo, Metcalf & Eddy, Engineers, Statler Building, Boston, Massachusette

Recent Observations on Nitrogen Fixation in Blue-Green Algae:
Richard C. Dugdale, Department of Biological Sciences,
University of Pittsburgh, Pittsburgh, Pennsylvania

1:30 - 4:00 METHODS OF PREVENTION OR CONTROL:

Chairman: M. A. Churchill, Stream Pollution Control Section, Division of Health & Safety, Tennessee Valley Authority, Chattanooga, Tennessee

Limitation of Nutrients As A Step In Ecological Control

The Madison Lakes Before and After Diversion:

Gerald W. Lawton, Hydraulic & Sanitary Laboratory, University of Wisconsin, Madison, Wisconsin

The Badfish River Before and After Diversion of Sewage Plant Effluent:

Theodore F. Wisniewski, Committee on Water Pollution,
Madison, Wisconsin

Spray Irrigation for the Removal of Nutrients in Sewage Treatment Plant Effluent as Practiced at Detroit Lakes, Minnesota:

Winston C. Larson, Winston C. Larson and Associates, Detroit Lakes, Minnesota

Chemical Methods for Removal of Phosphorus and Nitrogen from Sewage Plant Effluents:

Gerard A. Rohlich, Hydraulic & Sanitary Laboratory, University of Wisconsin, Madison, Wisconsin

Stripping Effluents of Nutrients by Biological Means:

George P. Fitzgerald, College of Engineering, University of Wisconsin, Madison, Wisconsin

The Use of Algae in Removing Nutrients from Domestic Sewage:
R. H. Bogan, Department of Civil Engineering,
University of Washington, Seattle, Washington

5:30 - 6:30

Social Hour, Hotel Sinton

6:30 - 8:30

Banquet

Do They Dig You, Daddy-O, Or Are You Way Out?

R. G. Lynch, The Milwaukee Journal, Milwaukee, Wisconsin

Friday, April 29, 1960

Chairman: Frank Woodward, Division of Environmental

Sanitation, State Department of Health,

Minneapolis, Minnesota

9:30 - 10:30

Use of Algicides

The Practical Use of Available Algicides and Modern Trends Toward New Ones:

Kenneth M. Mackenthun, Committee on Water Pollution,

Madison, Wisconsin

10:30 - 11:00 RESEARCH NEEDS

Research Needs in Water Quality Conservation:

Bernard B. Berger, Chief, Water Supply and Water Pollution

Research, SEC

11:00 - 11:05 Concluding Remarks:

A. F. Bartsch

11:15 - 12:30

Tour of SEC Facilities:

H. W. Jackson, Biologist, Water Supply and Water

Pollution Training Training Program, SEC