Bostwick H. Ketchum
March, 1949.

LIMNOLOGICAL ASPECTS
OF
WATER SUPPLY AND WASTE DISPOSAL

Publication of the American Association
for the Advancement of Science

Edited by
F. R. Moulton
and
Florence Hitzel

American Association for the Advancement of Science
1515 Massachusetts Avenue, N.W., Washington 5, D. C.
1949

PRINTED BY
THE HORN-SHAFER COMPANY
BALTIMORE 2, MD.

FOREWORD

By THEODORE A. OLSON, Chairman of the Symposium

SCHOOL OF PUBLIC HEALTH, UNIVERSITY OF MINNESOTA, MINNEAPOLIS, MINN.

The present period is characterized by much specialization in scientific work, often with resulting neglect of borderline fields of considerable importance. This lack of inter-science cooperation is often apparent even within relatively small subdivisions of scientific work. For instance, Steinhaus (1946) in his "Insect Microbiology" stated that the microbiologist and the entomologist greatly need an introduction to one another, although both are in the generally accepted sense biologists.

It is at once apparent that the lack of complete understanding between scientists in related fields may be even greater when one considers the border area lying between the engineer and the biologist. Dr. Paul Sears of Oberlin College, in discussing this matter (1947), suggested biology courses for engineers and pointed out that there are many ill effects which are unwittingly produced by engineers because they had too little knowledge of living things and their ecology. An industrial engineer may, for example, do an excellent job creating factories of great economic importance to the country yet fail to solve properly the waste disposal problems associated with the same industry. Thus valuable surface waters may be seriously polluted to the point where great economic, as well as aesthetic, losses are sustained. Problems of drainage and of irrigation often involve questions relating to biological principles as well as to engineering skills.

Conversely, the biologist may often fail to take advantage of the knowledge available in the field of engineering and of the sound advice he might receive from members of that profession. Carried sufficiently far, a plan for cooperation between the two groups, which necessitates working and thinking together, should lead to coordinated efforts which would be really effective and practical. Such action on the part of biologists and engineers should break down the invisible wall which at present seems to exist between their respective fields of endeavor.

In view of the general agreement that future progress must include cooperation and mutual understanding in fields of inter-science, the appearance of this volume is timely. The subject matter presented represents a sampling of the border area which lies within the scope of interests common to the engineer, the limnologist, and the oceanographer. On this common ground a better understanding and a fuller appreciation of the role played by each profession can be built. It is hoped, therefore, that these papers will stimulate further thinking and cooperative work in the fields of activity represented, and that ultimately they will lead to other as yet unexplored areas of common interest.

THEODORE A. OLSON
Chairman of Symposium

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LIST OF CONTRIBUTORS

Alfred F. Bartsch, Ph.D.

Biologist, Pacific Northwest River Basin Office, U. S. Public Health Service, Portland, Ore.; formerly Senior Biologist, Wisconsin Committee on Water Pollution, State Board of Health, Madison, Wis.

Margaret P. Briggs, M.A.

Graduate Student, Department of Agricultural Bacteriology, University of Wisconsin, Madison, Wis.

Cornelia L. Carey, Ph.D.

Associate Professor of Botany, Barnard College, New York, N. Y.

Shih L. Chang, M.D.

Assistant Professor of Sanitary Biology, Department of Sanitary Engineering, Graduate School of Engineering and School of Public Health, Harvard University, Cambridge, Mass.

Warren S. Churchill, M.A.

Biologist, Wisconsin Conservation Department, Northeast Fishery Area Headquarters, Woodruff, Wis.

Bostwick H. Ketchum, Ph.D.

Marine Microbiologist, Woods Hole Oceanographic Institution, Woods Hole, Mass.

James B. Lackey, Ph.D.

Editor, The Blakiston Company, Philadelphia, Pa.

John B. Moyle, Ph.D.

Aquatic Biologist, Fisheries Research Unit, Minnesota Department of Conservation, St. Paul, Minn.

Theodore A. Olson, A.M.

Associate Professor, School of Public Health, University of Minnesota, Minneapolis, Minn.

Clarence E. Taft, Ph.D.

Associate Professor of Botany, The Ohio State University, Columbus, Ohio.

Willis M. Van Horn, Ph.D.

Research Associate, The Institute of Paper Chemistry, Appleton, Wis.

John N. Wilson, M.A.

Associate Public Health Biologist, Minnesota Department of Health, Minneapolis, Minn.

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MICROBIOTA OF SEWAGE TREATMENT PLANTS AND POLLUTED STREAMS

By JOHN N. WILSON
MINNESOTA DEPARTMENT OF HEALTH, MINNEAPOLIS, MINN.

LATE LAST century there was some controversy among sanitary engineers in this country and in Europe concerning which of two methods of sewage treatment should be advocated—chemical or biological treatment. Those who favored the former tried to show that sewage could be treated successfully in much the same manner as surface water is prepared for drinking. However, the advocates of Mother Nature's method—biological treatment—had caught a preliminary glimpse of her seemingly miraculous powers of self-purification and were pressing ahead rapidly with their experiments in this virgin field. In 1890, a report from the Lawrence Experiment Station, in Massachusetts, stated that when sewage is passed intermittently through a filter of coarse gravel from which all sand has been washed, ". . . each stone was kept covered with a fine film of liquid, very slowly-moving from stone to stone and continually in contact with air in the spaces between the stones. The liquid, starting at the top as sewage, reached the bottom within twenty-four hours with the organic matter nearly all burned out" (Mills 1890). Shortly after this report an English investigator, Scott-Moncrieff (Anonymous 1892), experimenting with the treatment of sewage from his own home developed a filter plant using upward flow and demonstrated beyond any doubt the biological action as opposed to mechanical action alone. Although the early experiments in sewage treatment were conducted largely by engineers, representatives of other professions, such as bacteriologists, chemists, biochemists and biologists, began gradually to appear on the scene to contribute their share in the development of the methods of biological treatment in use today.

The purpose of this paper is to discuss sewage treatment from a biological viewpoint and to show some relationships that exist between the microbiota of sewage treatment plants and polluted streams. The term microbiota is used to designate those plants and animals which range from the higher bacteria through certain aquatic insect larvae. In a sense, microbiota embodies a synthesis of plankton (flowing sewage or polluted water of a stream) and benthos (biological film of trickling filter or similar treatment unit and bottom-dwelling organisms of a polluted stream). Only those organisms which can be identified by direct microscopic examination are included. The large group of bacteria which is commonly identified by culture methods is omitted.

To facilitate interpretation of the data, the microbiota of the sewage treatment plants included in these studies have been divided into three classes on the basis of function: The *binding* organisms, the *free-living* organisms, and the *scouring* or *grazing* organisms. The members of the binding group comprise the fibrous-gelatinous matrix of the biological film. The slime bacterium, *Zoogloea ramigera,* certain molds and fungi, and filamentous algae are among the representatives of this group. The free-living organisms include the higher bacteria, protozoans and rotifers. The rotifers and larger protozoans feed upon bacteria and solids in the sewage. Many smaller protozoans subsist largely upon dissolved nutriment in the sewage. Those which feed upon bacteria help to prevent overpopulation among the bacteria and some function as colloiders of dissolved material. The scouring or grazing group includes insect larvae, round worms, annelid worms, snails and adult insects (Collembola). As the name suggests, these organisms feed directly upon the biological film and sewage solids. But scouring organisms may sometimes create a nuisance or interfere with normal plant operation; consequently, control measures have been developed in recent years (Page 11). Nevertheless, they play an unquestioned role, particularly in low-rate trickling filters,* by consuming solids in the sewage, by reducing the volume of the film and by inducing sloughing, which opens the voids between the rocks, thereby enhancing aeration.

The studies on the microbiota of sewage treatment plants have been made intermittently over a period of five years. The following projects are included:

1. Investigations on the biology of high-rate trickling filters were made over a period of three months in 1942. The results of this work were published as a supplement to the report of the engineering investigations of filters of this kind (Walton 1943). This project was sponsored by five State Health Departments: Wisconsin, Minnesota, Iowa, Illinois and Indiana.

2. Studies on the biology of contact aerators (Hays Process) were made during a brief period in the winter of 1944. This work was under the auspices of the Repairs and Utilities Branch of the Seventh Service Command in Omaha and was performed as a supplement to engineering investigations. Five plants were included in the survey.

3. Investigations of the sewage treatment plant at South St. Paul, which handles a large volume of wastes from meat packing plants, were made this last summer (1947). The biological studies included the filter back-washing operation and a vertical section of a filter.

Most of the reactions which take place in a sewage treatment plant may also be observed to some extent in a polluted stream; therefore, let us first consider stream self-purification from the microbiotic standpoint.

Microbiota of Stream Self-Purification

In his book entitled "Stream Sanitation," Professor E. B. Phelps (1944) uses a financial simile in a most apt manner to describe the self-purification

* For the purpose of this paper, the following distinction is made between low-rate and high-rate trickling filters: Low-rate trickling filters are operated with intermittent application of sewage, whereas high-rate filters are dosed continuously. The total amount of biochemical oxygen demand applied per unit period of time on a high-rate filter is much higher than on a low-rate filter.

of streams. Mother Nature is a banker, the amount and strength of the sewage discharged to the stream are the debt, or liability, and the dissolved oxygen and other factors of self-purification are the assets. If the demand for oxygen is excessive in relation to that available, the dissolved oxygen is depleted and the stream goes into bankruptcy. At the opposite extreme, the stream may be so large in comparison to the amount of waste discharged into it that the sag in dissolved oxygen may be scarcely perceptible.

Let us consider a stream which has been polluted to a moderate degree so that it could be classed somewhere between the two foregoing extremes. Space will not permit a discussion of the physical, chemical and biochemical factors of self-purification; however, it is within the province of this paper to demonstrate the role of the microbiota of a stream in the amortization of the pollution debt. Mother Nature is an exacting banker. She expects the pollution debt to be paid off without any needless delay, but she is also resourceful in changing the composition of the microbiotic association to cope with the situation. The rigorous conditions of the environment caused by dumping sewage into the stream are met at first by eliminating the sensitive species from the microbiota. The increase in turbidity is a factor in limiting the growth of phytoplankton, and the deposition of putrescible sludge makes the bottom of the stream untenable for the growth of many insect larvae and other sensitive benthic organisms. As a rule, the zooplankters dominate the polluted stretches. These include the bacteria-feeding ciliate and flagellate protozoans as well as the saprophytic forms. Because of the greatly augmented supply of food present in the stream below the point of sewage discharge, the population of these organisms is usually large.

As the sewage solids settle to the bottom to form sludge deposits, the normal association of benthic organisms changes from a large number of genera and species to a few hardy genera whose numbers usually increase prodigiously. For example, it is not unusual to observe aquatic annelids (sludge worms) in such numbers on a slightly submerged sludge bank that they resemble the nap of a thick rug. The importance of these biological workmen and of other scavengers of the benthos in the process of self-purification of streams has been demonstrated repeatedly.

When recovery progresses to the point where there is some clearing of the water, algae and chlorophyll-bearing protozoans may be seen to thrive in the fertile water, sometimes to the extent of producing a water bloom. The stream returns to its normal condition when the fertility decreases below the point required for the maintenance of the algal bloom. At the same time, hosts of bacteria, after consuming nutriment which is available to them in the diluted sewage, starve and perish as they move down stream. In this manner, a stream purifies itself.

Sewage treatment plants are often called "condensed streams" where self-purification is carried on by a succession of different organisms, each utilizing the waste products of the preceding species. In such a system, one should be able to trace out the various zones of self-purification, namely: Recent pollution, active decomposition and recovery. While it is true that the development of processes of biological treatment of sewage was aided by observations on the self-purification of polluted streams, yet, in the process of contracting a stream to the dimensions of a sewage plant, forces other than those normally active in the self-purification of a stream are brought into play. Surface, contact, and interfacial forces become operative as a group. For example, in a trickling filter, the sewage disperses a thin film and passes from rock to rock with all substances in the sewage coming into contact with the biological film. Particles in the sewage are adsorbed by the film and digested particles are removed from the film to be washed out with the effluent from the filter. The reduction in the strength of sewage (biochemical oxygen demand removal) by biological contact processes at work in a sewage plant approaches 90%. At normal summer temperatures, this value is reached in the self-purification of polluted streams only after days of flow. However, the usual time required for the percolation of sewage through sand beds and trickling filters is 1 - 2 hours (Imhoff and Fair 1940). In spite of this contrast in the rate at which self-purification proceeds, many of the same organisms and many similar biotic relationships may be observed in streams and sewage plants.

Microbiota of Sewage Treatment Plants

Technique. Five series of samples were collected for the identification and enumeration of the microbiota from the sewage treatment plant at Owatonna, Minnesota, which was included in the cooperative survey of high-rate filters (Walton 1943). These samples were taken from the upper surface rock, one foot beneath the surface and the filter effluent. The samples of effluent from the filter were taken during extensive sloughing or unloading induced by stopping the distributor for a brief period. The rock medium was analyzed on the basis of numbers of organisms per square cm of surface area. The samples of effluent were enumerated on the basis of numbers per liter of the original samples which were examined without concentration. Quantitative determinations were made on samples preserved in formalin, but in order to facilitate the identifications the living organisms were examined. The standard plankton counting cell (Sedgwick-Rafter) was used with a maximum magnification of 210 diameters.

Samples from contact aerators were usually obtained by scraping growth from plates and sample boards in the aerators. Wherever possible, at each plant, one plate was lifted from near the influent to the primary aerator, one from near the effluent, and two additional plates from corresponding locations in the secondary aerators. This was done to determine the nature of the biological gradient as postulated by the proponents of the process. The samples were examined in the fresh state with the maximum magnification of 440 diameters. The results were qualitative, but the relative numbers of each organism were recorded for comparative purposes.

The method of quantitative analysis of the filter growth in the plant at South St. Paul differed from the technique described above. Instead of measuring the surface of several rocks from which the growth had been scraped, the volumes of representative samples of growth were measured in a graduate cylinder and diluted to a sufficient degree to permit examination in a standard counting cell. By this means a direct relationship is established between the quantity of growth and the number of organisms present therein. The vertical series of samples from the filter rock were collected at 6-inch intervals over a distance of 5 feet, where a filter had been opened for repair. In order to determine the microbiotic composition of the film and solids removed by backwashing a filter, samples were collected near the beginning and near the end of the operation. Before the backwashing started, however, a sample for control was collected from the combined filter effluent as it emptied into the final clarifier.

Reaction of Microbiota to Changes in the Operation of a Trickling Filter

The sewage treatment plant at Owatonna, Minnesota, was investigated 5 times from March 18 to April 24, 1942, to determine the effects of changes in plant operation upon the microbiota of the trickling filters. This sewage plant was designed to handle a large volume of waste from a canning fac-

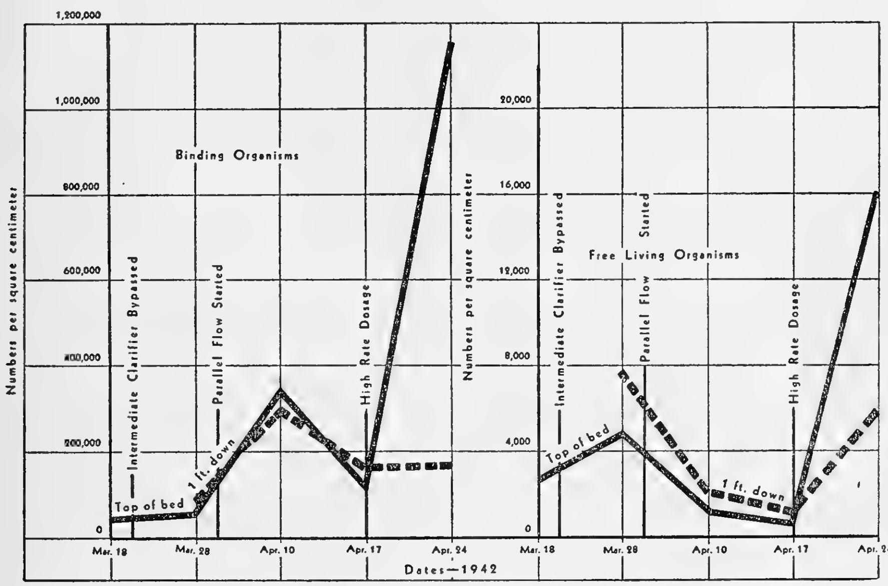

Fig. 1. Effect of changes in the operation of the sewage plant at Owatonna, Minn. upon the microbiota in the upper part of the second stage trickling filter.

tory in the fall in addition to the normal flow of sanitary sewage from the community of nearly 9,000 population. Provision was made in the design for coping with the wide variation in strength and volume of sewage. Among other devices for absorbing "shock loads," the design called for two trickling filters placed in series with an intermediate clarifier between. The microbiota in the secondary filter only will be discussed.

Analytical data obtained from earlier tests on this plant showed that during the 9 months of the year when the cannery is inoperative, the primary filter does most of the work and the secondary filter operates on a strictly rationed diet. The microbiota is partially "starved" during these slack months. Just as a patient in a condition of physical weakness must be strengthened before an operation, so it was considered advisable to enrich the secondary filter before the experiments were conducted. Consequently, the intermediate clarifier was bypassed and the effluent from the primary filter flowed directly to the secondary filter. This procedure was started on March 20 and continued 11 days (March 31), after which auxiliary piping was installed to provide parallel operation of the two filters with low-rate dosage. Three weeks later (April 17), the outer orifices of the distributors were plugged as a means of increasing the rate of dosage on the central portion of the filters. This schedule of operation is shown on the graphs (Figs. 1, 2). The samples which were collected on April 17 represented the second series taken after parallel flow was begun and they immediately preceded the change to high-rate dosage.

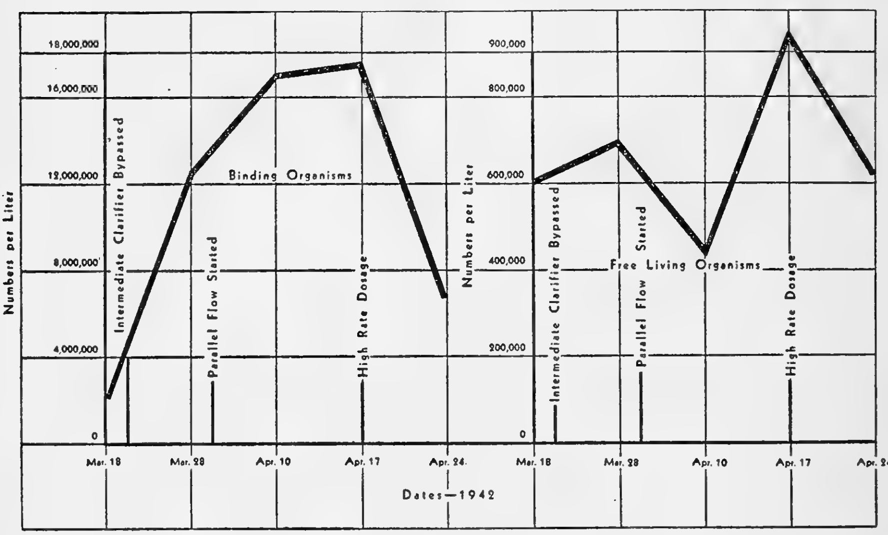

FIG. 2. Effect of changes in operation of the sewage plant at Owatonna upon the microbiota in the effluent from the second stage trickling filter.

These changes of operation caused quantitative as well as qualitative changes in the microbiota within the secondary filter, which is the one to be discussed (Figs. and Tables I and II). The initial enrichment process caused a noticeable increase both in binding and free-living organisms, but a decrease in the scourers. Among the binding components of the film, the white sulphur bacteria (*Beggiato alba*) almost doubled in numbers and fungi appeared on the surface rock where they were not found before. Almost all of the free-living organisms increased as a result of the more adequate diet of bacteria and organic solids. Predominant among these were two ciliate protozoans, *Opercularia sp.* and *Paramecium sp.,* and rotifers. Scouring or grazing organisms were not abundant at any time during the tests. Those present were minute round worms and a few sludge worms

TABLE I

Microbiota on the Top and at the One-Foot Level of the Secondary Filter in the Sewage Treatment Plant at Owatonna, Minnesota. (Numbers per square centimeter of rock surface)

Organism	March 18, 1942		March 28, 1942		April 10, 1942		April 17, 1942		April 24, 1942	
	Top	1 foot down	Top	1 foot down	Top	1 foot down	Top	1 foot down	Top	1 foot down
Binding forms										
Beggiatoa alba	39,233	Not abundant	59,619	86,247	328,464	291,438	104,026	165,600	1,097,600	170,660
Fungi (unid.)			360		4,526	360	2,190	585	50,600	2,200
Subtotal	39,233		59,979	86,247	332,990	291,798	106,216	166,185	1,148,200	172,860
Free-living and scouring forms										
Arcella sp.	238		87	406	12	437				
Amoeba sp.						45				
Opercularia sp.			540	1,624	60			234	5,175	2,340
Vorticella sp.	Present		87	56	158		18	117	575	
Chilodonella sp.	159	Occasional	24	406						
Euplotes sp.		Frequent	609							
Colpidium sp.			348				193	234	5,175	1,380
Metopus sp.				56						
Oxytricha sp.	79	Occasional	435	112		45				275
Paramecium sp.	715	Frequent	1,653	4,060	12	180	193	234		138
Stylonychia sp.	159					450				
Uronema sp.			261		79	360	64		4,025	413
Rotifera sp.	79	Occasional	132	280	432	225	9	234	360	550
Chaenia sp.										
Limnodrilus sp.		Occasional	3							
Nematodes	1,111	Frequent	444	784	264	360	70	148	720	765
Subtotal	2,540		4,623	7,784	1,017	2,102	547	1,201	16,030	5,891
Total	41,773	Not quantitative	64,602	94,031	334,007	293,900	106,763	167,386	1,164,230	178,751

TABLE II

MICROBIOTA IN THE EFFLUENT FROM THE SECONDARY FILTER AT OWATONNA
(NUMBERS PER LITER)

Organism	March 18, 1942	March 28, 1942	April 10, 1942	April 17, 1942	April 24, 1942
Binding forms					
Beggiatoa alba	1,726,200	12,727,300	16,033,100	17,686,030	6,446,300
Fungi (unid.)			1,150,800		115,080
Subtotal	1,726,200	12,727,300	17,183,900	17,686,030	6,561,380
Free-living and scouring forms					
Arcella sp.	172,600	28,000	86,310	30,000	12,000
Amoeba sp.		57,540	6,000		
Opercularia sp.	230,160	32,000		27,000	15,000
Vorticella sp.		4,000	4,000	28,770	28,770
Chilodonella sp.		8,000		57,540	
Euplotes sp.		230,160	16,000	28,770	28,770
Colpidium sp.					143,850
Lionotus sp.		28,770		3,000	3,000
Metopus sp.		28,770			
Oxytricha sp.		28,000	28,700	86,310	12,000
Paramecium sp.		60,000	230,160	287,700	86,300
Stylonychia sp.				86,310	6,000
Uronema sp.		86,300	28,770	28,770	201,390
Rotifera sp.	28,770	16,000	44,000	18,000	28,700
Chaenia sp.					3,000
Nematodes	172,600	92,000		287,700	57,000
Subtotal	604,130	699,540	443,940	969,870	625,780
Total	2,330,330	13,426,840	17,627,840	18,655,900	7,187,160

(Oligochaeta). The change to parallel flow modified the volume and character of the sewage sprinkled on this filter. There was a 50% decrease in volume of sewage but instead of receiving partially digested solids and sloughed film from the primary filter, the filter received fresh material from the primary sedimentation chamber. As a result, the binding organisms increased in the upper section of the bed as well as in the effluent from the filter; but the free-living organisms decreased steadily in the upper layer of rock and increased in the lower strata toward the middle of April, as evidenced by the samples of filter effluent on April 17.

On April 17, when the rate of dosage was increased on the central portion of the filter, there was a widespread unloading of the microbiota indicated by the effluent, while at the same time the numbers of organisms increased in the upper strata of rock. The last investigation (April 24, 1942) indicated that the filter had assumed the biological characteristics of a typical high-rate unit, whose microbiota are described as follows:

1. The *binding* group composed of the omnipresent slime bacterium, *Zoogloea ramigera,* large numbers of *Beggiatoa alba* (white sulphur bacteria) and fungi.

2. The *free-living* group composed principally of bacterial-feeding and saprophytic protozoans, such as *Opercularia, Vorticella, Colpidium, Uronema, Arcella vulgaris,* and *Bodo spp.*

3. The *scouring* group, with Nematode worms as the sole representative: This group plays a minor role in many high-rate filters, although there are some notable exceptions.

The trend of increase in the population of the entire microbiota toward the close of these investigations demonstrated the importance of an adequate supply of food in a sewage filter. This increase in numbers of organisms was accompanied by an increased efficiency of the filter as a treatment unit.

Contact Aeration (Hays Process)

This process of biological treatment is similar to activated sludge except that with activated sludge, the sewage-sludge mixture is churned freely in open aeration chambers, while in contact aeration large asbestos plates are suspended 2 inches apart throughout the aeration tanks to provide a substrate for the growth of the microbiota. Most plants of this type have two such aeration chambers placed in series with 3 sedimenation units arranged as follows: Primary sedimentation tank, primary aerator, intermediate sedimentation tank (clarifier), secondary aerator, and final clarifier.

The proponents of the contact aeration process claimed a real advantage for their process owing to the establishment of a "biological gradient" which apparently is akin to the succession of organisms in a polluted stream below a sewer outlet (Griffith 1943). This presupposed the maintenance of straight-line flow throughout the plant with no recirculation, as occurs in some other methods of biological treatment. Our studies showed that where plants of this type were operating beneath their designed capacity, the "biological gradient" could be demonstrated. Near the influent of the primary aerator in one instance, the growth was very heavy, filling the spaces between the plates in places. This condition was accompanied by an unhealthy growth of the bacterial slime, *Zoogloea ramigera,* and the presence of numerous anaerobic flagellate protozoans. Myriads of green and red sulphur bacteria as well as the green alga *Chlorella vulgaris,* and the diatom, *Navicula sp.* occurred where the light could reach them. Free-living bacteria were also abundant in this part of the aerator, but no ciliate or amoeboid protozoans were to be found. Near the outlet of this aerator, however, there were large numbers of ciliate and flagellate protozoans which were subsisting largely on bacteria in the sewage. In the secondary aerator, the microbiota showed continued improvement. The appearance of the slime on the plates indicated a healthy condition, and dense pink colonies of rotifers occurred on the upper portions of some plates. Moreover, large numbers of *Opercularia sp.* occurring in the secondary aerator, indicated that an advanced stage of purification of the sewage had been reached.

Among the plants of this type there were several instances where overloading caused a failure of the process, as was evidenced by oxygen depletion in the aerators and a septic condition throughout. Anaerobic bacteria and protozoans thrived in a manner similar to what is expected in a digestion chamber for sludge. According to Lackey and Dixon (1943) there was ". . . oxygen starvation in the midst of plenty." Sometimes, where such difficulty was encountered, the biological gradient was modified by interposing a trickling filter in the system or recirculating some of the flow. In every case, the results were good. We are to conclude from this that the maintenance of the aerobic activity among the microbiota is as important in a sewage plant as it is in a stream, if a condition of nuisance is to be avoided.

Trickling Filters Modified for Backwashing

The microbiota in the trickling filters at South St. Paul is abundant and varied. The filters are not housed; therefore the sunlight on the surface rock stimulates the growth of algae and diatoms. Chief among these are *Chlorella sp., Oscillatoria tenuis* and *Navicula spp.* Moreover, some organisms were found in a sample taken during backwashing operation that are rarely found in trickling filters. These are *Macrobiotus,* the water bear, *Aelosoma hemprichi, Pristina sp.,* and *Dero sp.* The first is a Tardigrade, the other three are aquatic annelids. Although snails have often been reported from low-rate beds with intermittent dosage of sewage, their occurrence on high-rate units, such as these at South St. Paul, is unusual. Several *Physa sp.* have been found at a depth of 1-2 feet. In addition to these rather unusual organisms, there are representatives of the higher bacteria, fungi, protozoa (rhizopods, flagellates and ciliates), rotifers and insect larvae.

Each filter is backwashed every two weeks with 450,000 gallons of plant effluent applied through the underdrains of the filters. The action of the reversed flow is enhanced by the introduction of forced air through openings in a pipe grid system imbedded near the base of each filter. The procedure requires about 12 minutes and is effective in removing huge quantities of sewage solids and biological film. Judging by the myriads of filter flies in all stages of development which are visible on the surface of the wash water leaving the filter, this procedure may be cited as a means of fly control.

The samples taken near the beginning of the backwash contained more than twice as many binding organisms as at the close of the operation, but the reverse was true of the free-living organisms and the scouring component. Apparently, the first masses of binding organisms were loosened quickly from the upper surface of the filter, but the majority of the other organisms resided deeper in the bed and required more time to be dislodged. The control sample from the effluent of the filters under normal operating conditions indicated some natural sloughing of solids with about one-third the number of binders, one-tenth as many free-living forms and but a trace of scourers in comparison to the average concentration of the effluent from the backwash.

The series of 13 samples which represent the vertical section of a trickling filter disclosed several interesting facts. But, first, it is to be noted that the concentrations given in Table III represent numbers per mm of film but do not give any clue to the thickness of the film or its mass per unit volume of stone media. Tests have shown, however, that efficiency of the film is a function of surface area and not mass. Table III indicates that among the binding organisms, *Zoogloea ramigera* is the predominant organism throughout the filter, but it is most abundant, and, from its appearance, most active in the section from 6 to 30 inches in depth. The large numbers of sewage fungus, *Sphaerotilus natans*, present on the surface of the filter on the underside of the upper rocks, may be attributed to the fact that the carbohydrates, upon which this organism feeds, are assimilated largely in the upper portion of sewage filters. The abundance of the white sulphur bacteria throughout the remainder of the section is consistent with previous tests on high-rate filters. Among the free-living organisms, predominance is shared by ciliates, rhizopods and flagellates alike. Although the minute nematodes outweigh the filter flies in numerical predominance, the latter are probably the more important component of the scourers. Moreover, the oligochaetes, such as *Dero sp.*, although few in number, exert a potent influence on the reduction and stabilization of sewage solids and assist in clearing the voids between the rocks to improve ventilation. The importance of these and other scouring organisms in sewage filters and some recent developments in methods for their control should not be overlooked.

Recent Developments in Selective Control of Microbiota in Sewage Plants

Among the experts on sewage treatment in England, there are many who advocate the cultivation of a balanced scouring fauna on sewage filters

TABLE III

Relation of the Microbiota Occurring at Different Levels in Trickling Filter No. 5 South St. Paul, Minnesota, August 9, 1947
(Numbers per ml of film)

Depth	Binding	Predominant organisms*	Free living	Predominant organisms	Scouring	Predominant organisms
Top	5,110,000	*Sphaerotilus natans*	24,589,000	*Chlorella vulgaris*	1,800	Nematodes
Beneath top rock	1,430,000	*Sphaerotilus natans*	4,057,000	*Chlorella vulgaris***	365	Nematodes
6-inch	11,800,000	*Beggiatoa alba*	746	*Euplotes sp.*	205	Psychoda alternata
12-inch	10,460,000	"	34,600	*Astasia sp.*	7,430	Nematodes
18-inch	9,460,000	"	152,000	*Euplotes sp.*	1,940	"
24-inch	9,250,000	"	25,500	*Euplotes sp.*	5,025	"
30-inch	16,730,000	"	6,920	*Chilodonella sp.*	865	"
36-inch	5,810,000	"	39,200	—	103	"
42-inch	5,170,000	"	36,990	*Euplotes sp.*	16,420	"
48-inch	9,330,000	"	41,240	*Amoeba sp.*	630	"
54-inch	3,180,000	"	17,700	*Hartmanella sp.* *Navicula spp.*	9	Psychoda alternata
60-inch	3,120,000	"	15,800	*Colpidium sp.*	—	—
66-inch	5,350,000	"	—	*Bodo sp.*	60	Nematodes

*Second only to *Zoogloea ramigera*.
**Faded.

in order to increase aeration, to prevent ponding and to assist in stabilizing sewage solids. Nuisance from adult filter flies *(Psychoda spp.)* is at a minimum where the population of other macrofauna such as chironomids is sufficient to control them. Nevertheless, a certain amount of fly trouble is considered to be inseparable from the low-rate filter (Lloyd 1945).

In this country, however, the benefits derived from these organisms seem, in the opinion of the majority of sewage plant operators, to be overshadowed by the nuisance which they create in the immediate vicinity of the plants. Moreover, from the southern part of this country Brothers (1946) reported such enormous numbers of filter flies on certain high-rate filters that not only was practically all of the growth consumed but the filter bed and pipe lines became clogged. Numerous methods of control had been tried through the years prior to World War II from chlorination to flooding the filters for extended periods. One operator perfected the unique, if hazardous, method of spraying gasoline on the surface of the filter and igniting it. Most of the earlier methods had the one common disadvantage of injuring the biological film.

During the war some significant experiments on the control of filter flies by the use of DDT were conducted under the sponsorship of the U. S. Army Sanitary Corps. Brothers' (1946) experiments at Camp Fannin, Texas, demonstrated the effectiveness of DDT when application is by the batch method and the chemical is introduced into the sewage as it is sprayed on the filter. The optimum concentration of one part per million was effective when a holding period of one hour was provided. The effectiveness of such a treatment should be apparent for about 30 days.

Although the investigation of Carollo (1946) in this field was conducted independently from Brothers' investigations, there is excellent agreement in the results. Carollo, however, applied the DDT emulsion to the filter with the sewage over a 24 hour period at the rate of 1 part per million based upon the 24 hour flow.

Examination of the microbiota of the test filters following the application of DDT confirmed the selectivity of the chemical. According to Carollo, snails became more abundant after each treatment, and sewage filter bacteria were not killed in concentrations up to 100 parts per million.

Since the time of these investigations, there have been others which confirm them in similar situations and extend the range of possibilities of DDT in the selective control of the microbiota in sewage plants. Our knowledge, however, is insufficient with regard to the residual effect of DDT on receiving waters.

Summary and Conclusions

1. The term microbiota is a collective one, including microscopic and near-microscopic organisms from the higher bacteria through insect larvae. For convenience in discussing the biology of sewage plants, the organisms are divided into the following 3 groups on the basis of the functions which they perform: *Binding, free-living* and *scouring* organisms.

2. Self-purification in streams and in sewage treatment plants is discussed. Many of the processes are similar, but in general the reactions observed in a sewage plant are accelerated. This is attributed to the action of surface, contact and interfacial forces which operate as a group in trickling filters, contact aeration tanks and other biological treatment units. The biological film, with its teeming life, removes food from the sewage with which it comes in contact and discharges waste products to be carried away with the effluent.

3. Many of the organisms which are important agents of self-purification in streams are also found in sewage treatment plants.

4. The importance of an adequate supply of food in a sewage filter was illustrated by the effect of changes in operation on the microbiota of the secondary filter at Owatonna, Minn.

5. Studies on the biology of contact aeration showed the importance of an adequate supply of dissolved oxygen in the aerators. Where plants were operating beneath their designed capacity, the biological gradient, which is apparently akin to the succession of organisms in a polluted stream, could be observed. Experience has shown, however, that where septic conditions have been created by overloading, the most effective means of correcting the condition is to alter the succession of organisms either by interposing additional treatment units or by recirculating some of the sewage.

6. An unusual opportunity for examining the abundant and diverse microbiota on trickling filters was afforded at South St. Paul, not only at the time of backwashing, but also when a filter was opened for repair. Examinations of the microbiota at the beginning and toward the end of the 12 minutes required for the backwashing process indicated some differential in the rate of removal of the organisms. A control sample of the combined effluent from the filters confirmed the efficiency of the process as a means of removing excessive microbiota and solids. Moreover, this was an excellent demonstration of control of filter flies. The examination of the vertical series of samples showed some noticeable variation in the concentration of certain organisms, as for example *Sphaerotilus natans, Zoogloea ramigera* and *Chlorella vulgaris;* otherwise, the population of microbiota was reasonably uniform throughout the section.

7. Recent experiments in the control of filter flies in trickling filters indicate that an amount of DDT emulsion equivalent to one part per million of the total daily flow of sewage is highly effective in controlling the flies (larvae, pupae and adults) in the dosed area. When applied with the sewage, DDT does not destroy the biological film or cause it to unload or slough off. There are, however, insufficient data on the residual effect of the DDT on aquatic life in receiving waters.

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SOME EPIDEMIOLOGICAL AND BIOLOGICAL PROBLEMS IN WATER-BORNE AMOEBIASIS

By SHIH L. CHANG

DEPARTMENT OF SANITARY ENGINEERING, GRADUATE SCHOOL OF ENGINEERING AND SCHOOL OF PUBLIC HEALTH, HARVARD UNIVERSITY, CAMBRIDGE, MASS.

Interest in the problem of amoebiasis in this country reached a climax in the few years following the Chicago epidemic of amoebic dysentery in 1933 *(Natl. Inst. Health Bul.).* Toward the end of the thirties, the problem was more or less tossed out of the window and attracted attention only from those interested in tropical diseases. Although it has been estimated that from 5 to 10% of the general population pass the cysts of *Entamoeba histolytica* (Craig and Faust 1943), the infection has not been considered a serious public health hazard. This is partly due to the fact that in most cases, the infection is in a quiescent state, relatively few cases developing clinical amoebiasis which requires medical care, and partly due to the fact that the highest incidence of even quiescent infections has been found in those localities where health problems have not yet received full attention of health authorities.

While it cannot be denied that in spite of the world-wide occurrence of quiescent amoebiasis, amoebic dysentery and liver abscess are usually diseases of the tropics and subtropics, the Chicago epidemic has clearly shown that under certain circumstances, severe clinical amoebiasis may occur in epidemic form even in the temperate zone. It may also be pointed out that while the carrier rates were high in the Southern States, e.g., 17.3, 11.4 and 36.4% in Tennessee and 25.9 and 14.9% in New Mexico, they were also impressive in some of the surveys made in the Northern States, e.g., 4.6, 7.7, and 10.7% in Minnesota and 4.1 and 11.1% in Pennsylvania (Craig and Faust 1943). These carriers not only constitute a potential source of infection to others, but may themselves develop amoebic dysentery when they enter the tropics or subtropics, or when certain changes take place in the alimentary canal.

The successful control of an infectious disease demands a thorough understanding of its mode of transmission. Unfortunately, it is an extremely difficult task to ascertain the mode of transmission of some infectious diseases. For instance, in spite of extensive epidemiological and experimental studies on human poliomyelitis in the last 15 years, we are still in the dark as to how this infection spreads in a community. Controversial opinions also exist on the mode of transmission of amoebiasis. In places such as mental institutions and orphanages where water supply is well controlled and where personal hygiene of an overcrowded population is usually bad, direct contact and contamination of food and drink by carriers are probably the only two routes by which amoebiasis spreads. However, as to the spread of the infection in a large community, two schools of thought seem to have prevailed, one of which emphasized the importance of contaminated water supply while the other stressed the presence of carriers among food handlers.

Many epidemiologists have not yet fully accepted the view that amoebiasis is water-borne. The skepticism seems to be based on the fact that amoebiasis in general does not follow the epidemiological pattern of classic water-borne bacterial diseases. For instance, amoebic infection is usually not directly traceable to water supply; it is not explosive in nature; and the cysts of *E. histolytica* have not been demonstrated in water. The Chicago epidemic, in the opinion of these epidemiologists, is so unusual that it should be considered as an exception rather than the rule.

In an attempt to ascertain the role played by water in the spread of amoebiasis, the author has endeavored in the first two sections of this paper to bring together and analyse the important reports pertaining to this subject and to discuss certain problems encountered in determining the mode of transmission of this protozoan infection.

Epidemiologically speaking, the mode of transmission of any infectious disease is generally detected by a close correlation of the disease in one way or another with its source of infection. In establishing this relationship, the following conditions are desirable:

(1) The infection should have a relatively short incubation period.
(2) The infection should give rise to certain distinct clinical symptoms that call for medical attention, thus establishing the diagnosis.
(3) The causative organism of the disease should be demonstrable in the transmitting agent, or agents.

Unfortunately, not all infectious diseases satisfy these above-mentioned conditions. This is particularly true in the case of amoebiasis which has certain features that make the establishment of the relationship of the infection to its source extremely difficult under ordinary circumstances. These features are discussed in the subsequent section of the paper.

I. Certain Peculiar Clinical Features of Amoebiasis

A. *Symptomless Infection.* One of the characteristics of the behavior of *E. histolytica* is its great tendency to produce asymptomatic infection, particularly in the temperate zone. Ingestion of the cysts of *E. histolytica* with subsequent appearance of the organism in the stool does not necessarily mean that the infected person is going to develop clinical symptoms. In fact, most of the infected cases develop into a quiescent state. This phenomenon is not only confirmed by the existence of carriers in various countries (Craig and Faust 1943) but also by the outstanding work of Walker and Sellards (1913) in the Philippines. These two authors fed a faecal suspension containing cysts of *E. histolytica* from a carrier to 20 volunteers and found that 18 became infected and 2 did not. Among the 18 parasitized, only 4 developed dysentery, respectively 20, 57, 87, and 95 days after the feeding. Some of the quiescent cases were followed for over 2 years and never showed symptoms related to the infection. Furthermore, Wenyon and O'Conner (1917) reported that of the 106 carriers of *E. histolytica* found among 1979 healthy persons in Egypt, only 16 gave any history of dysentery.

Hence, it seems that even in the tropics and subtropics, the majority of cases of amoebiasis exist in the native population in a quiescent state. For these asymptomatic cases, the determination of the source of infection would be extremely difficult, if not impossible.

B. *Secondary Factors in the Etiology of Amoebic Dysentery.* Although amoebic dysentery has been known to us since the end of the last century, we must admit that we are still uncertain about the factors concerned in the etiology of the clinical disease. As stated before, introduction of *E. histolytica* into the human intestine oftentimes results in a carrier state and dysentery may not develop until certain secondary factors are present. Evidently the factors involved in the development of dysentery are complex. Some laboratory investigators and clinicians (Dobell and O'Conner 1921; Wenyon 1926; James 1927; Westphal 1937; Deschiens 1938; Nauss and Rappaport 1940; Manson-Bahr 1947) have reached the conclusion that under normal conditions, *E. histolytica* is a harmless parasite in the human intestine and will not invade tissue and provoke dysentery or liver abscess until other factors exist, such as injury of the tissue by pathogenic bacteria or chemical agents. The fact that many of the German soldiers who had been carriers for years in Germany developed amoebic dysentery during outbreaks of bacillary dysentery in North Africa made Horster (1943) believe that it was the presence of pathogenic bacteria such as the shigellae that damaged the tissue and provoked clinical amoebiasis in those carriers. Very recently, Wenyon (1947) has suspected that the Chicago epidemic might have been an outbreak of bacillary dysentery among a population with a high carrier rate of *E. histolytica.*

The other group of investigators led by Craig (1944), Faust (1941) and Johnson (1941) believe that *E. histolytica* is pathogenic under all circumstances. While there are some reports in the literature of the occurrence of typical amoebic ulcers in the intestine of apparently symptomless persons, these observations have usually been made in the tropics and subtropics. Those who favor this belief frequently quote the careful observation of Faust (1941) made on 202 autopsies of accidental death in New Orleans in which *E. histolytica* was found in 13, with 8 showing bowel lesions but only 5 with amoebae in the lesion. Commenting on Faust's observation, Napier (1947) has very recently pointed out that in none of these autopsies was a typical amoebic lesion demonstrated, and the description of the lesions, particularly of the superficial erosions, do not distinguish them from post-mortem changes.

In a previous communication, the author (1946) showed that *E. histolytica* is an obligate anaerobe flourishing in natural or artificial media at E_h values between -350 and -400 millivolts. This strongly reducing condition is apparently provided by the growth activity of the accompanying bacterial flora. In another communication, the author (1945) pointed out the importance of ligating the rectum in the establishment of infection in kittens of 2 strains of *E. histolytica* which had lost much of their infectivity through prolonged *in vitro* cultivation, and felt that the ligation of the rectum favored the development of pathology not so much because of the retention of the inoculum, but rather because of changes induced in the intestine after ligation, particularly changes in the bacterial flora.

It is beyond the scope of the present paper to discuss in detail the problem of pathogenesis of *E. histolytica.* There should not be any doubt that *E. histolytica* is a pathogenic protozoa. However, parasitism in the human intestine by a pathogenic protozoön may not always be accompanied by pathological changes. Haemolytic streptococci are unquestionably pathogenic bacteria; but they are frequently found in the throat of healthy individuals. The same thing has been shown to be true of many other pathogenic bacteria.

Whatever may be the actual mechanism by which the bacterial flora affects the pathogenic activity of *E. histolytica,* it is probably a more complex phenomenon than the mere damaging of the tissue to favor invasion by the amoebae. The provision of anaerobic conditions by the bacterial flora, in the author's opinion, may not only favor the survival and multiplication of amoebae but may also play a leading role in facilitating the tissue invasion by the latter. In one experiment (unpublished data) in which the author fed culture-induced cysts of a strain of amoeba that had lost much of its infectivity for kittens to 6 kittens, 2 animals had the rectum ligated; 2 had the rectum ligated and also received 10 ml of a rich culture of *Clostridium perfringens* in gelatin; and 2 were control. The first 2 animals became sick after the 5th day and were sacrificed on the 9th day. At autopsy, the caecum of each animal was punctured with a platinum electrode and a capillary pipette containing saturated KCl in 2% agar, connected to a potentiometer. The potentials were measured against a calomel reference cell and the E_h values of the caecal contents of both animals were found to be -275 and -282 millivolts respectively. Both caeca showed a few patches of superficial ulcers of the mucosa with many trophozoites in the ulcers but outside the basement membrane. The second two animals that had received the clostridium culture were sick on the 3rd day and dying on the 6th day. They were sacrificed. At autopsy, the E_h values of the caecal contents were recorded as -375 and -392 millivolts respectively, and both caeca showed several typical amoebic ulcers with numerous trophozöites in the submucosa. The control animals remained uninfected and were sacrificed 7 days after feeding. The E_h values of their caecal contents were -185 and -198 millivolts respectively and no lesion was noticed at autopsy. Although the number of animals used was small, the unique results provide some basis for explaining the establishment of amoebic infection. It is anticipated that the ingestion of cysts of *E. histolytica* by some individuals who have an intestinal bacterial flora which does not provide conditions anaerobic enough for the perpetuation of the amoebae, may not produce any infection at all. In those persons whose intestinal bacterial flora furnishes a moderately anaerobic condition, the ingestion of cysts may produce an infection without apparent pathology. In those persons whose intestinal bacterial flora creates a profound anaerobic condition which may extend into the mucosa, the introduction of amoebic cysts will establish infection and produce amoebic lesions.

Furthermore, it is not entirely unlikely that the amoebae do secrete a cytolysin for their parasitic life in the tissue and that the secretion of this cytolysin may be facilitated by the profound anaerobiasis.

However, there may be other factors involved in the pathogenicity of *E. histolytica,* such as the virulence of the amoeba, the nature of the diet, the physiological condition of the intestine, particularly its ability to resist the changes produced by the normal bacterial flora, and the immunity status. All of these may affect in one way or another the development of clinical amoebiasis.

C. *Primary and Secondary Amoebic Dysentery.* Whatever the actual mechanism involved in the development of amoebic dysentery, from the epidemiological point of view, the clinical cases may be divided into two groups, namely: the primary and secondary dysentery. The primary cases are those that develop dysentery following the ingestion of infective material. They could be due to either introduction of amoebic cysts of a virulent strain into the intestine where conditions favorable for development of dysentery exist, or introduction of such amoebic cysts accompanied by a bacterial flora which is capable of altering the normal intestinal flora and favors tissue invasion. This type of case usually occurs during epidemics or in small outbreaks in poorly sanitated or unsanitated tropical or subtropical regions. Examples are the cases in the Chicago epidemic, and those developed after consuming grossly polluted water in the tropics and subtropics as cited by Strong (1944) and observed in the Burma campaign in World War II (personal communication). It is in this kind of cases that the incubation period can be reasonably accurately determined and source of infection established.

The secondary cases are those that have been carriers for months or years and develop amoebic dysentery upon introduction of certain secondary factors. The type of case in this group is illustrated by the carriers who developed amoebic dysentery during outbreaks of bacillary dysentery as observed among German soldiers by Hauer (1942) and Horster (1943) and among the Allied troops in North Africa (Coggeshall 1943) and the Near East (Fairley and Boyd 1943). In this type of case, the determination of the course of infection is very difficult, since the disease is not correlated with the primary source but with the occurrence of secondary factor or factors.

D. *Incubation Period of Amoebiasis.* Under natural conditions, it is impossible to know the incubation period of asymptomatic amoebiasis. In the quiescent cases in Walker and Sellards' experiment (1913) the average incubation period was about 9 days. Even if we take for granted that the incubation period of naturally infected cases is 9 days, it would not be of any help in ascertaining the date of infection unless the population is constantly examined for the presence of the amoebae in the stool.

In the secondary cases of amoebic dysentery, the incubation period, as stated before, is very obscure, since it is not the period between the ingestion of amoebic cysts and the development of clinical amoebiasis, but the period elapsed after the secondary factors come into operation.

Hence, from a practical view-point, it is only in the primary cases of amoebic dysentery that the incubation period can be determined with a fair degree of accuracy. In the four cases of dysentery observed in Walker and Sellards' experiment, the incubation period was 20, 57, 87, and 95 days respectively. The incubation period of the cases involved in the Chicago epidemic has been considered by Wenyon (1947) as too short for amoebic dysentery. Wenyon stressed in particular the few cases having an incubation period of two days and thought that, in order to produce amoebic dysentery with so short an incubation period, the cyst-contaminated water must have been so heavily polluted that it would have shown physical impurities to arouse objections on the part of the guests. In the author's opinion, there must have been misdiagnosed cases in that epidemic, in view of the fact that physicians and technicians did not become aware of the existence of the epidemic until it was over and most of them were probably not too familiar with the diagnosis of amoebiasis. Hence, it is not unlikely that those few cases with an incubation period of less than one week might have been cases of bacterial enteric infection; but there is not much doubt that the epidemic, as will be shown later, was itself amoebic dysentery. Furthermore, the incubation period of the 4 cases in Walker and Sellards' experiment cannot be regarded as universally applicable since the virulence of the amoeba, the susceptibility of the host, and the nature of the intestinal bacterial flora may vary from strain to strain, person to person, and place to place.

In fact, the incubation period of the majority of cases in the Chicago epidemic was not too different from that observed in other studies. Of the 216 selected cases who resided in the two hotels involved for a short enough time to allow the determination of incubation period, 25% gave an incubation period of less than 11 days, 25% between 12 and 20 days, 25% between 21 and 36 days, and the remaining 25% between 37 and 120 days. It may be interesting to point out that, because of the relatively long incubation period in the majority of cases, the Chicago epidemic was not recognized during the outbreak as there were only 15 cases which were connected with the two hotels reported to the Chicago Board of Health, and that the epidemic was only disclosed by the questionnaire survey made later when suspicion was aroused by a report on amoebic dysentery at the American Public Health Association Convention held in Indianapolis *(Natl. Inst. Health Bul.)*.

II. The Role of Polluted Water as a Transmitting Agent of Amoebiasis

As early as 1926, Craig mentioned the possibility of water-borne amoebiasis. Clark (1925) reported the marked reduction in post-mortem incidence of amoebic infection in Panama following the introduction of a modern water supply; but the evidence has been considered as too non-specific to support the view that amoebiasis is water-borne, since many other factors, such as improvement of personal hygiene and change of eating habits, could have altered at the same time and might also have affected the incidence of amoebic infection. Nevertheless, there have been accumulated a few substantial facts that provide sufficient ground to believe that polluted water may be one of the most important sources of amoebiasis.

A. *The Chicago Epidemic.* In spite of the skepticism expressed by some epidemiologists and eminent tropical disease experts, the Chicago epidemic of amoebic dysentery, in several of its essential features, still stands out as an example of water-borne amoebiasis. As stated before, Wenyon (1947) expressed skepticism on the basis that the incubation period was too short for amoebic dysentery; but the number of cases having an incubation period of less than one week was so small that they should carry no weight in judging the nature of the epidemic.

Although the finding of *E. histolytica*-like cysts in the cross-connection pipe removed at the time of inspection *(Natl. Inst. Health Bul.)* served only as weak evidence for the water-borne nature of the epidemic, there were a few essential observations that strongly supported the water-borne theory. First, if the epidemic were bacillary dysentery, there should have been a very large number of cases of bacillary dysentery noticed during the early part of the epidemic. On the contrary, the epidemic was reported to be uncomplicated by the existence of outbreaks of bacterial enteric infections *(Natl. Inst. Health Bul.)*. Second, the fact that amoebiasis, including the asymptomatic infection, has been successfully controlled in the two Chicago hotels after the sanitary defects were corrected is also strong evidence that the source of infection of that epidemic was of water origin. Third, the recent outbreak of bacillary dysentery in Kansas (Kinnaman and Beelman 1944) in which 3000 cases were reported was not complicated by cases of amoebic dysentery.

B. *The Chicago Stockyard Fire Outbreak.* In the Chicago Stockyard fire in 1934 (Hardy and Spector 1935), at least 11 cases of amoebic dysentery were noticed along with over 300 cases of dysentery and 78 cases of typhoid fever among the firemen and some spectators who drank water from a pipe which was used to supply water to the stock and fed with sewage-polluted water and sewage effluent. It is of particular interest to note that among the 216 firemen who developed enteric infection of various degrees of severity, 57% showed cysts of *E. histolytica* in their stools, while only 15% of 161 firemen who did not drink the polluted water and consequently were not infected, had the cysts. While it could be argued that the 11 cases of amoebic dysentery might have been secondary cases developed in carriers, the much higher percentage of cyst passers among the firemen who drank polluted water than among those who did not, serves as good evidence that many of those cyst passers among the higher percentage group were infected by the polluted water they drank.

C. *Discovery of Cysts of E. histolytica in Sewage and Sewage Effluent.* Recently, Gordon (1941) reported that the effluent of the Moscow sewage discharging into a river had been found to contain *E. histolytica*-like cysts constantly during a period of 3 years of examination, and that storage of the effluent for 6½ hours in a tank failed to remove all cysts from the supernatant. Cram (1943), in this country, reported that *E. histolytica*-like cysts were occasionally found in the sewage sludge both from municipalities and from army camps, and that storage and sewage treatment failed to remove all the cysts from the sewage effluent. In a previous report, the present author (1945a) showed that the cysts of *E. histolytica* have a specific gravity of about 1.06 and that even in perfectly quiet, pure water, it would take 2 or more days for the cysts to settle through a 5-foot depth at 10-28° C. These results imply that in places where carriers exist, the sewage will contain the cysts, and some of these cysts are likely to remain in the effluent after treatment. Pollution of water with this sewage or sewage effluent will render the former a source of amoebic infection.

III. Biological Problems in Water-Borne Amoebiasis

Having shown that polluted water supply may constitute one of the most important sources of amoebic infection, this section of the paper attempts to bring out some of the biological problems related to the cysts of *E. histolytica* in order to furnish information on the purification or disinfection of water for removal or destruction of the amoebic cysts that may be present.

A. *Cultivation of E. histolytica in Artificial Media.* To detect the presence of cysts in possible agents of transmission, or to determine the viability of cysts in survival tests requires cultivation of the cysts in artificial media. Excystation of the mature cysts and multiplication of the trophozoites result in a positive culture. Although several good entamoeba media have been successfully used by protozoologists, obtaining positive culture of the organism is not simple. As shown in a previous report by the author (1946), *E. histolytica* requires a moderately anaerobic condition for excystation and a profound anaerobic condition for perpetuation, and these anaerobic conditions are furnished by the growth activity of an accompanying bacterial flora. This bacterial flora must also not produce unfavorable pH range in the culture. These growth requirements of the amoebae are not provided by every kind of bacterial flora. Therefore, unless the cultures are handled by experienced persons, cultural results obtained in tests for the presence or survival of amoebic cysts may be misleading.

B. *Survival of Cysts of E. histolytica in Water and Sewage.* In a previous report by the author (1943), it was shown that culture-induced amoebic cysts may survive in sterile normal saline, distilled water, river water, or raw sewage for a few days to almost 3 months depending on the temperature of the suspending medium. With a cyst concentration of about 1 million per ml of fluid, the survival time may be expressed by the equation: $t = 87.2 \times 10^{-.0048T}$, where t is the time in days and T temperature in °C. The survival time is essentially the same whether the suspending fluid is sterile normal saline, river water, or raw sewage.

C. *Sedimentation of Cysts of E. histolytica in Water or Sewage.* As stated before, the specific gravity of amoebic cysts was found to be about 1.06. Assuming that the modal diameter of amoebic cysts is 15 microns (except in the small race which may have an average diameter of 10 microns), the settling rate in perfectly quiet, pure water is about 1 foot in 16 hours at 10° C and in 10 hours at 25° C. Under natural conditions, the settling of cysts in polluted water is bound to be much slower, since the water is usually flowing and has convection currents and a higher specific gravity than pure water. This, of course, refers to the isolated cysts only. For those that are embedded in solid matter, the settling rate would be much faster. Hence, while the embedded cysts may be settled out in polluted water, one cannot depend on storage of water or sewage for removal of cysts unless a long period of storage is used.

D. *Removal of Amoebic Cysts by Filtration, Flocculation and Sedimentation, or by Both Methods.* Spector, Baylis and Gullans (1934) have shown the effectiveness of the rapid sand filter in removing cysts from water. In the preparation of purified cyst suspensions for cysticidal studies, the author (1944) has used a small sand filter composed of a 2-inch layer of sand of size between 100 and 140 mesh to separate cysts from starch grains and found that such a sand filter removes practically all cysts from the suspension if the sand is fresh and undisturbed and if no pressure is exerted on the filter. Brady and Black (1945) reported that the portable pressure filters used by the Army removed 88% of the cysts from the water filtered at a rate of 15 gallons per minute. The cyst removal was increased to 99% if flocculation was applied to the water before filtration. These authors also reported that the diatomaceous earth filter developed by the Army removed almost all the cysts from the treated water.

Brady and Black (1945) reported that flocculation with good floc formation followed by sedimentation for 2 hours removed over 99% of the cysts from the supernatant. In a few tests made by the author (unpublished data), it was found that good flocculation of a river water by the use of alum followed by sedimentation for 2 hours removed practically all the cysts (in water containing 100 cysts per ml) from the supernatant of 10 cm in depth, and that the settled flocs contained cysts in lumps and unharmed. However, with poor flocculation, only 90% to 95% of the cysts were removed. Hence, it seems that this method of water purification, if properly done, may remove all the cysts in water.

E. *Resistance of Cysts of E. histolytica to Acids and Bases and Various*

Disinfectants. In the last 5 years, numerous experiments on the destruction of amoebic cysts in water by various chemicals have been conducted in our laboratory. A few reports by other investigators have also appeared in the literature. It is beyond the scope of the present paper to deal in detail with the data on this subject, but it is felt desirable to present here a brief account of the conclusions reached in our studies and by other investigators. The early studies in which the eosin staining method was used (Wenyon and O'Conner 1917; Mills, Bartlett and Kessel 1925; Baylis, Gullans and Spector 1936) for determining the viability of treated cysts and the more recent study made by Stone (1937), in which the viability of the treated cysts was tested by the culture method without restoring the bacterial flora are not included in this presentation because these methods of testing the viability of amoebic cysts are unreliable.

1. *Acids and Bases.* Cysts of *E. histolytica* are very resistant to acids and bases. The cysticidal effect seems to be chiefly a function of the hydrogen or hydroxide ion concentration. In our studies on water disinfection (Fair, Chang and Morris 1945), it was found that the culture-induced cysts were not destroyed by acids at a pH value of 0.5 with a contact time of 2 hours. With bases reaching a pH value of 14.0, the cysts were destroyed in 10 minutes; but a pH value of 13.0 showed no cysticidal effect in 2 hours. Hence, it seems that the OH-ions are more cysticidal than the H-ions. Unfortunately, this cysticidal pH value is too high to be of practical use in ordinary water treatment.

2. *Halogens and Halogen Compounds.* In an earlier report by Chang and Fair (1941), it was shown that amoebic cysts were definitely more resistant to active chlorine than vegetative bacteria, such as *Escherichia coli* and that the cysticidal efficiency of active chlorine increased with lowering of pH value, rise of water temperature, and increase in contact time, and, to a less extent, with decrease in cyst concentration. It was concluded that for complete destruction of amoebic cysts that may be present in water, doses of active chlorine in the lower range of superchlorination would be required provided that the pH of the water could be held below 7.5 and the chlorine demand was not too high. Brady and his associates (1943) also reported that superchlorination is necessary to destroy the cysts; but they also noticed that the contact time is more important than the chlorine dosage, since increase in chlorine dosage sometimes resulted in a lowering of the cysticidal efficiency. In view of the fact that these investigators used calcium hypochlorite in their tests and that the increase in chlorine dosage by the use of calcium hypochlorite automatically raises the pH of the chlorinated water (Fair, Chang and Morris 1945), thus decreasing the amount of more cysticidal hypochlorous acid (HOC01) and increasing the amount of less cysticidal hypochlorite ions ($OC1^{-}$), this phenomenon observed by Brady *et al.* (1943) was to be expected. It is regretted that these authors did not determine the pH value of their treated water.

Becker, Burks and Kalieta (1946), working with amoebic cysts obtained from carriers in India, reported that even as much as 10 ppm of residual chlorine failed to destroy all cysts in water of relatively low organic content with a contact time of 60 minutes. These authors used bromthymol blue as an indicator in regulating the pH of the chlorinated water to neutral. Since organic dyes like bromthymol blue react rapidly with active chlorine to give false colors, it is apparent that they were working at unknown pH values. In view of the fact that these authors used calcium hypochlorite for active chlorine and NaOH for regulating the pH, it is probable that these authors were working at high pH values.

In two recent reports, the present author (Chang 1944a; 1944b) brought out the fact that in chlorination studies, the chemistry of chlorine and chlorine compounds must be thoroughly understood. The effect of pH on the cysticidal efficiency of chlorine is profound and lies in the alteration of the proportions of chlorine present as hypochlorous acid and hypochlorite ions. In the pH range up to 9.0, the cysticidal activity seems to be entirely due to the HOC1 concentration. At 25-28° C and with a contact time of 10 minutes, about 2 ppm of residual chlorine as HOC1 destroyed all cysts when a cyst concentration of 30-60 per ml was used. More recently, we showed that the cysticidal efficiency of hypochlorite ions is only about 1/300 of that of HOC1 (Chang, Fair and Morris 1945; 1946). In another report (Fair, Chang and Morris 1947), it has been shown that the cysticidal efficiency of HOC1 can be expressed by the cysticidal concentration-time relationship which is represented by the equation: $tC^n = k'$, where k' is the disinfection constant, t the disinfection time in minutes, C the concentration of disinfectant in ppm, and n the concentration coefficient of the disinfectant. Substituting the values for HOC1 into the equation, it was found that,

$$t = 205C^{-1} \text{ at } 3° \text{ C.}$$
$$t = 39C^{-1} \text{ at } 23° \text{ C.}$$

From the value of n, it is seen that the concentration affects the cysticidal efficiency of HOC1 just as much as the contact time. In the same report (Fair, Chang and Morris 1946), it has been shown that the temperature affected the cysticidal efficiency of HOC1 to give a Q_{10} of about 2.2.

The chloramines and chloramides are definitely less cysticidal than HOC1. Among the monochloramines and chloramides, ammonia-monochloramine is more cysticidal than the organic compounds or hypochlorite ion. This is believed to be due to the small molecular size of ammonia-monochloramine, which penetrates more readily into the cysts than the larger molecules of organic chloramines and chloramides and negatively charged hypochlorite ions. Among the organic monochloramines and chloramides, succinchlorimid seemed to be the most cysticidal. At 23° C and with a 10-minute contact time, 10 ppm of residual chlorine as ammonia-monochloramine destroyed all cysts, while the same efficiency was reached by 80 ppm of residual chlorine as succinchlorimid, or 140 ppm of residual as monochloramine-T or azochlorimid. It must be pointed out that the results obtained with succinchlorimid were oftentimes irregular, due apparently to its unstability in solution.

The dichloramines are more cysticidal than the monochloramines and among the dichloramines, ammonia-dichloramine seemed to be the most cysticidal. About 6 ppm of residual chlorine as ammonia-dichloramine destroyed all cysts in 10 minutes at 23° C while the commonly used organic dichloramine, Halazone, reached the same efficiency at a residual chlorine concentration of 12 ppm (Fair, Chang and Morris 1945; 1946).

Chlorine dioxide as tested in our laboratory, seems to be less cysticidal than HOC1 on a basis of ppm as titrable chlorine, and we were uncertain as to the method for determining the amount of active chlorine in this compound.

Bromine, in its elemental form, has also been tested in our laboratory for its cysticidal efficiency. In a small number of experiments, it has been found that this halogen is slightly less cysticidal than HOC1 on a weight basis and slightly more cysticidal molecule for molecule (Fair, Chang and Morris 1945). This is believed to be due to the fact that bromine, having a smaller hydrolysis constant, exists to a greater extent in elemental form than chlorine, the elemental forms of the halogens being thought to penetrate cysts more easily than their compounds. However, bromine showed a considerably greater loss to the halogen demand of the water than HOC1.

Iodine has been extensively studied in our laboratory. Its cysticidal efficiency, as reported by us (Fair, Chang and Morris 1945; 1946) is less than that of HOC1 on a weight basis but slightly more on a molar basis. This is again believed to indicate that elemental iodine penetrates cysts more easily than HOC1. The advantage of elemental iodine as a cysticidal agent over HOC1 lies in the fact that it is less affected by changes in temperature, does not form iodomines with ammonia or amino compounds, and does not impart as much objectionable odor and taste to the treated water as does HOC1 at the cysticidal level. The cysticidal efficiency of elemental iodine has also been expressed in concentration-time relationship as follows:

$$t = 171.5C^{-1.4} \text{ at } 3° \text{ C}$$
$$t = 118.7C^{-1.4} \text{ at } 10° \text{ C}$$
$$t = 57.4C^{-1.4} \text{ at } 23° \text{ C}$$

The effect of temperature on the cysticidal efficiency of iodine has also been calculated to give a value of Q_{10} of 1.6 (Fair, Chang and Morris 1946).

Ozone was reported by Kessel and his associates (1944) to be more cysticidal than active chlorine. These investigators found that a residual of 0.1 to 0.3 ppm of ozone (using orthotolidine test) destroyed all the cysts in water in a few minutes.

The cysticidal efficiency of synthetic detergents has been studied by Kessel and his associates (1946) and also in our laboratory. In the report of Kessel *et al.* (1946), it is noticed that some of the cationic detergents were cysticidal at a concentration of less than 12.5 ppm. In our reports

(Fair, Chang and Morris 1945), we showed that anionic detergents were noncysticidal at a concentration as high as 20,000 parts per million. Neutral detergents, such as Hexyl-resorcinol, were cysticidal at a concentration of 75 ppm in 10 minutes to 30-50 ppm in 120 minutes. Cationic detergents were cysticidal at lower concentrations but some were considerably more cysticidal than others. For instance, Fixanol, Sapamine, Ceepryn, Zephiran, Ortho-7 and Nopco-QCL were cysticidal at 5-10 to 30 ppm in 10 to 120 minutes, while others, such as Emulsol-660B, were not cysticidal even at 75 ppm in 10 minutes to 50 ppm in 120 minutes. The cysticidal efficiency of cationic detergents was found to be affected by the presence of proteins, lipoids, and probably also by greasy and soapy substances. Anionic detergents were found to be good neutralizing agents for cationic detergents (Fair, Chang and Morris 1945; Kessel and Moore 1946).

IV. Discussion

Is amoebiasis an important infection in this country? Besides the nationwide distribution of cases of quiescent infection, about 3 to 4 thousand cases of amoebic dysentery have been reported annually in the Public Health Reports of the U. S. Public Health Service in the last 10 years. Of these reported cases, about one third have been from the State of Mississippi. Hence, it would seem that if amoebiasis is not a serious infection in the country as a whole, it is so in Mississippi.

While it would be absurd to think that polluted water is important in spreading amoebic infection in areas where water supply is sanitarily controlled, it would be equally absurd to think that polluted water is of no importance in establishing the infection in areas where water supply is still inadequately controlled. Judgment as to the more important mode of transmission of this infection must be based on the local conditions existing rather than on biased opinion.

Since the present paper deals only with the problems in water-borne amoebiasis, emphasis has been laid on this route of transmission. The author, of course, has no intention of minimizing the other routes of transmission when circumstances that favor the spreading of the infection by such routes exist. From the epidemiological problems presented in the paper, it is apparent that any argument for or against the water-borne theory is circumstantial in nature, and so are the arguments for or against the theory of other routes of spreading the infection. If diseases like typhoid and bacillary dysentery result from unhygienic habits of eating and drinking, so does amoebiasis. A very careful study of the epidemiology of the amoebic dysentery as well as quiescent amoebiasis and of the effectiveness of various methods of control in Mississippi may bring out valuable information and throw more light on the subject of the mode of transmission of amoebiasis.

The biological characteristics of the cysts of *E. histolytica* brought together in the present paper provide us sufficient information as to the problems to be encountered in the control of water-borne amoebiasis. From these characteristics, it is apparent that if polluted water is to be used as a source of public water supply, it must be considered as a potential source of amoebic infection and must be treated as adequately as is the practice in a modern municipal water works, namely, by storage, flocculation and sedimentation, filtration, and chlorination. The cysts that may be present in the water are completely removed by the combined effect of the first three methods but will not be killed by chlorination as normally practiced in water works. If chlorination is the only method available for destruction of cysts in water, then superchlorination or breakpoint chlorination must be employed. For emergency treatment of water to destroy cysts, superchlorination at pH 5.0 to 6.0 followed by dechlorination or treatment with elemental iodine to give a residual of about 4 to 5 ppm in 10 minutes at pH 5.0-7.0 would be satisfactory. The use of 30 to 50 ppm of some of the best cationic detergents is also feasible, but this treatment would produce a bitter and slightly soapy water. Ozone is a powerful cysticidal agent, but its use is bound to be handicapped by its low solubility and the difficulty of distributing it evenly in a large body of water.

It goes without saying that one of the best control measures of amoebiasis is a thorough treatment of the cases of amoebic dysentery to prevent them from swelling the army of carriers and a thorough treatment of all carriers. While the former can be effectively carried out, the treatment of all carriers, particularly in areas where the carrier rate is high, would certainly encounter many difficulties.

V. Summary

In this paper, some of the clinical features of amoebic infection have been discussed to bring out the difficulties involved in the study of the epidemiology of this infection for establishing the mode of transmission. These features are quiescent infection, primary and secondary amoebic dysentery, incubation period, and the not-too-well-understood factors involved in the provoking of amoebic dysentery.

The water-borne nature of amoebiasis has been discussed. Evidences such as the Chicago epidemic, the Chicago Stockyard fire outbreak of enteric infections, and finding of *E. histolytica*-like cysts in sewage and sewage effluent are brought out and analysed to show the probable role played by polluted water in the spread of amoebiasis.

The biological characteristics of the cysts of *E. histolytica* have been presented, such as the cultivation of amoebae, the specific gravity and settling of amoebic cysts in water and sewage, the removal of amoebic cysts from water by flocculation and sedimentation and by filtration through sand or diatomaceous earth filters, and the resistance of amoebic cysts to various water disinfectants, particularly the halogens and halogen compounds.

At the end of the paper, the thought is expressed that if amoebiasis is not a serious public health hazard in the United States as a whole, it is

so in the state of Mississippi. It is suggested that the study of the epidemiology of amoebic dysentery and the quiescent infection, and of the effectiveness of various methods of control in Mississippi, may throw more light on the subject of the mode of transmission of amoebiasis in a free community.

The author is grateful to Dean G. M. Fair, to his colleague Professor E. W. Moore, and to his friend Dr. D. Weinman for reading this manuscript and making helpful suggestions.

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BIOTIC RESPONSES TO STREAM POLLUTION DURING ARTIFICIAL STREAM REAERATION

By A. F. BARTSCH

WISCONSIN COMMITTEE ON WATER POLLUTION

AND

WARREN S. CHURCHILL

WISCONSIN CONSERVATION DEPARTMENT, MADISON, WIS.

Introduction

During lumbering days, the Flambeau River carried logs of majestic pine and hardwood, and even now the water is shaded occasionally by virgin timber. Its natural beauty is an annual attraction to the fisherman and vacationist. Aside from these values, the river is efficiently washing away pulp and paper mill wastes equal in strength to the domestic sewage from a city about the size of Omaha, Richmond or Oklahoma City. This report is primarily an account of the way in which the living things in the stream are affected by this natural clean-up job.

Hydrographic and Cultural

The Flambeau River is located in the north-central part of Wisconsin (Figure 1) and drains southwesterly into the Chippewa. The drainage area of the North Fork, considered in this report, is about 1,150 square miles with 720 square miles above the city of Park Falls. This area is mostly cut over land, relatively undeveloped and with a low human population. The most prominent cultural feature is Park Falls with a population of about 3,200.

Domestic sewage is treated efficiently by the activated sludge process (Figure 2). Daily B.O.D. population equivalent from this source is 150. Critical pollution of the North Fork by waste sulphite liquor originating at a Park Falls mill has been a serious problem for more than 10 years. The daily B.O.D. contribution from this source was about 189,000 population equivalent during the summer of 1946. In years of low stream flow, conditions became severe as far downstream as Oxbo—25 to 30 miles from the pollutional source. Dilution by river water alone as a source of dissolved oxygen has been adequate only when flows approach 2,000 c.f.s.—almost three times average flow.

These conditions led to selection of the Flambeau River as the subject for experimental artificial reaeration. The project was undertaken as a cooperative one by the Sulphite Pulp Manufacturers' Committee on Waste

Disposal, the National Council for Stream Improvement and the Committee on Water Pollution, State of Wisconsin.

The reaeration procedure, still going on, consists of pumping air under pressure through carborundum diffusers located in the head and tail races of Pixley Dam. This location—6.3 miles from the pollutional source—was selected as the point where dissolved oxygen approaches depletion.

Fig. 1. Drainage area of Flambeau river. North Fork shown in black.

Four summers of artificial reaeration study have shown the procedure to be valuable in disposing of B.O.D. more rapidly, raising dissolved oxygen levels and shortening the zone of critical conditions. That was the setting under which a biological study was carried out during the summer of 1946.

The aims were three in number: (1) to determine the responses of downstream biota to the presence of pollutants tributary to the river; (2) to locate the stream level or levels of biological recovery, and (3) to determine if artificial reaeration visibly accelerates the biological recovery.

Biological Considerations

It is generally recognized that the living organisms in a polluted stream are intimately concerned with many of the transformations that lead to

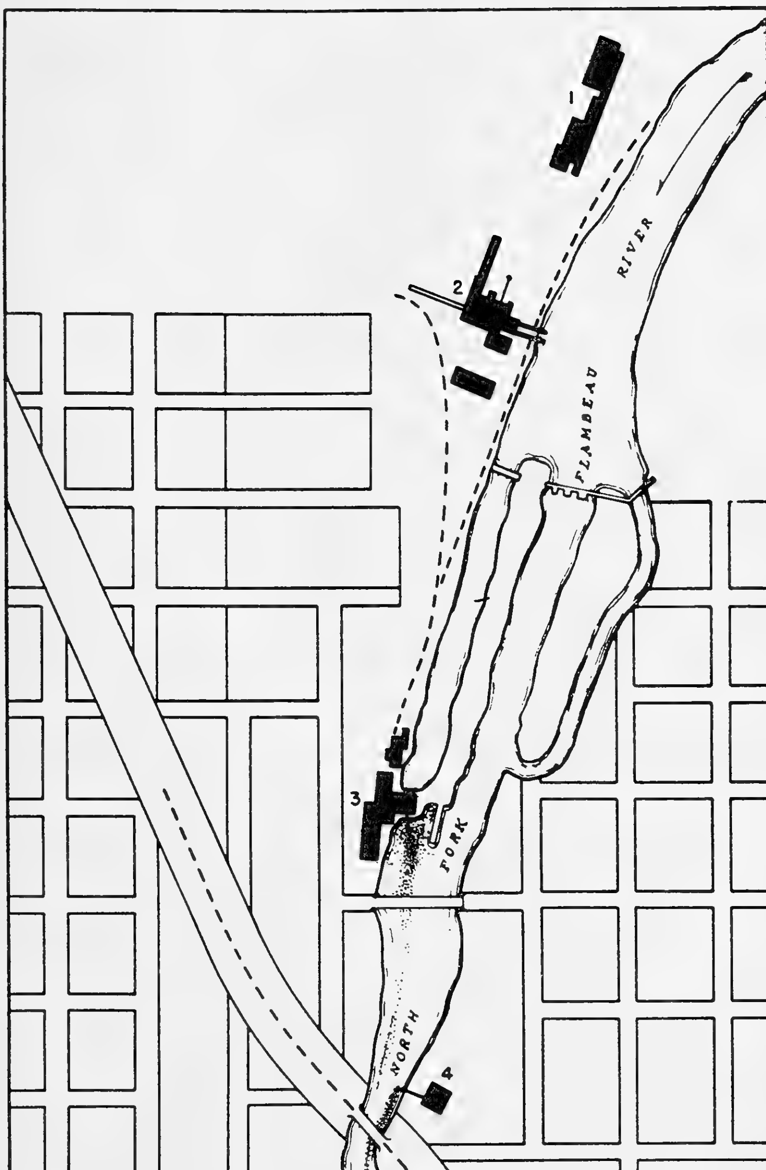

Fig. 2. Sources of pollutants, city of Park Falls. 1 and 2, lumber mills. 3, pulp and paper mill. 4, sewage treatment plant.

sanitary recovery. At the same time, their linear distribution is influenced by the intensity of pollution, while population magnitude is determined by degree of suitability of the habitat. Some organisms find pollutional conditions ideal for their livelihood and characterize these conditions by their presence. They are sometimes called index organisms. Others are inhibited by these same conditions, and their absence may be significant.

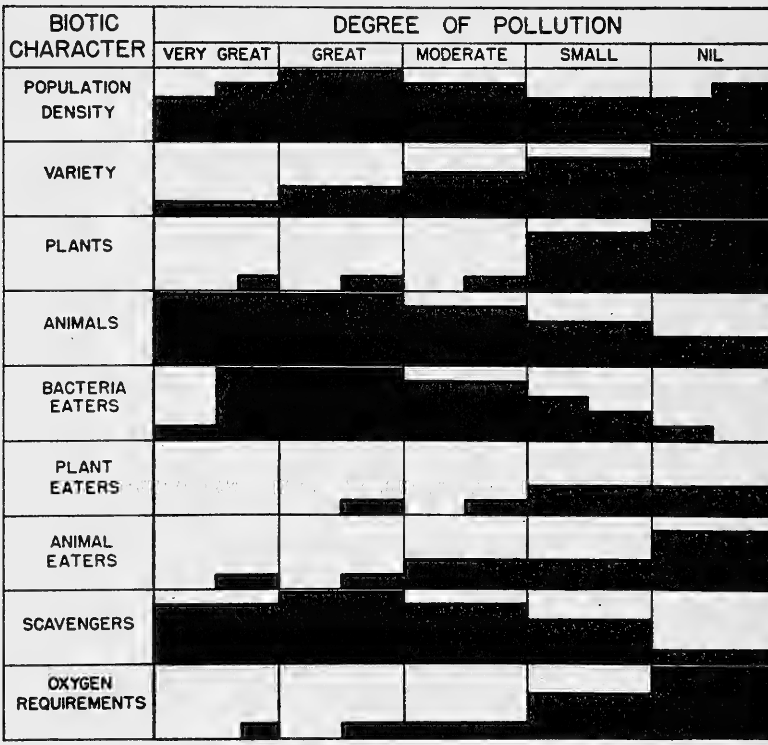

FIG. 3.

Such biotic responses are the basis for biological criteria of stream condition as summarized in Figure 3 modified from Hentschel.[1] Some of these are as follows:

(a) Population density generally is in direct proportion to degree of pollution except at the two extremes.

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[1] Hentschel, E. 1923. *Abwasserbiologie.* Handbuch der biologischen Arbeitsmethoden by Emil Abderhalden. Sec. IX, Part 2, Book 1. Berlin-Vienna: Urban und Schwarzenberg.

(b) Variety of organisms is inversely proportional to the degree of pollution.

(c) Scavenger populations may be tremendous in extreme pollution and decrease rapidly with stream recovery.

(d) The more serious the condition of pollution, the less are the oxygen requirements of the existing biota.

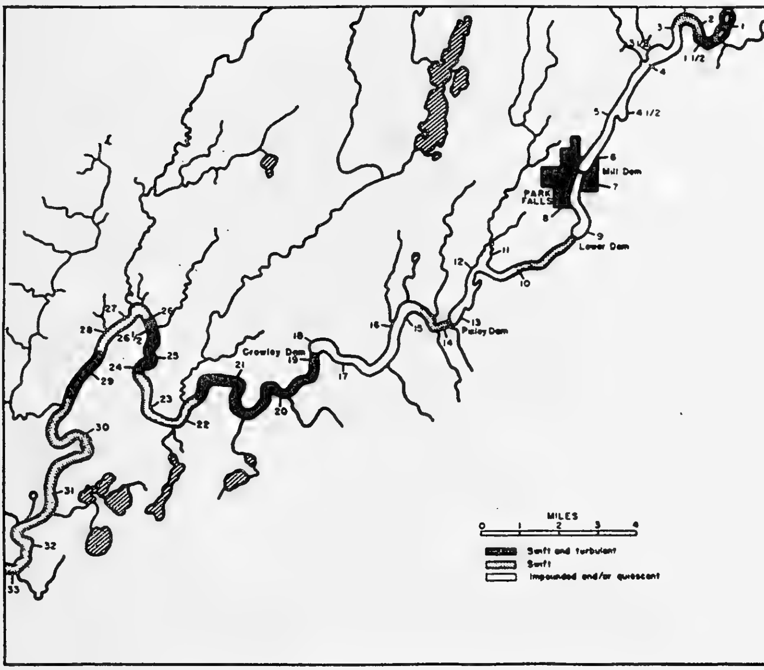


Fig. 4. Sampling stations, north fork of Flambeau river.

(e) Biotic responses to stream pollution are superimposed upon the responses to natural stream conditions.

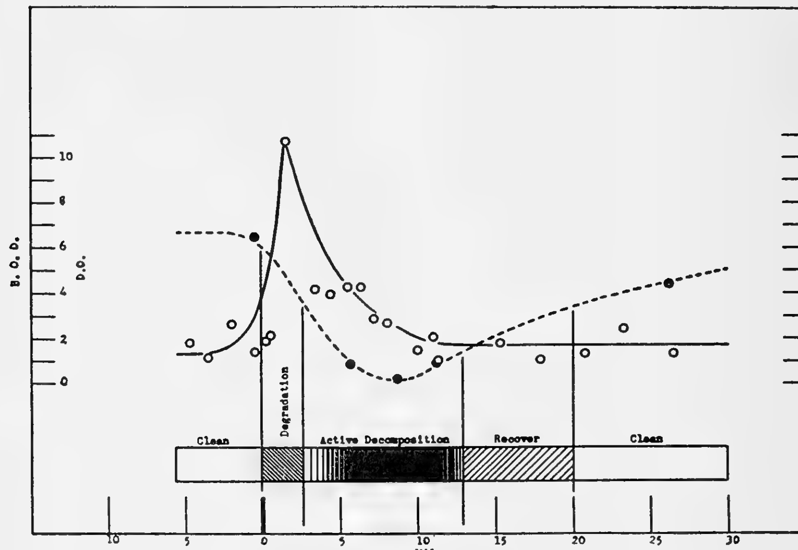
(f) The value of these criteria is jeopardized by the presence of toxic types of trade wastes.

With this summary as a brief review, we are ready to examine the sanitary and biotic status of the Flambeau River.

Procedure and Data

Sampling stations were selected at various stream intervals as shown in Figure 4, beginning 7.1 miles above Park Falls and extending 39.1 miles downstream. All sampling was done by boat, since the river is not otherwise accessible. Samples were collected for data on B.O.D., apparent color, physical character of stream bed, aerobic and anaerobic plate counts, *B. coli* index, plankton and benthos. Not all of these data, although available, will be reported here.

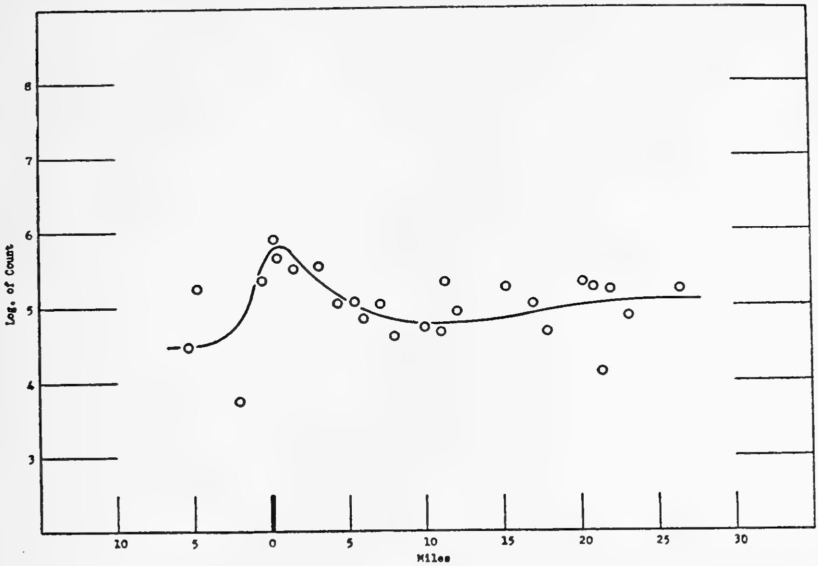
In the region under study, available bacterial foods are waste sulphite liquor, wood fiber, domestic sewage and other organic substances of natural origin. Graph 1 shows that the food supply, expressed as B.O.D., increases abruptly as a result of the entry of waste sulphite liquor at level zero. Bacterial oxidation plus a non-biological affinity of this trade waste for



Graph 1. ———o——— Five day biochemical oxygen demand ppm at 20° C. ------•------ Dissolved oxygen in ppm. Average for August 8, 9, 10.

oxygen reduces the supply to less than 1 ppm within 10 miles. A correlative relationship will be shown between these environmental conditions, high food and low oxygen, and the biotic density and distribution. It goes without saying, of course, that other factors also are influential.

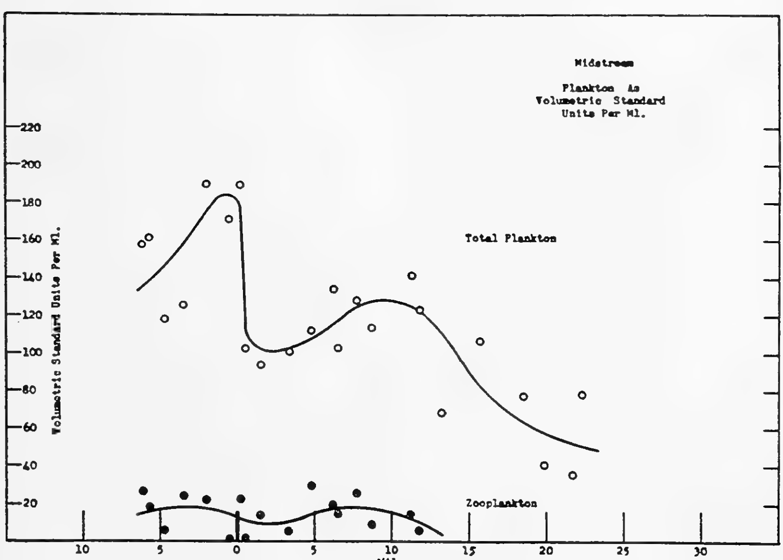
Twenty-four hour, 22° C aerobic plate counts are plotted as log of the count per milliliter in Graph 2. Field conditions necessitated storage of the samples just above the freezing point for 48 hours before plating, so the dependability of the data is questionable. At the same time, they do suggest an inhibitory influence of waste sulphite liquor. Anaerobic counts tended to increase erratically downstream but are not considered significant. As would be expected, the *B. coli* index reached a peak of 100,000 below Park Falls and gradually decreased to 1,000 twenty-seven miles downstream.



Graph 2. Aerobic bacteria per milliliter plotted as logarithm of the count.

Two and one-half liter plankton samples were concentrated by means of a continuous-flow centrifuge and analyzed in accordance with Standard Methods of Water Analysis so that results could be expressed as population per milliliter and as volumetric standard units.

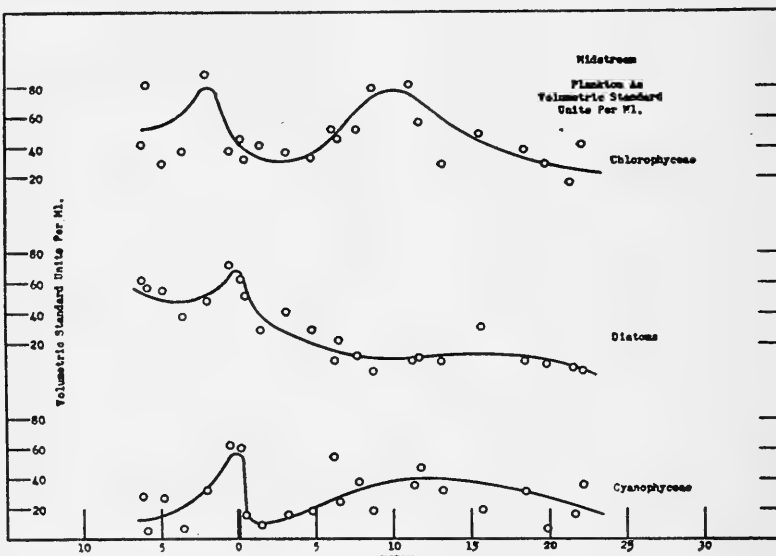
The trend curve for total volume (Graph 3), expressed as volumetric



Graph 3.

standard units (8,000 cu. micra), shows an increasing quantity above Mill Dam. Following introduction of mill wastes, the quantity drops within 1.5 miles from 190 to 95 volumetric standard units. Plankton again increase in the quiescent water behind Pixley and Crowley Dams although never reaching the volume peak above Mill Dam. The decrease from the 11 mile level downstream shows the destructive and inhibitory influence of swift and turbulent water. If quiescent water persisted downstream, it is possible that the plankton volume would increase beyond the peak at Mill Dam.

Volume of zooplankton is erratic but shows the same depression as total plankton. As a group, they appear relatively scarce which may be due, in part, to the difficulty of recognition in the preserved condition. These



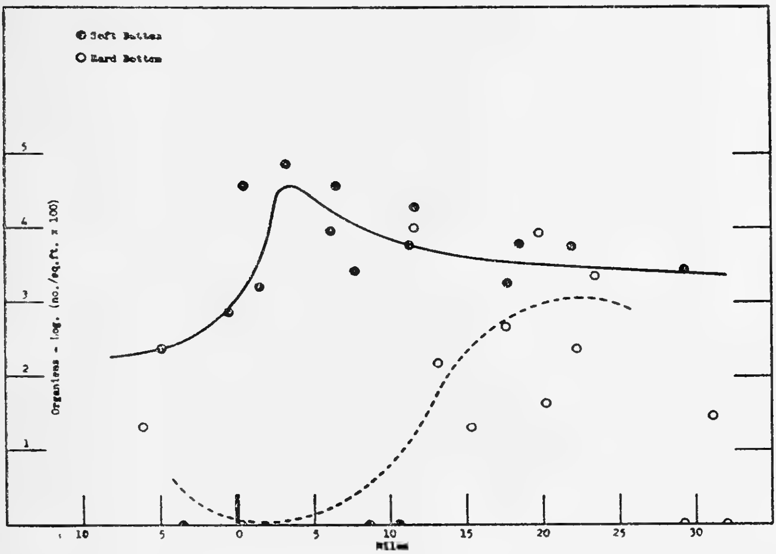
Graph 4.

totals, however, do not include immense populations of *Carchesium* and *Vorticella* attached to waving masses of *Sphaerotilus natans* along the shore.

The main constituents of the phytoplankton show a trend of the same pattern as that of total volume (Graph 4). The green and blue-green algae and the diatoms all show a decline within ½ mile of the pollutant entry point. Volume recovery is rapid from the 3 to 11 mile levels for both green and blue-green algae, whereas the diatoms continue to decline to the 10 mile level and then increase only slightly.

The plankton data fail to locate a point or zone where recovery is complete. It is concluded that the decreases in plankton in the downstream vicinity of Park Falls reflect the toxic influence of industrial effluents—perhaps from SO_2 in waste sulphite liquor. The following rising volumes are interpreted as a positive response to increased food and food material supplies made available through stream recovery processes. Further decreases downstream apparently result from the swiftness and turbulence of the stream.

While it would have been valuable to study the plankton for species



Graph 5. Total Oligochaeta, log. (no./sq. ft. x 100)

that may be characteristic of waste sulphite liquor pollution, no attempt could be made to do so. That information is lacking at the present time.

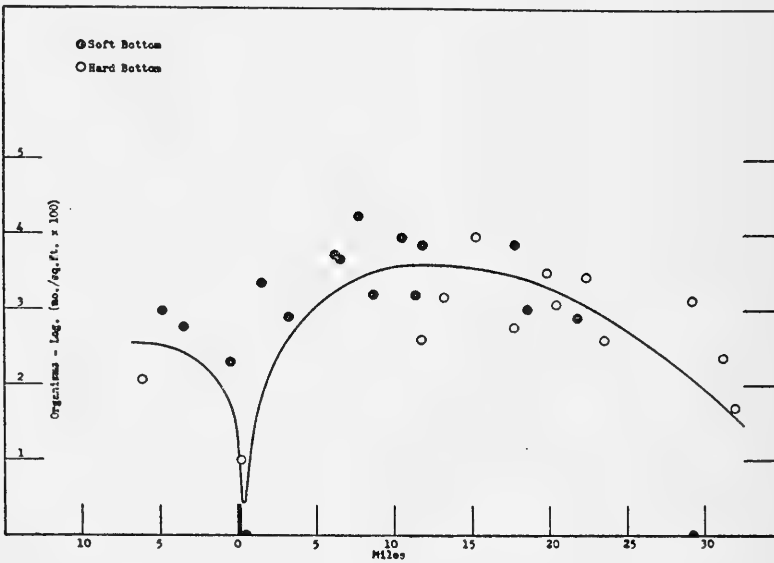
Samples of the bottom deposits and their organisms were collected with the Eckman or Peterson dredges or with the square-foot sampler with downstream net. Samples were examined for physical quality of the substratum and determination of quality and populations of bottom organisms.

Appreciable deposits of bark, chips or wood fiber were found as far as 6.1 miles downstream, and intermittent deposits of fiber 11 miles downstream.

Bottom organisms, as referred to here, are those restricted in their habitat to a position on the surface of, or imbedded in, the accumulated deposits or natural bed of the stream. They obtain their food from the supernatant medium in which they are bathed or feed as they burrow through or creep over the beds of deposited substances. Inhabitants of such substrata in pollutional areas must be able to tolerate low oxygen supply and toxic products of anaerobic processes. Some of the larger inhabitants are nematodes, flatworms, annelids, crustaceans, molluscs and insect larvae.

Since organisms vary in their powers of pollutional tolerance, it is to be expected that some species will find suitable conditions for existence at points where natural recovery has not yet progressed sufficiently for the livelihood of others. For this reason population peaks of various species will be arranged in linear order downstream. Certain species, though often occurring in clean water, are able to tolerate and frequently thrive under severe pollutional conditions. Notable of these are tubificid worms and chironomid larvae. Certain naiad worms, sphaeriid mussels, some snails and the Isopod, *Asellus communis,* thrive in regions of low and improving oxygen content. The presence of appreciable numbers of these, especially in the absence of less tolerant species, is evidence of serious pollution. Some of these biotic relationships and responses to pollution will be shown in the following graphs.

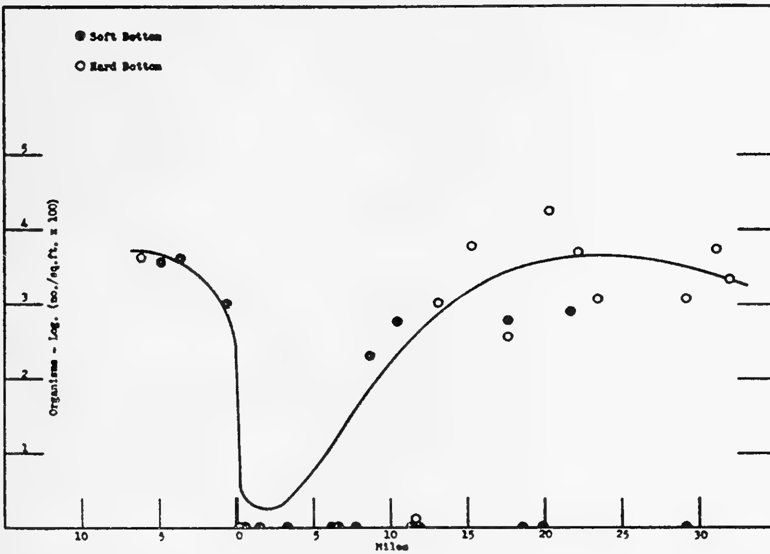
Total *Oligochaete* population, and especially the tubificid worms (Graph 5), shows a positive response to the presence of pollutants. They are notorious for this type of response, and their population trend follows the B.O.D. trend quite closely. Even hard bottom populations, which are normally low, increase considerably downstream.



Graph 6. Total Chironomidae, log. (no./sq. ft. x 100)

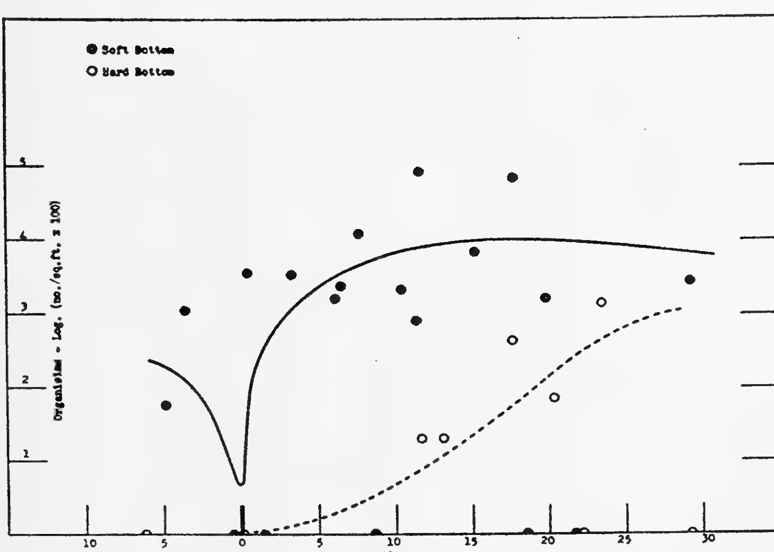
Chironomid larvae (Graph 6) are absent from the immediate downstream vicinity of Park Falls but respond quickly to pollutional conditions by producing an appreciable population within 2 miles. They reach a population peak at about 11 miles and then decrease gradually downstream.

The distribution of all other insect larvae (Graph 7) is in contrast to the Chironomidae. A substantial population of these clean water forms was present above Park Falls but dropped to zero following the introduction of pulp and paper mill effluents. The bottom was devoid of insect larvae, other than chironomids, for a downstream distance of 8.6 miles, and some habitats were still unsuitable beyond that point.

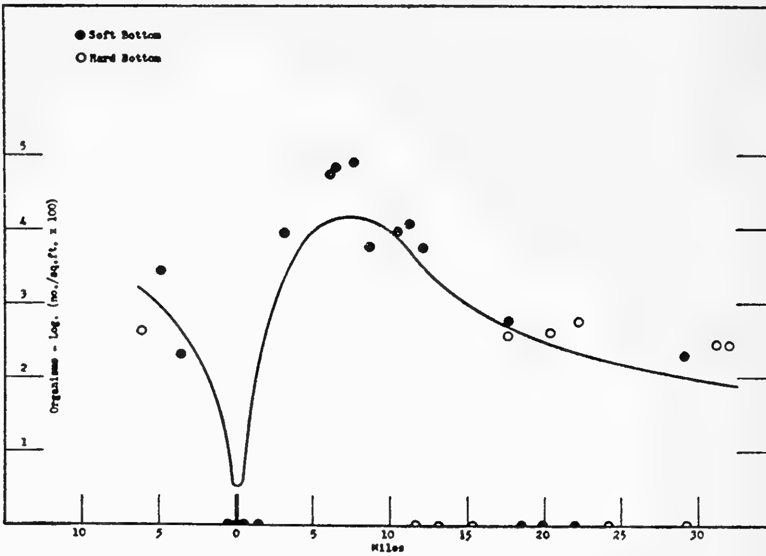


GRAPH 7. All insect larvae minus Chironomidae, log. (no./sq. ft. x 100)

The snail populations (Graph 8) consisted of 4 different species with *Campeloma integrum* the dominant one. Three of them are pulmonates which may breathe air by means of a lung and therefore are not dependent upon dissolved oxygen values of the stream. For this reason, their distribution is governed mainly by available food, suitable substratum and con-

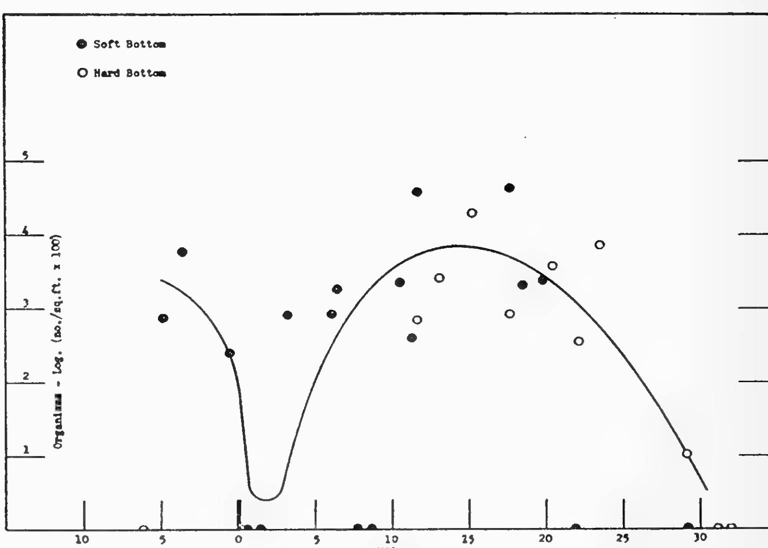


GRAPH 8. Total snails, log. (no./sq. ft. x 100)



Graph 9. Total Sphaeriidae, log. (no./sq. ft. x 100)

centration of toxicants. While they apparently resist high concentrations of waste sulphite liquor, they do not inhabit the zone immediately below the industrial sewer outlet. Within 3 miles, they reappear in concentrated population and persist beyond the 32 mile station.

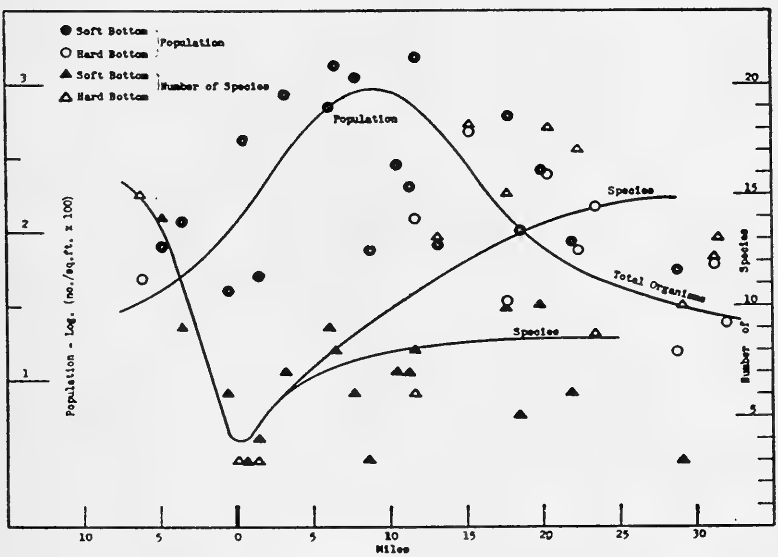


Graph 10. Population of Asellus, log. (no./sq. ft. x 100)

The sphaeriid mussel, *Sphaerium rhomboideum,* also responds positively to intense conditions of pollution (Graph 9). Although immediately inhibited by trade waste, the rapid population increase downstream resembles that of the Oligochaetes.

The sow-bug, *Asellus,* is recognized as an indicator of mild or improving conditions. They reach their population peak near the 15 mile level (Graph 10) and are infrequent beyond 25 miles.

Population peaks of the prominent bottom organisms have the following linear order: Oligochaeta—3.2 miles, Sphaeriidae—7.7 miles, Chironomidae—11, *Asellus*—15, snails—22, and various insects as a group—26 miles.



Graph 11. Relationship of populations and numbers of species.

The trend curve of total bottom population (Graph 11) is in contrast to station variety expressed as number of species. It will be noted that variety begins to decrease above the zero level. This appears to be due, at least in part, to decreased velocity above Mill Dam and to the presence of bark and chips accumulated on the bottom. Variety is decreased further with the entry of trade wastes with only 3 species found in the distance of 1.8 miles to Lower Dam. Variety increases somewhat below this point. On soft bottom, the pre-pollutional variety is not regained at any downstream level examined, but on hard bottom variety is normal between the 10 and 15 mile level.

While there is considerable station-for-station fluctuation in total population, the trend is for a striking population peak near the 9 mile level. The following progressive decrease apparently results from declining food supply and changing physical conditions.

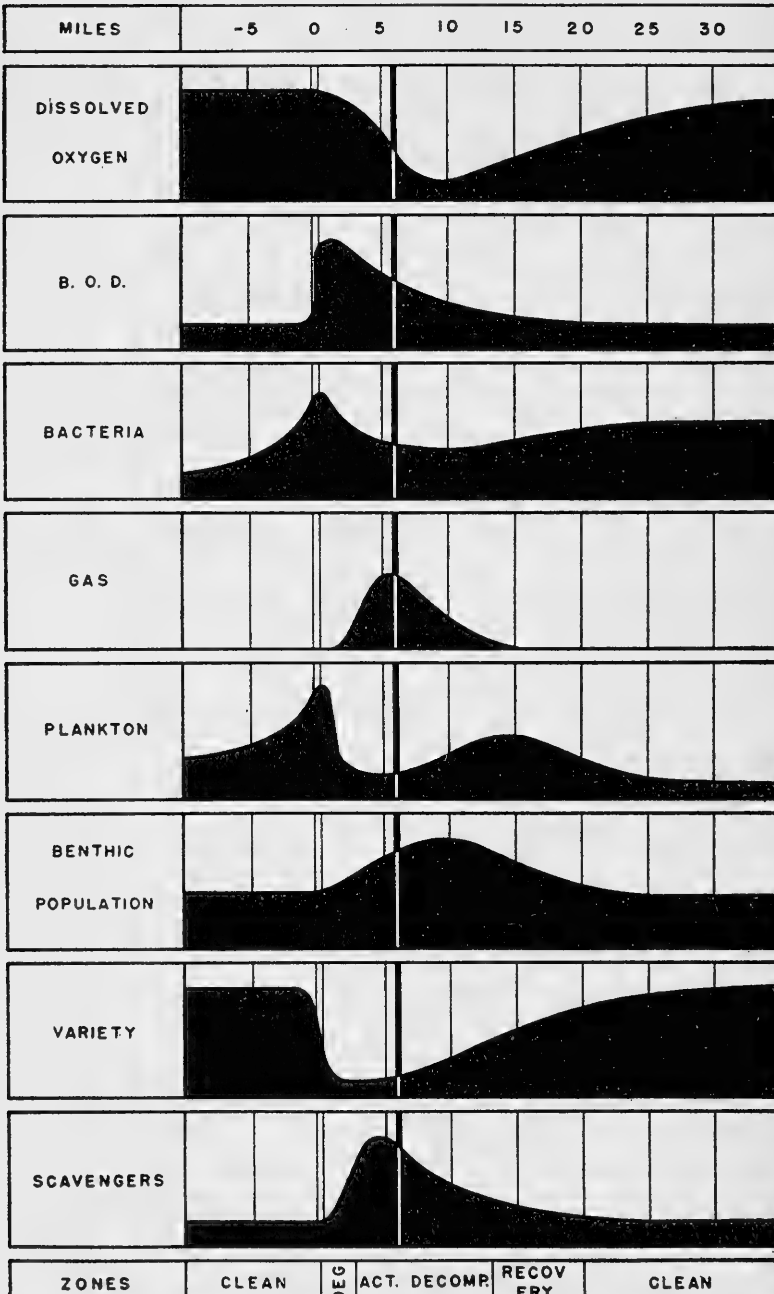


FIG. 5. Diagrammatic summary of data.

Conclusions

The pertinent data on stream condition are diagrammatically summarized in Figure 5. Without question, there are definite biotic responses to industrial pollution and the environmental conditions that result. Decreases in population of bacteria and plankton and abrupt loss in variety of bottom organisms below the industrial sewer suggest the presence of a toxic ingredient. Gas production and the rising of bottom fiber mats occur in the region of oxygen depression. This region is unsuitable for many kinds of organisms, but the persistent ones thrive, and scavengers are numerous.

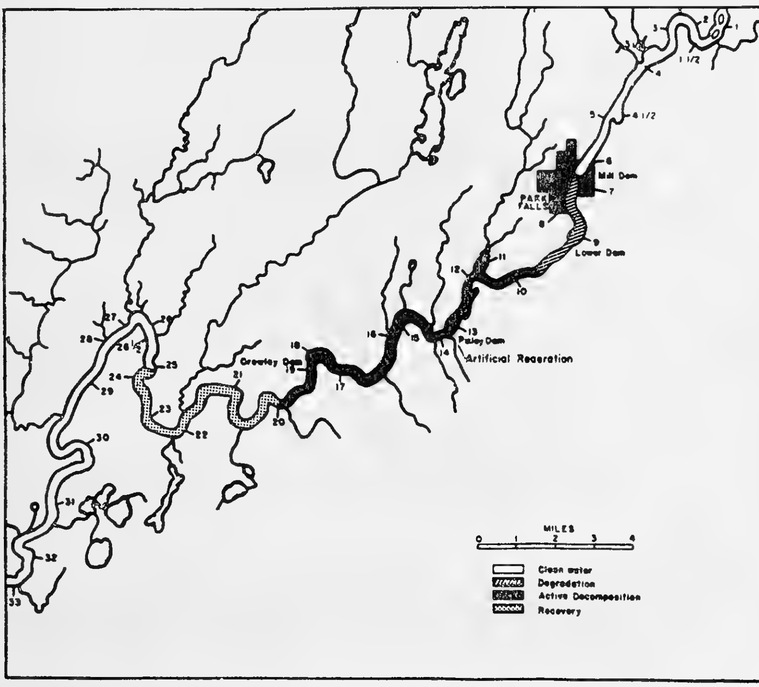


Fig. 6. Stream zones, north fork of Flambeau river.

Abrupt improvement of the biological picture below the site of artificial reaeration was not apparent. At the same time, improvement in the recovery zone may be accelerated so that a normal, clean water biota extends farther upstream.

The available data show that conditions for biotic existence below the 20 mile level are similar to those above Park Falls. These are the zones of clean water as shown in Figure 6. Thirteen miles of stream are seriously affected by pollution and have little recreational or other value. For 2.5 miles below Park Falls, in the zone of degradation, dissolved oxygen decreases to 40 per cent saturation and inhibitory substances are present. Below this zone oxygen conditions are poorest for aquatic life. Dissolved oxygen approaches or reaches zero in the zone of active decomposition and then slowly climbs to a level sufficient for a variety of organisms. Improvement continues in the recovery zone with normal biotic populations at the 20-mile level.

Biotic responses to stream pollution lead to the intense biochemical activities that assist in sanitary recovery. Bad as it may seem, localized stream defilement is a necessary part of the process. Only by this sacrifice are the recreational downstream areas preserved.

Summary

During the summer months, since 1944, diffused air has been pumped into the North Fork of the Flambeau River near Park Falls, Wisconsin, in an attempt to alleviate the serious conditions resulting from sulphite pulp and paper mill pollution. While four years of study have shown benefits from the artificial reaeration procedure, biotic responses to pollution under these conditions were unknown.

Investigation has shown no abrupt biological improvement near the artificial reaeration site, but improvement in the downstream recovery zone is accelerated. The introduction of these industrial pollutants has an immediate toxic influence upon most organisms and alters the biological picture for a distance of 20 stream miles. In this region the societies of organisms increase, reach a population peak, and decrease in linear order as their biochemical activities change the degree and character of pollution. Action upon the pollutants in the 20-mile zone produces local critical conditions but, in so doing, protects downstream recreational areas.

A STUDY OF KRAFT PULPING WASTES IN RELATION TO THE AQUATIC ENVIRONMENT

WILLIS M. VAN HORN

THE INSTITUTE OF PAPER CHEMISTRY, APPLETON, WIS.

Introduction

Of the several methods for the manufacture of chemical wood pulp, the kraft process now occupies a major position in the industry. Tonnage-wise, the amount of this type of pulp produced in the United States exceeds that produced by the sulphite and soda processes combined. In the present discussion, it is well to distinguish clearly between the kraft and the sulphite processes.

In the sulphite process, the liquor used for digesting the wood is an aqueous solution of sulphurous acid in which lime, or some other base, has been dissolved; the final result is, therefore, a solution of a bisulphite of the base containing an excess of sulphurous acid. The nature of this liquor, where calcium is used as the base, as well as the mechanics of the sulphite process, makes it economically unfeasible to recover the spent liquor; it is accordingly wasted to the river and is commonly known as sulphite waste liquor.

The kraft process, on the other hand, is an alkaline process. The principal constituents of the cooking liquor are sodium hydroxide and sodium sulphide, the latter comprising up to 45% of the total. From the present point of view, the important aspect of the kraft process is that the spent liquor is recovered, for the most part, by a process involving evaporation and combustion. In this treatment, the chemicals are recovered and heat energy is generated as well.

In order to understand the problem at hand, it is desirable to consider briefly the principal features of the kraft pulping process.

The wood is carefully prepared and cut into small chips, after which it is introduced into large receptacles called digesters. The cooking liquor is added and the digester securely closed. Heat is then gradually applied and the cooking process is started.

The cooking cycle of any type of chemical pulp production consists of three phases: a period during which the liquor is penetrating the chips and the internal pressure is being brought up to cooking level, a full pressure period during which the wood is actually being cooked, and a gassing-down period during which the pressure in the digester is lowered slowly to the point where the pulp can be blown therefrom.

At the start of a cook, there is a considerable amount of air in the digester which must be removed before the start of the second phase.

After the digester is brought up to pressure, therefore, it is relieved; that is, the air and other accumulated gases are permitted to escape. This initial "blowdown," as it is called, is usually condensed and its condensate is one type of waste from a kraft mill. Similarly, at the completion of the second, or cooking, phase of the operation the digester is again relieved, these "final blowdown" gases are condensed, and the condensate becomes waste.

When the digesters are blown (or emptied) at the conclusion of the cook, the pulp is blown to a pit and the spent liquor (or black liquor) is collected and sent to the recovery plant. The pulp is washed by a series of waste waters used in washing previous batches of pulp. The black liquor concentration of these waste waters is increased with every batch of pulp washed and, eventually, when the concentration is high enough, they are sent to the recovery plant along with the concentrated black liquor. In the final washing processes, however, the wash waters may be so dilute that their chemicals cannot be recovered economically; for that reason, they may be sent to the sewer.

The spent black liquor and the concentrated wash waters are subjected to evaporation under a vacuum to a consistency of approximately 50% solids. The vacuum is produced by a barometric leg and in its effluent may be found in solution some of the noncondensable gases from the process of black liquor evaporation. This effluent is a waste material.

The concentrated black liquor containing most of the nonfibrous portion of the original wood, as well as the spent chemicals, is then burned in recovery furnaces; the chemicals and heat energy are thus recovered.

When the black liquor from the blow pits is sent to the recovery plants, it is usually stored in large receptacles for varying periods. As the material stands in these tanks, a thick foamy material, called sulphate soap, rises to its surface. Actually, it is composed of the sodium salts of resin and fatty acids which were in the wood. Currently, this soap is recovered and sold, but small amounts of it may be found in the dilute wash water. It is interesting to note that, although eastern and southern woods have a relatively high resin and fatty acid content, the woods of the Pacific Northwest contain very little. For that reason, the kraft mills in the northwest are not ordinarily faced with a serious soap problem.

In summary, it can be stated that the major stream polluting products from the normal operation of a kraft mill are: (*a*) initial blowdown condensate, (*b*) final blowdown condensate, (*c*) evaporator condensate, and (*d*) soaps and other material in the weak wash waters.

It is the purpose of this paper to describe the possible effect of these wastes on the aquatic environment and to consider measures which may be employed to prevent their reaching the stream.

Kraft Wastes and the Stream Environment

The relief gases referred to above have been studied rather widely in Sweden and Germany. It was demonstrated by Klason and Segerfelt (1911) that up to 1000 gm of mercaptans may be produced per ton of wood pulped. It was noted that pine yielded approximately twice as much mercaptans as spruce. Falk (1909) analyzed the condensates from the kraft cooking process and found that the oily and aqueous portions contained the following per ton of wood pulped (Table I):

TABLE I

ANALYSIS OF KRAFT COOKING CONDENSATES

	In Oil Portion, kg	In Aqueous Portion, kg
Mercaptans	0.062	0.06
Dimethyl sulphide	0.927	0.17
Dimethyl disulphide	0.103	0.05
Turpentine	8.487	0.92
Methyl alcohol		5.00
Ammonia		0.18

The black liquor which is drained from the pulp and the small amount of which may find its way to the sewer, has been analyzed by Klason and Segerfelt (1911). According to them, its organic matter consists of 54.3% lignin, 2.5% fatty and resin acids, 3.7% formic acid, 5.2% acetic acid, and 30.3% lactonic acids. In addition, Cirves (1930) made the analyses shown in Table II.

TABLE II

BLACK LIQUOR ANALYSIS

Chemical	Amount, g/l
Sodium bicarbonate	14.78
Sodium hydroxide	1.43
Sodium sulphide	3.01
Sodium sulphate	14.70
Sodium sulphite	6.64
Sodium chloride	0.88
Sodium thiosulphate	3.98
Sodium salts of organic acids (soaps)	14.52
Organic acids	92.49
Water, etc.	846.05

It becomes apparent that many of the materials listed might have a serious effect on the aquatic environment if present in sufficient quantities. That this is the case has been demonstrated by several workers. Ebeling (1931) found that the greatest poisonous action of the nonsulphur compounds was exhibited by the sodium salts of resin and fatty acids. Hagman (1936) established the minimum lethal concentration of these acids to fish at 2 ppm. He also pointed out that the sulphur components (mercaptans, sulphides) of these wastes may be toxic in concentrations as low as 0.5 ppm. Hagman's results have been substantiated by Bergström and Vallin (1937), who made a special study of the relief and evaporator condensates described above.

Several investigators have studied this problem in our own country. Cole (1935) investigated the effects of whole black liquor on perch, bluegills, large-mouth black bass, and rock bass, and found that it would kill these species in concentrations above 5000 ppm. Extrom and Farner (1943), working on the premises of a typical kraft mill located on a medium-sized stream, were able to calculate the concentration, under ordinary circumstances, of the wastes in question in the stream. Using these concentrations in continuous flow experimental aquaria, they determined that bass, bluegills, and sunfish were not adversely affected by such concentrations of black liquor and blowdown condensates. They found, however, that some of the chemicals listed above, particularly the sulphur compounds and the soaps, were present in the mill sewer in concentrations high enough to kill fish. They concluded, therefore, that, if there was stream volume discharge to adequately dilute the wastes, the stream environment would not be adversely affected, but that it was possible (particularly at periods of low stream flow) for the wastes to be present in dangerous concentrations.

Generally speaking, therefore, it can be concluded that, of the components found in a typical kraft pulp mill waste, the sulphur compounds (particularly the sulphides and the mercaptans) and the resin and fatty acids (and their sodium salts) are, potentially, the most dangerous to the aquatic environment.

Remedial Measures

Studies on toxic effect of kraft pulp mill wastes on stream environments have progressed to the point where a well-directed effort can be made to materially abate such pollution.

One of the most effective methods of abating kraft pulp mill pollution embodies simply efficient and careful operation of the plant. It has been pointed out that, normally, most of the spent liquor is recovered; indeed, the economic feasibility of the kraft process depends on adequate chemical and heat recovery. If the plant is efficiently operated at all times, the loss to the sewer of harmful chemicals can be greatly reduced. However, because of inadequately trained or careless operating personnel, or because of unavoidable mechanical difficulties, it is not always possible to effect top-rate performance. For that reason, it is desirable to examine additional methods of abatement. For purposes of discussion, this can be done most satisfactorily by considering each waste element separately.

The soap problem. No well-operated mill permits the escape of much of the soap material to the sewer. Formerly, it was burned along with the rest of the black liquor and its chemicals and heat values were recovered. More recently, however, its chief constituent, tall oil, has been separated and marketed in considerable quantities. The fat scarcity in recent years has made its recovery especially profitable and it is expected that, even after the scarcity ends, there will be a ready market for it. From the stream pollution abatement point of view, the recovery of tall oil removes essentially all the fatty and resin acids from the wastes.

The digester relief condensates. It was noted above that these wastes contained considerable amounts of turpentine. Formerly, most of this material was wasted. In recent years, however, the economic advantage of its recovery has become so apparent to mill operators that most mills recover it and find a ready market for it.

In addition to the turpentine, the digester relief condensates contain sulphur compounds; in the present case, those of primary interest are the sulphides and mercaptans. The amounts of these materials in these wastes, which may be relatively large from the point of view of stream pollution, are too small to be recovered to economic advantage. For that reason, not a great deal of attention has been directed to their removal from the wastes. The problem, therefore, is to provide a method by which these wastes may either be removed or neutralized before they are passed to the stream.

One of the complicating factors in removing the mercaptans and sulphides lies in their inherent odor characteristics. Anyone who has spent much time around a kraft mill knows that there is, ordinarily, a typical smell associated with the process. Many people think that this smell is unpleasant, not to say foul, and there is some justification for this opinion. The basic source of this smell is in the presence of the very substances under consideration. In considering ways and means of eliminating these compounds from the stream, attention must be given, therefore, to the problem of air pollution as well.

In conducting experiments on the toxicity of kraft mill waste components to fish and other aquatic organisms, it was discovered that the toxic properties of any given concentration may be significantly reduced if a finely divided stream of air is passed through the solution. It is unlikely that the phenomenon is caused by any other means than the action of the air-flushing or washing the materials from the water, although it is possible that some oxidation occurs. This observation suggests the possibility that the wastes in question could be detoxified simply by subjecting them to a process of thorough aeration and/or agitation.

The easiest and most economical method of achieving this end is the one that has been and is now employed—namely, the use of the receiving stream. In normal circumstances, if adequate volumes of water are available for dilution, the stream will absorb or dissipate these sulphur compounds rapidly enough so that the danger to aquatic fauna is eliminated. The possibility always exists, however, that, in periods of low flow and high production, the absorbing capacity of the stream will be taxed beyond a safe limit and, under those conditions, the need for some other method of treatment becomes apparent.

The problem is complicated by the relatively large amounts of water in which the sulphur compounds may be carried. A treatment process must take into account these water volumes with the resulting complications relating to economics.

Various attempts have been made to impound these wastes in reservoirs, sometimes for extended periods. For example, Crawford (1946) was able to provide adequate stream protection by storing in a lagoon (for periods up to 90 days) the more concentrated wastes from a typical kraft mill. The objective of this installation was to provide storage space during periods of low water for subsequent release when the river was high. The work demonstrated that, during the time the wastes were impounded, their pollutional properties were greatly reduced. A somewhat similar installation, described by Gehm (1947), has been made in Texas with satisfactory results.

The construction and successful operation of lagoons involve a number of requirements not always available on a pulp mill premises. For example, in order to operate successfully, the contents of a lagoon must be held at a level higher than that of the receiving body of water. In mills located on or near tide water, this primary requirement cannot be met without great capital outlay. Furthermore, lagoons, over a sustained period of operation, will fill with solids and must be dredged or otherwise cleared. Depending on the type of operation, this might have to be done often enough to make lagooning operations unfeasible.

Several studies have been made to remove the sulphur compounds from kraft wastes of kraft mills by special processes. Bergström and Trobeck (1945) have suggested that water pollution can be abated by removing the sulphur compounds in wooden absorption towers. The towers contain fillers of a type to afford a maximum absorbing surface. The wastes are passed downward over the filler and waste stack gases are forced upwards through the tower. It is claimed that this process will remove most of the sulphides contained in the condensates. If this type of installation can be made to work, it will reduce considerably the amount of sulphides and other sulphur compounds which ordinarily pass to the stream. One objection, however, becomes apparent. The apparatus may prevent the sulphur compounds from passing to the stream and pass them to the air instead. This would result in an air pollution problem which might be considered worse than possible effects on the stream.

Actually, the feasibility of the Bergström and Trobeck development remains to be demonstrated in our country.

Summary

Under normal circumstances, kraft pulp mill operation depends, for its economic feasibility, on the recovery and utilization of the components of its spent liquor. If the mill is well operated and adequate stream discharge is available, the effluent from the mill should not cause a serious stream pollution problem. Certain sulphur components in the wastes, particularly those in the condensed blowdown and evaporator condensates,

have been shown to be toxic to fish and other aquatic fauna. Methods of preventing these components from reaching the stream are discussed.

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PLANKTON AS RELATED TO NUISANCE CONDITIONS IN SURFACE WATER

By JAMES B. LACKEY
THE BLAKISTON COMPANY, PHILADELPHIA, PA.

Man has never been able to do without water very long. Preferably clear, cool, virtually tasteless and odorless; not like the "arf-a-pint o'water—green" that crawled and stunk, which Gunga Din gave the British Tommy. But, like the Tommy, we are grateful for even that kind if no other is available. Gunga Din's water must have been taken from a pool in full bloom, well exemplifying those nuisances which blooms can cause. For they all too frequently turn surface waters into nauseous soups of some green, brown or red shades that truly stink. Hence the reason for an article on "Plankton as Related to Nuisance Conditions in Surface Water."

Plankton may be defined loosely as suspended aquatic small plants and animals. A bloom is an unusually large number of plankters, usually one or a few species, per unit of the first few centimeters of surface water. An arbitrary definition (Sawyer, Lackey and Lenz 1944) has set 500 individuals per ml as constituting a bloom. Extremely small algae, such as Chlorella or Gleocapsa, are not crowded at such an aggregation, but Pandorina would be. There are, of course, plankters of which a single individual exceeds 1 mm in diameter, or 1 ml in volume.

Nuisances due to peak occurrences of these organisms are many. A partial list includes: Nauseous tastes and odors in drinking water; lakeshore decay with odor and debris; interference with bathing, boating and fishing; killing of fish; prevention of stock watering; shortening of filter runs in water purification plants; effects on industrial water use, such as growths in cooling systems; poisoning of waterfowl, stock, and possibly man; poisoning of mussels; secondary fish depletion because copper sulfate has been used to control blooms; secondary increases in mosquito nuisances by increasing larval food.

This list could be extended by specific instances. For example, during the war a sewage plant effluent was turned into an otherwise dry stream bed near a small Texas town. The effluent, highly purified, produced heavy growth of filamentous algae which, in turn, produced pooling. In the stagnant waters, mosquitoes—some of them anophelines—bred abundantly. This is a highly specialized case and it might be questioned, academically, that either surface waters or plankton were concerned. The complaints of fishermen concerning the vast mats of filamentous algae in some Wisconsin lakes are similar, but there is no doubt that these algae *can* be planktonic or that they interfere with both bathing and boating as effectively as a bloom of Anabaena or Microcystis.

Tastes and odors in drinking water are only too familiar to water plant operators, as are shortened filter runs. Almost any group of algae may cause them, but blue-greens, brown flagellates (such as Synura or Uroglenopsis) and diatoms are the chief offenders. Increased operating costs accrue in such cases, but equally bad is the loss of revenue when summer cottagers leave because an onshore wind piles their beach with a loathsome mass of decaying algae. And according to newspaper and other reports (Staff, Univ. Miami Marine Laboratory 1947) the recent red waters near the Florida shores were simply a huge bloom of dinoflagellates which not only hurt commercial fishing, but also left fish carcasses along shore in such numbers as to cause vacationists to leave.

It has been observed frequently that stock will not drink while a heavy red bloom of Euglena sanguinea is present. Stock deaths have been attributed to poisonous blooms in the U. S. (Fitch *et al.* 1934), Africa and Australia; and in 1946 (Hervey) waterfowl deaths in Utah were ascribed to huge numbers of dinoflagellates. Mussel poisoning along the Pacific Coast is familiar, and its appearance along the Atlantic Coast should be carefully watched for, as we increase the species of shellfish we eat. There are numerous instances of enteric diseases in man, apparently waterborne, and not satisfactorily accounted for, but which seem to travel downstream—in one instance, at least, paralleling the downstream travel of an algal bloom.

The Nature, Occurrence and Causes of These Blooms

Blooms are typical of shallow lakes in the late summer, but may occur in almost any body of water at any time of the year. They are not generally noticed by laymen until surface waters are obviously discolored. Blue-green algae are perhaps the most common producers of blooms in stagnant water, but in streams a much wider variety of organisms is encountered in bloom densities. Colors range from a rather pale yellow-green or blue-green, such as caused by Anabaena in Prospect Park lake in Brooklyn, in 1924, to yellow (Gleotrichia, Mendota, 1942), brown (Peridinium, Chillicothe, Ohio, 1936), or red, Euglena sanguinea, North Alabama, 1935; Azolla, North Mississippi, 1940). Table I shows the organism groups which reached bloom proportions (i.e., 500 organisms per ml of raw water) (Lackey 1943-44), a total of 509 times during a two years' survey of 16 southeastern Wisconsin lakes and three rivers in 1942-43. It should be clearly understood that not all this impressive list of 509 blooms were of the nuisance type.

There were actually more blooms, 509, than the 478 samplings. Evidently high populations in Monona, Waubesa, Kegonsa, Wingra, Geneva, and the three rivers were mixtures of two or more species and their normal biota was rich and varied. Except Geneva, all had nuisance conditions. Blooms in Geneva were due largely to very small organisms; the layman would never have noticed them. Mendota had one extremely bad condition, a particularly nauseous population of Gleotrichia.

Table I also indicates to what group these abundant organisms belonged. The only animal blooms were flagellates and ciliates although no account of copepod populations was taken. Animals were not a nuisance.

TABLE I

THE NUMBER, COMPOSITION AND OCCURRENCE OF BLOOMS DURING A TWO-YEAR SURVEY

	Number samples	Total no. blooms	Blue-Green algae	Diatoms	Dinoflagellates	Cryptophyceae	Chrysophyceae	Volvocales	Green algae (other)	Flagellata	Ciliata
Mendota	59	22	2	10		3			6	1	
Monona	64	86	10	28		15	1	13	19		
Waubesa	64	112	12	52	1	18		16	11	1	1
Kegonsa	63	80	7	29		20	1	18	3	2	
Wingra	52	91	15	34		6	12	2	19	3	
Como	10	7		4			3				
Delevan	13	5		2		2			1		
Geneva	11	13	3	7		1			2		
La Belle	13										
Lauderdale	13	1					1				
Nagawicka	13	1		1							
Nemahbin	13	2		1		1					
Oconomowoc	14	4	4								
Okauchee	13	1					1				
Pewaukee	12	6	1	4			1				
Rock Lake	12	5		1		2	2				
Crawfish River	10	20	3	12			1	2	2		
Koshkenong River	13	33	9	11		5	2	2	4		
Rock River	14	20	2	9			1	1	7		
Totals	478	509	68	205	1	73	26	54	74	7	1

There were only 68 high occurrences of blue-green algae, generally the most troublesome group, while diatoms, rarely obnoxious, were high 205 times. Obviously the table indicates that any of the groups of algae may pass bloom proportions if conditions are right. No chronology is shown here, but these peak populations occurred at any time; two very dense swarms of Volvocales (Chlamydomonas and Diplostauron) occurred under ice cover in Kegonsa. Diatom abundance was noted through the year and in these lakes was due most frequently to small naviculoid forms and to bandbox forms, such as Cyclotella: small, and not productive of color in surface waters. In rivers, forms like Synedra occur in huge swarms and overnight may make their presence felt in the filter runs. In Lake Michigan Tabellaria and Asterionella have been conspicuous. Diatoms, however, are rarely troublesome except to water supplies.

Dinoflagellates, while frequent in these Wisconsin lakes, were not a nuisance. In oceanic waters their blooming is well known, and their oily inclusions could be very troublesome in water supplies. Cryptophyceae have not been reported as troublesome, but because they sometimes occur in huge numbers, may contribute to tastes and odors. Centrifuging a virtually pure population of Cryptomonas produced almost no whole cells; but the olive-green slime adherent to the centrifuge walls was highly offensive in odor, and these organisms readily disintegrate on sand filters. They tend to be especially abundant in rivers. Chrysophyceae seem to occur in winter and spring or late fall. In this survey they were not troublesome, but are notorious to water works men. They are especially characteristic of non-calcareous waters; many genera, abundant along the Atlantic seaboard or in Lake Michigan, are totally lacking in the Ohio Valley. Volvocales probably rank next to blue-green algae as obvious trouble makers, and together with other green flagellates, such as Euglena, are often conspicuous in waters whose organic content is above normal. But they do not occur in such numbers as to form windrows on the beaches.

Other bloom-forming organisms such as Azolla, Lemna or small arthropod water fleas are either rarely troublesome or do not belong to the plankton.

The causes of blooms have long been debated. Highly special or local causes may occur at times, but there seems little reasonable doubt at present that one or more optimal conditions—light, temperature, pH, food—produce them, and perhaps the most compelling of these is the nutrient content of the environment. Many laboratory studies have indicated that nitrogen and phosphorus relationships are perhaps the most critical. Trace elements should be sufficiently abundant in natural waters from large drainage areas to supply the infinitesimal amounts presumably needed. But nitrogen and phosphorus demands are comparatively large. Drainage areas may supply these demands from four sources: Original, virgin land; agricultural (fertilized) land; sewage; trade wastes.

Table II shows inorganic nitrogen relationships in two small Ohio creeks draining small areas; these determinations were made when light

TABLE II

INORGANIC NITROGEN—ORGANISM RELATIONSHIPS IN TWO SMALL OHIO CREEKS

Station	Nitrates p.p.m.	Nitrites p.p.m.	Number of species	Number of organisms
Lytle Creek				
1	.10	.01	40	826
2	.44	.02	32	1,629
3	.80	.08	31	954
4	.08	.01—	22	1,097
5	.08	.01—	39	25,189
Crown Creek	.04	.01—	40	313

and temperature were optimal for many species, and stream flows average. Both creeks showed small amounts of nitrites and nitrates but admission of a sewage effluent below Station 2 in Lytle Creek changed that condition. Station 1 was above the town, some of whose street drainage was responsible for the increase at Station 2. Within about four miles (Station 5) nitrates had decreased to normal, nitrites had dropped nine-tenths, and organisms had multiplied twenty-five times; there was a dense bloom of Euglena, Chlamydomonas and Phacotus.

Table III shows that temperature may keep populations low even if sufficient nitrogen and phosphorus are present; but rising temperatures result in a sharp increase of organisms, until the available nutrients are lowered past a critical point, when organisms also decrease sharply. This table does not indicate that most of the organisms in Lake Delevan in January and February were quite small, whereas in April and May the organisms were much larger and of different species.

TABLE III

NITROGEN-PHOSPHORUS ORGANISM RELATIONS IN LAKE DELEVAN

Date	Inorganic nitrogen in p.p.m.	Inorganic phosphorus in p.p.m.	Number of species	Number of organisms
January 24	0.87	0.07	21	819
February 29	.59	.35	26	361
April 8	.36	.025	32	996
May 9	.32	.03	34	1,826
June 6	.22	.01	24	184
July 31	.09	.01	33	223

Detailed studies of these Wisconsin lakes gave the following average inorganic nitrogen and phosphorus values over a year's cycle, shown in columns one and two of Table IV and averaged from analyses of samples collected during one year.

TABLE IV

INORGANIC NITROGEN AND PHOSPHORUS IN WISCONSIN LAKES

Lake	Inorganic N, ppm	Inorganic P, ppm	No. blooms
Mendota	.17	.018	22
Monona	.33	.041	86
Waubesa	.79	.38	112
Kegonsa	.35	.33	80
Wingra	.26	.012	91
Koshkenong	.39	.019	7
Delevan	.31	.023	5
Geneva	.10	.01—	13
Como	.14	.01—	0
Lauderdale	.13	.01—	1
Pewaukee	.15	.01—	1
Nagawicka	.37	.016	2
Upper Nehmabin	.24	.013	4
Okauchee	.13	.01—	1
Oconomowoc	.12	.01—	1
Lac La Belle	.19	.01—	6
Rock	.10	.01—	5

On the basis of this and other data Sawyer reasons: "In general, it may be concluded that lakes showing average annual concentrations of inorganic nitrogen and phosphorus in excess of 0.30 and .015 ppm, respectively, will produce frequent 'nuisance blooms.'" Table IV generally bears this out, but of course there are exceptions. Mendota produced one nuisance bloom in the two years' study, unless the masses of filamentous algae be so called despite its low nitrogen average. Wingra and Geneva, despite low phosphorus averages, produced many blooms. But virtually all of these were very small organisms, and the term bloom applied only when there were more than 500 of some species per ml of raw water. No discoloration or nuisance would have been evident.

Positive results are equally evident in Monona, Waubesa and Kegonsa. Here a large amount of contributed fertilizing minerals came from properly functioning sewage plant effluents, although agricultural drainage and industrial drainage were also contributors. Blooms in these lakes were so abundant and so frequent as to evoke legislative action on the part of the shore residents. Most of the nuisance conditions here were due to blue-green algae, Microcystis and Anabaena being the chief offenders.

But there is far too little evidence as to the causes of specific swarming growths of organisms. In shallow, warm lakes, accumulating nitrates and phosphates seem to produce blue-green algae; but agricultural drainage into streams of moderate hardness seems to produce swarms of euglenoid flagellates, small non-motile green algae and green flagellates belonging to the Volvocales. Sulfuric acid drainage from exposed coal seams produces, particularly, Euglena mutabilis (Lackey 1939). Distillery wastes have shown most abundantly, Volvocales: the first United States record of

Chlorobrachis gracilla was a bloom of them in whiskey distillery wastes at Lawrenceburg, Indiana (Lackey 1942). Cedar swamp acid waters along the Atlantic coast have produced blooms of the green flagellate Gonyostomum semen at Woods Hole, in New Jersey and near Savannah, often with an accompanying huge population of Euglena polymorpha. Reservoirs in areas underlain by granitic rocks may be troubled by yellow-brown flagellates, such as Uroglena, Uroglenopsis, Synura, Chlorodesmus and similar forms. Flagellates of these types are common to waters whose pH tends toward acidity and are almost lacking in waters which are prevailingly hard. The pattern behind many blooms is reasonably clear, but specific causes are not clear and probably causes are due to combinations of conditions. An accumulation of nutrients is certainly necessary. Small streams draining sparsely inhabited areas along the Columbia River had as small plankton populations as have been found despite summer temperatures and low, clear water stages. Positive evidence is found in the increased plankton populations of fertilized fresh water fish ponds, as well as marine experiments at Milford, Connecticut (Loosanoff and Engle 1942); Woods Hole; and lochs in Scotland (Gross *et al.* 1946).

Control of nuisance conditions has followed three general lines. In the Wisconsin lakes a sewage treatment plant was put into operation and control of some industrial wastes was initiated. Further control by copper sulfate was used. An attempt was made to secure legislative action for diversion of sewage plant effluent. Storm sewer wastes and agricultural drainage were neglected. None of these schemes achieves more than partial success. Treatment of sewage reduces the B.O.D., and by maintaining oxygen and decreasing putrescible matter, changes the nature of blooms from saprophytic organisms (mainly bacteria) to presumably altogether holophytic ones. This probably decreases the nuisance, but leaves an abundance of available N and P in food form. Copper sulfate treatment is temporarily successful, but since copper accumulates in the bottom mud, where it apparently destroys clams, worms and insect larvae, its long continued use is questionable. The same question should be raised for recently developed algacides of other natures which upset biologic balance. Diversion of sewage or sewage plant effluents from a lake to a stream merely begs the question. The streams near Madison are already extremely rich in plankton, and additional food might cause nuisances therein.

Control or elimination of bloom nuisances thus far is purely temporary. The most promising investigations would seem to aim at the removal of phosphorus. Nitrogen might at times be available by fixation from the atmosphere. But before phosphorus removal is attempted, further studies of the amounts and mechanism of contribution from agricultural drainage should be made. In some instances, as when fertilizer is spread on iced-over fields, it appears the farmer is paying for fertilizer which, on the first thaw or rain, at once washes into streams or lakes. The biology of industrial wastes needs much more study. The wastes of tanneries have a B.O.D. averaging 1200 ppm; those of milk and distillery wastes are far greater. Many wastes, such as those of paper and chromium plating industries, are indirectly or directly toxic, even though they leave the oxygen content of water largely unaffected. Such toxic effects may likewise be nuisances. Prevention of pollution or poisoning of shellfish areas, bathing beaches, lakes, and streams is better than cures. And if the biochemist or chemical engineer can achieve prevention by recovery of useful by-products as an economic measure, very little legislation will ever be necessary.

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PRELIMINARY STUDIES ON THE VIABILITY AND DISPERSAL OF COLIFORM BACTERIA IN THE SEA*

By BOSTWICK H. KETCHUM, CORNELIA L. CAREY, AND MARGARET BRIGGS
WOODS HOLE OCEANOGRAPHIC INSTITUTION, WOODS HOLE, MASS.

The disposal of sewage in the sea is widespread and increasing. There is, however, little information to indicate how much sewage a given body of water will accommodate and even less on the fate of the pollution bacteria in the sea. The economic effects of polluted estuaries are already evident, since large areas have been closed for the taking of shellfish and many beaches throughout the country have been posted as unsatisfactory. These problems will probably become more acute, because the disposal of sewage is essential and the sea provides an efficient means of dispersion.

Harbors and estuaries frequently contain many thousands of bacteria per ml, a large proportion of which may be enteric species. In the open sea, however, the bacterial counts normally range from 50-200 per ml, and the coliform bacteria are never found in open, unpolluted sea water. This tremendous decrease in numbers occurs within a short distance from the mouth of the harbor or estuary (Calif. State Dept. Pub. Health 1943; Knowlton 1929; Mass. Dept. Pub. Health 1936; Warren and Rawn 1938; Weston 1938; Winslow and Moxon 1928). It is clear, therefore, that the introduced bacteria do not persist for extended periods in the sea. The relative importance of dilution of the polluted water by sea water, of the death of the coliform bacteria, of sedimentation and predation by animals has never been clearly assessed in the marine environment.

Our studies on this problem have included laboratory investigations of the viability of *Escherichia coli* in sea water and surveys of some polluted areas selected in the hope that the various factors in the disappearance of pollution bacteria could be evaluated. The results described here must be considered of a preliminary nature.

The results of our laboratory investigations of the death rate of *Escherichia coli* in sea water will be described first. Previous investigations of this problem have given widely divergent results, varying from death rates much more rapid than are found in fresh waters (Calif. State Dept. Pub. Health 1943; Carpenter, Setter and Weinberg 1938; ZoBell 1936, 1946) to the conclusion that sea water is neither antiseptic nor inimical to enteric bacteria (Dienert and Guillerd 1940). We have found that the laboratory treatment of the sea water influences the results greatly. The use of artificial, synthetic or diluted sea water cannot be expected to give results which will correspond to the natural phenomenon.

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* Contribution No. 446 from the Woods Hole Oceanographic Institution.

When natural, unpolluted sea water is brought into the laboratory and stored in the dark a tremendous growth of the bacterial population takes place. This growth and the effect of adding *E. coli* to the water are shown in Figure 1.* The total population of the raw sea water increases to a maximum in three days, then decreases to a more or less uniform population of about a million cells per ml. A slight initial increase in bacterial numbers is also detected in the water to which the coliforms were added. This period of growth of the mixed population is followed by a decrease in numbers so that the final populations are approximately the same whether *E. coli* were added or not.

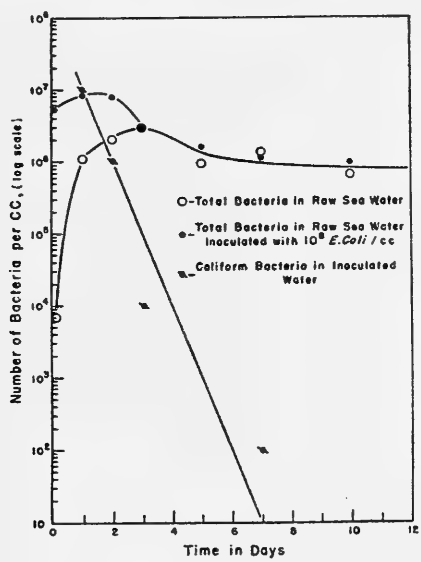

FIG. 1. Growth of marine bacteria and the viability of *Escherichia coli* in untreated sea water.

The concentrations of *E. coli* were estimated independently in this experiment by inoculating lactose broth fermentation tubes. The indicated numbers of *E. coli* thus obtained are also shown in Figure 1. They decrease regularly from the initial inoculum of 10^8 cells/ml and after approximately seven days only a millionth of this population persists. Clearly, the conditions which are suitable for the growth of marine bacteria are inimical to the growth or persistence of the coliforms. It appears that the final populations in both the raw sea water and in the water inoculated with *E. coli* consist of the normal sea water organisms.

If, instead of using untreated sea water, *Escherichia coli* are intro-

* The numbers were determined by plate counts after 7 days' growth on a medium containing 1 gm glucose, 1 gm peptone, 0.05 gm NaH_2PO_4, 15 gm agar in 1 liter of aged sea water.

duced into water sterilized by autoclaving or by boiling, the results shown in Figure 2 are obtained. In the autoclaved water the death rate of the coliform bacteria is very slow. The maximum decrease observed was to one-fifth of the total population in a period of seven days. In the boiled water the death rate is more rapid and is similar to the rate found in the untreated sea water. It is clear from these results that the bactericidal action of sea water is destroyed by the heat of autoclaving, but is unaffected by the milder boiling treatment.

Another observation shown in this figure is that a second or subsequent inoculum of *E. coli* dies off more rapidly than the first. In these experi-

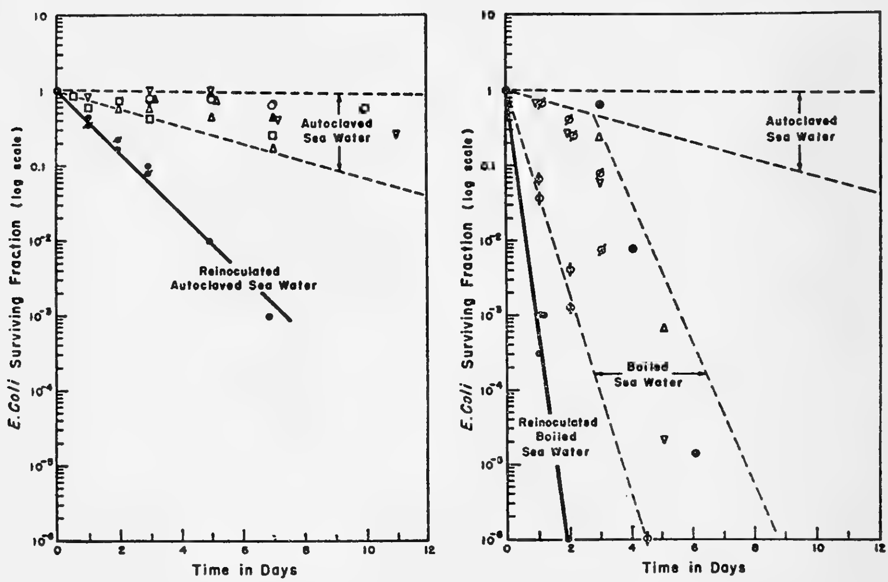

FIG. 2. The viability of *Escherichia coli* in autoclaved and in boiled sea water. The original inoculation of E. coli provided a population between $5x10^7$ and $5x10^8$ cells/ml. The surviving fraction is plotted. Different symbols represent different experiments.

ments the water receiving the first inoculum was stored until no viable cells were found. This took about 20 days for the autoclaved sea water, 5 to 10 days for the boiled water. When another inoculum of *E. coli* was added to the water, the bacterial counts decreased as shown by the heavy lines. The death rate in the reinoculated autoclaved water is eight times as great as the rate observed for the first inoculum. A threefold increase in rate was observed with the boiled water. It may be presumed that the greater death rates observed with reinoculated water correspond to what would be obtained in polluted estuaries.

The coefficients of death rate, as shown in Table I, summarize the results of these laboratory investigations. This coefficient is the reciprocal of the time (in days) for the population to decrease to one-tenth its original

TABLE I

AVERAGE COEFFICIENTS OF DEATH RATE OF ESCHERICHIA COLI IN SEA WATER TREATED IN VARIOUS WAYS

Treatment	Coefficient of Death Rate (k)	
	First inoculum $days^{-1}$	Subsequent inocula $days^{-1}$
None	1.00	
Boiled 5 min.	1.15	3.48
Autoclaved 10-15 min.	0.04	0.35

value. Thus, a coefficient of 1.0 means that a tenth of the population dies daily; the coefficient 0.04 indicates that 20 days are required for an equivalent mortality. These experiments show that sea water has a potent bactericidal action. The activity is decreased greatly by autoclaving, but not by boiling the sea water. It is increased by previous "pollution" of the water with *E. coli.*

Further investigations are necessary to determine how the bactericidal activity of sea water varies with natural conditions. Is there a seasonal variation which might be correlated with variations of the normal population of the sea? Is the bactericidal activity greater in polluted harbors than in the open sea, and what are the effects of dissolved organic matter, oxygen supply and other variables associated with pollution? What is the identity or nature of the bactericidal activity?

Some of our experiments suggest that antibiotic substances produced by marine organisms may be responsible for the death of the pollution bacteria. They do not exclude, however, the possibilities that bacteriophage or autolytic or degenerative products of the coliforms themselves may also be involved. It is possible that all three contribute to the final action. It is significant, however, that Rosenfeld and ZoBell (1947) have recently described the production of antibiotic substances by several species of marine bacteria. None of these antibiotics was inimical to gram negative species and *E. coli* was not included among their test organisms. In our experiments pour plates of the normal sea water bacteria were made and the population allowed to develop for 48 hours. The surface of one of each pair of plates was then flooded by a suspension of *E. coli,* the excess being poured off. After a total time of four days the plates were inspected and clear areas were found surrounding some of the sea water bacteria. These results suggest that some of the sea water forms produce substances inimical to *E. coli.*

It is pertinent to inquire whether the results of these laboratory experiments bear any relation to the phenomena which occur in nature. In the sea the mortality of the bacteria may be completely obscured by the circulation and mixing of water masses. The dilution of the polluted water with sea water disperses the bacteria, and this effect must be accounted for in order to observe the death rate. In complicated estuarine situations, where there are several sources of contamination, it becomes especially difficult to differentiate between mortality and dilution. The recent development of an instrument for the continuous recording of salinity and temperature, however, has made it possible to conduct more rapid and accurate surveys of hydrographic conditions. From the data thus obtained the rate of dilution of the introduced contaminated water can be estimated. It is this possibility which has stimulated our interest in the field.

A comparatively simple picture of the fate of sewage bacteria in sea water may be found at the Sewer Outfall at New Bedford, Massachusetts. Here the sewage is introduced from a seven-foot diameter outfall pipe at a depth of 30 feet, 1100 yards offshore into water that is relatively uncontaminated. During the rising tide the flow of sewage is decreased and sometimes stops completely. During the falling tide the sewage wells

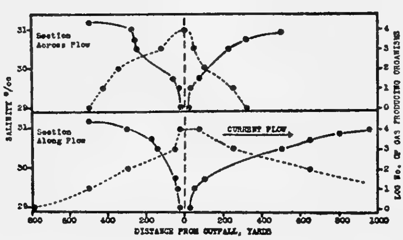

Fig. 3. The salinity and the indicated numbers of organisms producing gas from lactose broth at various distances from the New Bedford Sewer outfall.

out of the pipe in sufficient volume so that it is readily detectable. It is, indeed, difficult to obtain samples directly in the upwelling column since the boat's course is deflected by the rising and spreading current. A tidal current flow of about half a knot sweeps past the location of the outfall, and, on the ebb, carries the polluted water seaward. Several series of observations on the distribution of salinity and coliform bacteria have been made at this location.

The surface distribution of salinity and bacteria along the axis and across the axis of the tidal current are both shown in Figure 3. As would be expected, the salinity of the outfall water is low compared to the surrounding sea water of Buzzards Bay. The introduced sewage is diluted with 13-14 volumes of sea water by the time it appears at the surface. In crossing the axis of current in the neighborhood of the outfall, the bacterial numbers increase to a maximum at a location near the outfall and then decrease again on the other side. The distribution of salinity and of bacteria along the axis are similar except that the downstream distances required to reach a given concentration are greater.

The bacterial numbers are, however, always lower than would be predicted on the basis of dilution alone. To illustrate this point the dilution, expressed as percent outfall water in the sample,* and the surviving percentage of bacteria in the sections across and along the axis of current flow are plotted in Figure 4. If the bacterial count decreased only because of the dilution of the water the two sets of data would be superimposable. The surviving percentage of bacteria, however, is always less than the percent dilution of the water.

The extent by which the numbers of bacteria are less than the expected numbers is shown in Figure 5, where the numbers of bacteria found are

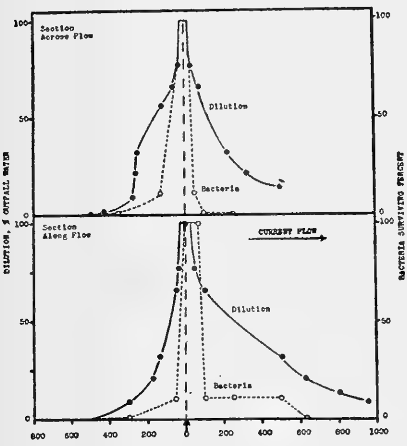

FIG. 4. The dilution of the outfall water, as calculated from the salinity change, and the percentage of bacteria surviving at various distances from the New Bedford Sewer outfall.

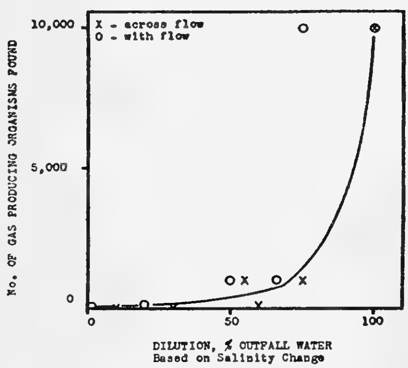

FIG. 5. The number of gas-producing organisms found plotted against the dilution of outfall water as calculated from the salinity change.

plotted against the dilution of the water. If the bacteria diminished in direct proportion to the dilution of the water a straight-line correlation would be expected since dilution of the water by 50% would lead one to expect half of the original bacterial population. The bacterial numbers are, however, substantially lower than can be accounted for by dilution alone. In the simplified situation at the New Bedford Sewer outfall, therefore, it is clear that the coliform population disappears much more rapidly than would be expected on the basis of simple dilution by sea water.

* Each sample of water is considered a mixture of the polluted, fresher water with sea water, i.e.

$$XS_0 + (1-X)S = S_1$$

in which X is the fraction of polluted water of salinity S_0, and S and S_1 are the salinities of the sea water and of the diluted water sample.

A more complicated picture is found in polluted estuaries where the circulation of the water masses play a more important role. The water exchange in an estuary includes a net outward movement of surface water contributed by rivers at the head of the bay and a net inward movement of the denser more saline sea water at mid-depth or near the bottom. Along the length of the estuary vertical mixing tends to increase the salinity and the volume of the outflowing surface waters with the result that the total surface outflow at the mouth of the estuary is greater than the flow into the estuary from the rivers. An imporant corollary of this generalization is that introduced pollution can be removed only in the surface waters, since any material which sinks to the deeper water moves in a net "upstream" direction.

It may be pointed out that the float tests, which have long been standard techniques in the study of such situations, give only a small part

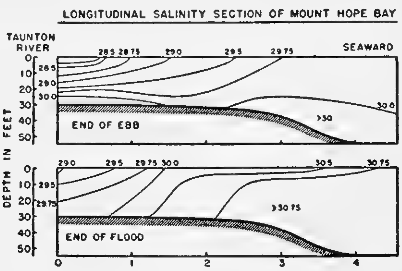

FIG. 6. The distribution of salinity in a longitudinal section of Mount Hope Bay at the end of the ebb and flood tides.

of this complicated picture. The floats show only the net flow of the surface waters, and give no information concerning the rate of vertical mixing. In studying the effects of various wind conditions the use of floats is especially deceptive. The wind drives the float more rapidly than it drives the water. The same wind, furthermore, increases vertical turbulence to such an extent that the pollution is dissipated more rapidly instead of being carried farther as suggested by the float results. Measurements of the salinity of the water provide the most useful tool in studying the exchanges and dilution of various water masses.

Typical salinity contours in Mount Hope Bay, below Fall River, Massachusetts, at the end of the ebb and flood tides are given in Figure 6. These data, collected by the continuous salinity-temperature recorder, illustrate the general principles of estuarine circulation described in the Survey of the River Tees (1931, 1935, 1936). The picture at low tide shows relatively flat, elongated salinity contours demonstrating the greater surface flow of the less saline and consequently lighter river water. The deep water retains much of its dense, high salinity character. Following the flood tide all of the contours are shifted upstream, and the gradients of salinity distribution with depth are more gradual.

An important contribution of water circulation to the disposal of pollution is the rate of dilution of the contaminated water. This occurs,

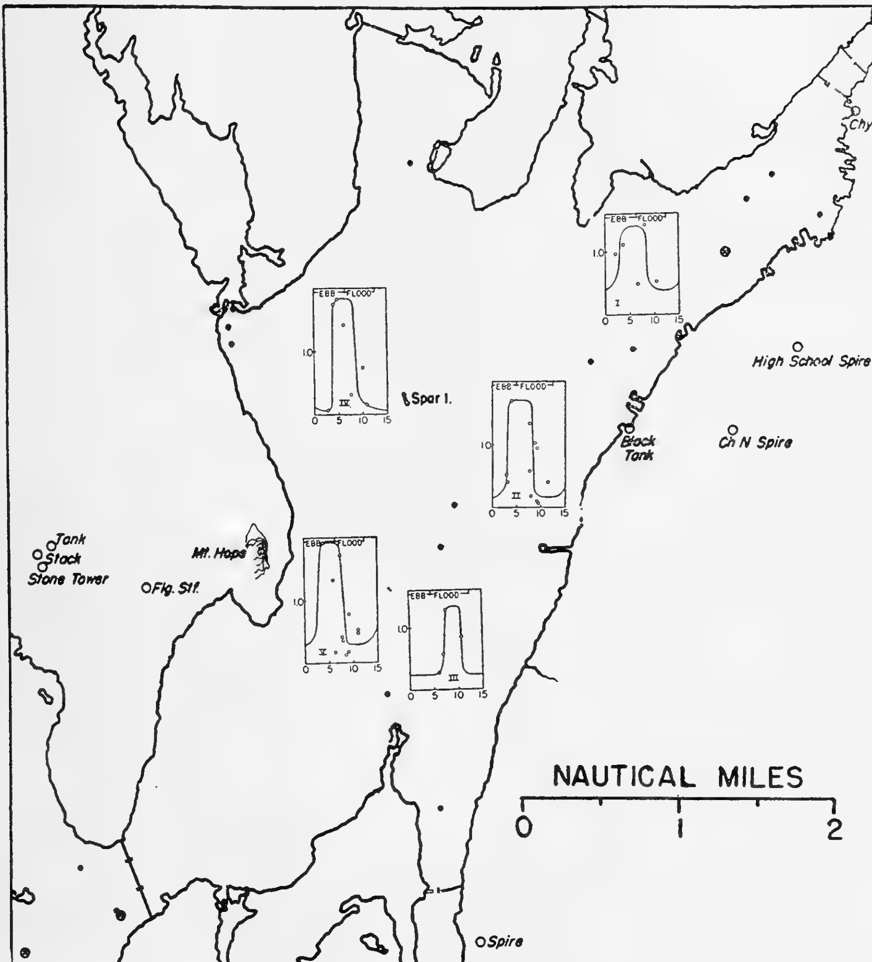

FIG. 7. The ratio between the observed and the expected bacterial counts at various locations in Mount Hope Bay at different stages of the tide. The expected numbers are computed by correcting the numbers introduced in the Taunton River (N.E. corner of chart) by the degree of dilution with sea water.

not only by horizontal mixing, but also by the vertical mixing of the surface water with the more saline deeper water. The rate of this mixing is increased as the density gradient between the surface and deeper waters decreases. As shown by the salinity contours in Figure 6 vertical mixing will be most rapid at the end of the flood tide, since at this time the vertical distribution of salinity and density is most uniform. Any increase in wind force will increase the rate of this mixing, which will tend to diminish the salinity gradients still further.

The distribution of bacteria in relation to the phase of the tide in Mount Hope Bay is shown in Figure 7. In this figure the bacteria are represented by a ratio between the number actually found and the number expected. The number expected is calculated by correcting the number introduced in the river water at the head of the bay by the degree to which this water has been diluted by mixing with sea water. The ratio thus obtained is plotted against the time after high water at each of the locations shown in this figure. If dilution were the only factor operative in this area all of these curves would be flat and show no variation with the tide. The fact that all of them increase above unity indicates that there are contributions of bacteria from sources other than the river mouth. This is to be expected since the area is densely populated and several sewerage systems empty directly into the bay. The interesting fact, however, is that in spite of this additional pollution, the numbers at each location fall far below the expected numbers during the period of high tide. Since the effect of dilution has been cancelled out it is clear that this diminution is the result of other processes which deplete the bacterial population.

To summarize, our investigations have indicated that the coliform bacteria disappear rapidly from normal sea water under laboratory conditions. This disappearance is not related in a simple way to the chemical content of sea water since autoclaving the water eliminates its bactericidal activity. The lethal factors, or substances, are apparently organic in nature and heat labile.

That the bactericidal property of sea water is important under natural conditions is indicated by the fact that the disappearance of coliform bacteria in the sea is much more rapid than can be accounted for by the dilution of contaminated water with sea water. It is fortunate indeed that this is the case, else our heavily polluted harbors would be unbearable.

Several additional problems must be studied in order to complete the picture. It would, of course, be desirable to determine the nature of the bactericidal substance or factor in sea water. The distribution of bactericidal activity in waters and muds collected from polluted areas and at varying distances from shore should aid in identifying its character. The potency of a given body of water may be expected to have an important relation to its ability to accommodate introduced pollution. Finally, considerably more fundamental information is needed concerning the principles which govern the mixing of water masses. When this information is available it should be possible to plan marine outfalls so that the introduction of pollution will lead to the minimum interference with the fisheries and economy of the area.

Acknowledgments

The authors gratefully express appreciation for the interest and advice of Dr. Selman A. Waksman, who initiated our laboratory studies, and of Dr. Alfred C. Redfield, who has encouraged our investigations of sewer outfalls and estuarine conditions. The contributions of Dr. William L. Ford, who conducted the hydrographic investigations and collected the salinity data used in this report, and of Mr. C. M. Weiss, who made the bacterial counts at New Bedford, are gratefully acknowledged.

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THE ALGOLOGISTS' PART IN CITY AND INDUSTRIAL WATER SUPPLY PROBLEMS

By CLARENCE E. TAFT
THE OHIO STATE UNIVERSITY, COLUMBUS, OHIO

For the past ten years I have had the privilege of being in charge of the Algae Studies in connection with the Columbus City Division of Water, Columbus, Ohio. During that period many problems have been met and, I hope, solved through the practical application of the accumulated knowledge concerning the algae. With this somewhat limited background in a science which was not new even at the turn of the century, I approached the preparation of this paper with two questions in mind. Their answers I consider fundamental to the understanding and solution of our problems.

The first question is, why is the Algologist peculiarly qualified to aid in Water Supply Problems? The second question is, what part does he actually play in the solution of these problems? The answer to the first is basically one of fundamental training, while the second is answered by the degree to which he applies his knowledge. Let us return for a moment to the first question where we must consider the qualifications of the Algologist. These qualifications are several in number. He must recognize the fact that algae are living organisms, unicellular or multicellular, and that their physical and chemical properties are such that they may influence the potability of water, either by mechanical or chemical means. He must recognize that in the algae, as is true of any plant which has specific hereditary complexes, the environment determines the growth and reproduction through its influence upon the physiological processes within the living cell. He must understand the action as well as the interrelationships of these environmental factors so as to know how and where to take samples in order that his final results will be more than representative of microhabitats. Also, he must be able to interpret the samples after they have been prepared for analysis. This last I consider of the greatest importance. It involves not only a knowledge of the algae, but a familiarity that will allow their identification with medium power magnification. With the counting chambers used it is impossible to use the higher magnifications of the microscope, so if one is not familiar with most of the common algae and cannot at least call them by their generic names as one would speak of an old friend, then that person is at a decided disadvantage, or rather completely lost. This familiarity can only be gained by hours of looking, painstaking key-work, and a thorough understanding of the life histories of all common algae. This last is of decided importance because much of the material encountered in some samples is undergoing reproduction and to the uninitiated has very little resemblance to the mature individual.

I find that it is this last, or the identification, that is the most difficult for people to understand. A summer seldom passes when I do not have a call or letter from some individual who asks if I can give him a couple of hours so he can learn to recognize the algae that may be in the water supply for which he is responsible. Although I feel that these requests may constitute a part of the Algologist's work, especially if he is a member of an educational institution, I know it cannot be done in the few hours available. Such a procedure, if attempted, can only lead to inadequacy and questionable results.

Briefly, we can now summarize the answer to the first question in one statement. The Algologist not only knows the names, but also some of the physiological and ecological relationships, of the organisms with which he works.

Let us now proceed to the second question by assuming that the individual has the necessary qualifications. The success which he will attain in the solution of City or Industrial Water Supply Problems will be proportionate to the degree of successful application of the above principles. He has a choice of two procedures, either of which may lead to the solution of the problem immediately at hand. One is by a complete and detailed survey of the water supply involving chemical and physical analyses as well as qualitative and quantitative analyses of both phyto- and zooplankton. Here the plankton data may be extracted for immediate use at any point during the survey, if such is necessary. The other data may be used immediately, or they may be incorporated in the records and later put to use in future studies of aquatic environments, which in turn lead to a better understanding of water supplies. Such a study as this usually necessitates the services of a permanent Staff Member who in reality can be rated as a highly trained Limnologist. Today I do not intend to deal with the problems of the professional Limnologist, but only with the occasional and varied problems which concern the consulting Algologist.

For those systems where such detailed and elaborate records are considered non-essential by those in charge, then the second procedure is applicable, and I presume is the most widely used. Possibly we can consider Columbus, Ohio, as a fair example. Its water supply lies mainly in the Scioto River, which is a drainage system in the west central part of the State. Two reservoirs were created by Griggs Dam about four miles west by northwest of the City, with a capacity of approximately 1,627,000,000 gallons, and by O'Shaughnessy Dam twelve miles north, with an approximate capacity of 5,000,000,000 gallons. In both cases the dams were constructed across narrow, rocky gorges, so that much of the impounded water is in vertical walled reservoirs. This is especially true of Griggs Reservoir. Because of the nature of the watershed which is almost entirely farm land the water often maintains a fairly high turbidity following storms which result in severe run-off. Spring thaws also contribute turbidities which may continue well beyond the middle of June. Clear water, with intermittent periods of turbid water caused by rains, then continues until the advent of the autumn rains.

During the summer of 1937, Mr. Charles P. Hoover, Chief Chemist for the Columbus City Division of Water, asked me to carry out some sort of an algal survey for the Department. This type of work was new to me at the time, but I felt that a weekly survey for one year would be valuable as a base plan. The samples were taken at Griggs Reservoir and proved their worth because we found the winter months to be especially unproductive. Little beyond sporadic sampling was done during the summers of 1938, '39, '40, and '41. These fortunately did not show any serious algal problems. I say fortunately because such occasional sampling would have been of little value if a "bloom" of some particularly bad alga had started. It was not until the spring of 1942 that we went on a basis of regular samples beginning with the clearing of spring turbidity and extending to the period of fall turbidity, or of periods of low temperature and overcast sky. We are very fortunate (algologically speaking) in having spring and fall turbidity as it reduces our danger period to about four months, July to November. Two stations were established at Griggs Reservoir, one near the Dam crest, and one about 300 yards above. Two stations were established at O'Shaughnessy and two more about two miles farther north. The four stations above O'Shaughnessy Dam fulfilled a dual purpose. They were located so as to check the possible influence of sewage effluent on plankton production and as a warning of what we might expect down river at Griggs Reservoir.

Next in importance was the method of collecting. Consideration of this factor was definitely colored by the needs and desires of those in charge of the Water Plant. Should the collections be extremely accurate, should they show absolute total plankton, should counts be in standard units, or would fairly accurate quantitative counts be satisfactory. The judgment that the Algologist exercises here will determine to a great extent what the Chemist will later consider success or failure. Furthermore, to be perfectly frank, here is where the problem of relative cost enters, if this is a problem. For a trial period we took each sample by putting 25 gallons of river water through a Number 20 Bolting Silk Plankton Net. This sample was made up to 50 cc during preservation and then standard procedures used to effect a count, although the "Standard Unit" was not adopted. The advantages are that it requires a minimum of equipment, that most any of the Water Plant personnel can be readily trained to make the collections, and also that all organisms of any considerable size are retained. A few of the very small organisms do go through but these as far as we know have little effect on the water.

Counts of the organisms in these samples are made and the results translated directly into organisms per gallon of raw river water. Where random samples through the summer would have little value, these weekly samples present an integrated picture of the plankton concentrations of any one station. All that is now necessary is for the algologist to analyze them on the basis of particular genera present or upon the basis of total numbers of organisms. In general the first is of greater importance. Knowing that only certain genera are real trouble makers, the algologist watches for them especially. If one should happen to appear, he is on his guard and when the next samples come in his attention is directed to it. If the number of individuals of this organism is great when first seen, then control may be essential immediately. One variation of the rule is for *Synura*. If one colony appears in the collections, then treat and treat fast. It may be that no organisms of especial significance appear, but that the combined total of all organisms rises steadily and rapidly. Here one must use his judgment as to the best procedure; in other words, he must be able to predict the probability of a "bloom." As there is a definite correlation between "water blooms" and environmental conditions the quality of one's predictions depends largely upon the understanding and interpretation of the environment. In the case postulated above if the weather continues to be warm with bright sunshine and little wind, I would recommend treatment. Only because of these regular collections of algae and with a knowledge of the probable effects of environment, can one anticipate plankton trouble and prevent it. At Columbus we have seldom found it necessary to apply copper more than twice during any summer since 1942.

The preceding discussion illustrates what is probably the best known, if not the most important, part played by the algologist. His position is somewhat that of a "watch-dog." He anticipates trouble and recommends precautionary measures before the troubles reach serious proportions.

Along with these regular surveys, the algologist sometimes can be of assistance in settling disputes that may arise in connection with water plant operation or policy. An example of this occurred in Columbus where some wanted to use Griggs Reservoir as a power boat course. The City was to build and rent docks to accommodate these boats. Although it provided an additional recreation area to the City Park System, as well as some additional income, a few individuals felt that maybe boats should not be in the water supply. My opinion was that these boats might be beneficial in the dispersion of algal blooms. Subsequent surveys tend to show that this heavy, and may we say somewhat wild boat traffic, has done much to disperse algal concentrations during the critical July and August days.

Another case occurred in which suddenly and for no apparent reason the water entering the mains had a violent odor and flavor. A quick check at the Reservoir disproved the possibility that it was of algal origin, and turned the investigation to the river between the Reservoir and the pumping plant. It was soon discovered that run-off from a new black top pavement was the cause.

Let us now assume that everything possible has been done and that water of as near perfect quality as can be reasonably expected has entered the mains. As a reward one would expect that they could sit back and accept the plaudits of the community. Through my contacts with Mr. Hoover and the Columbus Water Department, I find that this is not always the case. Let something go wrong with a manufacturing process in which City water is used and the first finger of suspicion is directed at that water. The algologist is not always the individual to finally clear the situation, but at times his knowledge will aid the plant personnel in clearing the water supply of suspicion. For instance, I was called in on a case where the bottle of the office water cooler had acquired a brilliant green film. Examination proved definitely that it was the alga *Chlorella.* The management was positive that it came in with the City water and demanded that the situation be corrected. I found that the empty bottles "just stood around" and that the cooler occupied a position in the corridor in front of a large window. The solution of the problem was now relatively simple. First was to wash and stopper all unused bottles. Second, scrub the inside of the cooler so as to eliminate this as a source of infection. Third, move the cooler into an area of reduced light where *Chlorella* cannot grow as well.

Of a more serious nature are the problems of those industries that use the same water repeatedly in their cooling systems after it has passed over cooling towers or through cooling basins. Algae develop at astounding rates on these towers or in the basins, and unless they are removed before the water re-enters the cooling system they leave gelatinous or other organic debris inside pipes and fins. This results in a perfect culture medium for bacteria and fungi and subsequently in clogging. Such clogging is often blamed on impurities supposed to have been in the original water used, or on the premise that algae must have gotten in through this medium. Few realize that an open dish of water exposed for a few days will soon have a very nice culture of many algal genera. Again the algologist can do more in preventing these situations than in their cure.

The algologist also has a part to play in water supplies involving wells. The question was raised as to the purity of water from a well in Northern Ohio. Samples which I examined and which came directly from the pump contained several genera of green algae, as well as several individuals of the zooplankters. Knowing that light is necessary for chlorophyll development, and that green algae continue to live only in light, and that zooplankters usually have algae somewhere in their food chains, I could safely say that the stream tapped by the well headed up in some nearby lake or pond, or that somewhere there was seepage. Either situation could result in polluted water. Another case superficially similar to this, but with a totally different ending, occurred near Columbus. The driller had brought in a well in the gravel drift south and west of the City, but samples from the pump showed a great deal of debris. Upon examination this debris proved to be fragments of moss leaves, some algae, and bits of zooplankters. But, and this was important here, these were fossils. The well had acidentally been drilled into an inter-glacial bog which had been covered by gravel following the retreat of the last glacial ice. Although not desirable in the water supply, the condition of the material definitely indicated that it was not the result of surface water seepage.

THE USE OF COPPER SULPHATE FOR ALGAL CONTROL AND ITS BIOLOGICAL IMPLICATIONS[1]

By JOHN B. MOYLE

FISHERIES RESEARCH UNIT, MINNESOTA DEPARTMENT OF CONSERVATION, ST. PAUL, MINN.

Introduction

During the last 40 years coppers sulphate ($CuSO_4.5H_2O$) has been used widely and effectively for the control of objectionable algal growths in lakes and reservoirs. This salt is also poisonous to fish and aquatic invertebrates. However, experimental work (Moore and Kellerman 1905; Marsh and Robinson 1910; Ellis 1937) and the general observations of many field workers show that usually plankton algae can be destroyed with concentrations below those toxic to aquatic animals.

Most users of copper sulphate and investigators like those already cited have been principally concerned with the comparative toxicity of the salt to different animals and plants and with the immediate practical results of algal control. To the conservationist there is another and a wider viewpoint which is worthy of consideration. Evaluation of algal control with copper sulphate in terms of immediate toxicity or in terms of sanitary or esthetic gain is not enough. The long-time effect of repeated copper sulphate treatments on the biological productivity of a water, especially on fish production, should also be taken into account. In an increasing number of lakes algal growths are being controlled for such reasons as elimination of obnoxious odors, improving conditions for swimming and maintaining the value of shore property. For these waters, consideration of the effect of copper sulphate on fish production is essential. Many municipal water supplies also are used for public fishing. In these recreational values should not be overlooked.

Growth of most objectionable algae can be controlled with concentrations of copper sulphate between .12 and .50 ppm. Fish, on the other hand, tolerate considerably greater amounts. In a Minnesota hard-water lake, concentrations as great as 1.2 ppm have been used without damage to a mixed game and rough fish population. Surber (1943) reports that 2 ppm copper sulphate did not kill small-mouth bass in hard-water ponds. Much higher local concentrations have been used for snail control in hard waters without fish loss (McMullen 1941) and Nichols *et al.* (1946) found that in the hard water of Lake Mendota (alkalinity about 170 ppm) the lethal concentration for large-mouth bass was "about 200 parts of applied copper

[1] Investigational Report No. 76, Fisheries Research Unit, Minnesota Department of Conservation, St. Paul.

sulphate per million." In soft water, fish and aquatic invertebrates are much more susceptible to copper poisoning. The addition of 3 ppm to a soft-water Nova Scotia lake by Smith (1935) resulted in nearly a complete kill of fish and fish food.

It should be noted that fish kills may result from the use of copper sulphate and not be due to copper poisoning. If treatment is delayed until a heavy algal crop is present, decay of the algae killed may so reduce the supply of dissolved oxygen that fish die of asphyxiation.

Algal Control and Fish Yields

In the simplest sense algae are the grass of our waters. They, with the larger aquatic plants, are the synthesizers of organic material on which all other aquatic life depends. This general statement is an over-simplification, since the interrelationships within the aquatic "microcosm" are immensely complex, but it does pose a very pertinent question. Will the reduction of algal crops through the use of copper sulphate seriously affect the potentialities of a water for raising fish and waterfowl?

Approaches to this problem in the literature are few and of an observational nature. Huff (1923), after considering 6 years of algal control with copper sulphate in Lake Vadnais, near St. Paul, observes that "Fish, . . . are apparently as abundant as they ever have been." Domogalla (1935) in reviewing 11 years treatment of Lake Monona, at Madison, does not commit himself to a definite answer but remarks "the opinions of fishermen on that subject (effect of copper sulphate on fishing) vary a good deal." This problem has recently been brought to the attention of sportsmen by Schoenfeld (1947) in an article in *Field and Stream,* in which he makes a general condemnation of the use of copper sulphate for algal control. He cites an unnamed Illinois pond which has been treated with copper sulphate for 30 years and is "now completely sterile." He states that perch fishing in Lake Monona, which has been treated, is poor compared to untreated Lake Mendota. There is also negative evidence which perhaps should be considered. With the exception of Schoenfeld's article, no account has been found in which a decline in fishing has been proven to be the result of continued use of copper sulphate for algal control.

The longest history of copper sulphate treatment in Minnesota is that of the Fairmont water supply. Here since 1921 algal growths have been controlled in Amber, Budd, Hall, and Sisseton Lakes. These lakes are part of a chain lying in an ancient glacial river valley in Martin County. They range in size from 84 to 513 acres, in average depth from 5.4 to 11.4 feet, are very hard and of high chemical fertility. *Aphanizomenon* is, and has been since 1921, the principal objectionable algae (Huff 1922; Moyle and Wilson 1946). *Microcystis* is also present and has at times been troublesome. Poisoning of livestock drinking from Hall Lake has been attributed to it (Fitch *et al.* 1934).

The Fairmont water supply lakes, together with untreated waters in the same chain are quite heavily fished by anglers. All have large rough fish populations, mostly buffalo and carp. Rough fish have been removed regularly since 1923.

Five untreated lakes in this chain have been selected for a comparison of rough fish yields from treated and untreated waters. The untreated lakes, North Silver, South Silver, Wilmert, Martin, and Iowa, range in size from 186 to 443 acres. They are shallow, fertile, and very similar to the treated waters. As far as can be ascertained these lakes, with the exception of South Silver in 1921, have never been treated with copper sulphate. Because most of the rough fish removal has been carried out in the winter by seining under the ice, the fishing records are considered by seasons rather than by calendar years. In all there have been a total of 137 fishing seasons on the 9 lakes, 64 on treated and 73 on untreated waters. The number of rough fish removal operations on any one lake ranges from 12 to 22, with an average of 15 for all.

For the last 24 years (1923-1947), the average rough fish yield per fishing season was 132.1 pounds per acre from the 4 treated lakes and 117.7 pounds per acre from the 5 not treated. On the average, the yield of the untreated lakes has been 9% less than of those treated with copper sulphate. If fishing records are broken down into two long periods, 1923-1933 and 1934-1943, to show trends, and a shorter period, 1944-1947, to evaluate present conditions, a similar decline in rough fish production will be observed for both treated and untreated waters (Table I). It appears,

TABLE I

ROUGH FISH YIELDS IN POUNDS PER ACRE PER FISHING SEASON IN TWO GROUPS OF SOUTHERN MINNESOTA LAKES [1]

	1923-33	1934-43	1944-47
Four treated lakes	143.6 (27)	127.9 (22)	76.8 (9)
Five untreated lakes	137.7 (39)	115.4 (39)	79.7 (12)

1 Parenthetical figures are the number of fishing seasons on which the average is based.

therefore, that most of the decline in the catch of rough fish in the treated lakes must be attributed to causes other than copper sulphate—to such factors as repeated rough fish removal, fishing skill, weather, and the price of fish. The somewhat greater decline in catch from the treated lakes may be due to copper sulphate but there are so many variables involved that the statistical validity of difference in yield cannot be proven.

From such information as we have on the Fairmont lakes, it appears that the continued treatment with copper sulphate has not seriously affected angling. Creel census carried out during the months of July, 1941, and July, 1942, on 2 of the treated lakes, Amber and Budd, showed an average catch rate of 2.28 fish per hour of fishing. The average catch rate during these months for all Minnesota lakes was 1.61 fish per hour of fishing (Hiner 1943).

Algal Control and Production of Fish Food

It has long been recognized by fish culturists that maximum fish production is usually obtained with the aid of fertilization. Good results have been obtained with inorganic fertilizers and with such organic fertilizers as cottonseed and soybean meal. The aim of fertilizing with inorganic fertilizers is to produce a plant plankton crop. These minute plants are of little direct importance as fish food, but by their growth and decay, organic material is released into the water. This promotes the growth of water bacteria and the smaller animals which are the food of fish. The role of organic fertilizers is more direct. Such material is available for immediate bacterial decay and may even serve directly as food for some aquatic animals. The application of either type of fertilizer usually results in an increase in both plant plankton and fish crops (Smith and Swingle 1940). Because plankton and fish production are known to be related in ponds, the question arises as to whether or not the elimination of part of the plankton crop with copper sulphate will seriously affect fish production. To answer this question, it is necessary to consider a more basic one. Is there a direct and proportionate relationship between plant and animal production in waters?

Huff (1923) found that the growth of the plant plankton crop or its reduction with copper sulphate had no effect on the population trends of microscopic animals in Lake Vadnais near St. Paul. Pennak (1946) in reviewing his work on the plankton of Colorado lakes concludes that "a relatively low plankton crop . . . may support populations of grazers which range from very low to very high and that dense phytoplankton populations are not necessarily associated with dense populations of grazers." Similarly, in natural Minnesota ponds used for the rearing of pike-perch from fry to fingerling size, a concentration of nutrients was found above which additional natural fertility, and presumably the amount of organic matter produced by that fertility, did not increase the yield. In a series of such ponds, having an excess of calcium and nitrogen, those in which total phosphorus concentrations were between .02 and .05 ppm yielded an average 11.4 pounds of fingerlings per acre; those within a total phosphorus range of .051 -.1 ppm yielded 71.2 pounds per acre; those within a range of .11-.2 yielded 65.5 pounds, and those with a total phosphorus concentration greater than .21 yielded at the average rate of 67.8 pounds per acre (Moyle 1949). It appears, therefore, that above a certain optimum level, added fertility and the production of organic material by algal growth does not greatly increase the production of aquatic animals. Above an optimum level, it is likely that other biological and spatial factors become more important than basic food supply.

The main purpose of pond fertilization is to make certain that enough organic material is present for maximum fish production. To be sure of this, ponds are fertilized heavily enough to raise an excess of algae. In algal control with copper sulphate, the reverse is accomplished. Excess organic producing power as represented by algae is removed by chemical treatment. In the Fairmont lakes, it appears that the removal of such an excess has had little effect on the yield of rough fish. Expressed another way, organic production has not been reduced below, or much below, the critical optimum level despite a long history of copper sulphate treatment.

There are several reasons why such a result might be expected. First, waters which have objectionable algal crops are very fertile and produce an abundance of other aquatic life besides specific bloom formers. Second, destruction of an algal crop by copper sulphate does not destroy the organic material that this crop represents. The organic material is released into the water upon decay of the algal cells. Often this decay is accompanied by a great bacterial increase (Whipple 1927). Third, usually following a copper sulphate treatment, rapid growth is made by copper tolerant forms which were not killed. After the destruction of a heavy *Aphanizomenon* bloom in Hall Lake on June 3, 1946, the population of planctonic diatoms and green algae rose from 300,000 to 41,000,000 per 100 liters in 5 days. During this same period, the population of animal plankters and euglenoids rose slightly from 34,000 to 40,000 per 100 liters (Moyle and Wilson 1946).

Schoenfeld (1947) concludes that since copper precipitates from hard water as insoluble copper carbonate, this salt accumulates on the bottom to the detriment of fish-food animals living there. This conclusion is evidently based on the work of Nichols *et al.* (1946) on the accumulation of copper in the treated lakes at Madison, Wisconsin, and the statement of Hasler (1947) that Lake Monona, a treated lake of this chain, had 800 bottom fauna organisms per square meter in contrast to 9,000 for untreated Lake Mendota.

It is well known that copper carbonate is poisonous to snails and its toxicity is utilized for controlling swimmer's itch. McMullen (1941) found that when high concentrations of copper sulphate or mixtures of copper sulphate and copper carbonate were applied to the shallow waters in Michigan, "crayfish, leeches, tubificides and insect larvae were usually killed." He also notes that the use of 20 ppm copper sulphate resulted in the accumulation of 78.6 mg of copper per square foot of bottom 4 hours after treatment.

It is certain from this and other similar observations by McMullen and also from the experimental work of Whipple (1927) that following a copper sulphate treatment, copper does precipitate onto the bottom mud. It is also certain that high concentrations of precipitated copper are poisonous to aquatic invertebrates. There is, however, an important question to be answered. Do these precipitated copper compounds remain insoluble and accumulate on the lake bottom to such an extent that production of fish food is affected?

Whipple (1927) noted that copper carbonate may be decomposed into copper "hydrate" (hydroxide?) and carbonic acid, and that although the hydrate is insoluble, the carbonate is slightly soluble in the presence of carbonic acid. Since carbon dioxide is being continually generated from bottom muds, it is likely that at least some of the precipitated copper carbonate goes into solution again. McMullen (1941), when eradicating snails in a hard-water Michigan lake, found that there were 75.6 mg of copper per square foot of bottom 4 hours after treatment; 30 mg at the end of 24 hours, and 4.8 mg at the end of 54 hours. Whipple cites a conclusion of Hale (without exact reference) that at least during the winter months all copper fed into the New York water supply came through the distributing system. Conversely, he notes another case where .3% copper was reported in the bottom mud of a reservoir that had been treated.

The most conclusive evidence on the accumulation of copper in the bottom muds of treated lakes is that of Nichols *et al.* (1946) who analyzed muds from three Wisconsin lakes with a long history of copper sulphate treatment. In the treated lakes a range of 18 to 1093 mg of copper per kilogram of bottom mud was found in Lake Menona; 18 to 595 mg in Lake Waubesa and 18 to 595 mg in Lake Kegonsa. In contrast, untreated Lake Mendota showed a range of 32 to 135 mg of copper per kilogram of bottom mud. These workers conclude that ". . . *it appears* that by far the greatest amount of that copper applied remains as a deposit in the mud of the lake."

The amount of siltation is also important. In lakes, such as those of the Fairmont chain, which are continually receiving silt from adjacent land, any copper precipitated to the bottom would be buried and its possible toxic effect on bottom fauna thereby greatly lessened.

Algal Control and the Growth of Larger Aquatic Plants

Larger aquatic plants are quite tolerant to copper sulphate and there is no record of injury to them from algal control measures in Minnesota. The higher algae are more susceptible than the aquatic seed plants and the growth of such forms as *Chara* and *Hydrodictyon* has been controlled with copper sulphate in fish ponds. Greater concentrations are necessary than those ordinarily used for plankton algae (Surber 1943; O'Donnell 1945). In reviewing the literature on the effect of copper on terrestrial seed plants, Miller (1931 p. 284) states that although copper is usually toxic, low concentrations of .02 to .2 ppm "not only increased the length of life of various plants but also their dry weight."

Generally, there is an inverse relationship between plankton production and that of larger aquatic plants. Minnesota lakes with little plankton may have aquatic weeds to a depth of 25 feet. In those with moderate plankton production, the littoral zone usually extends to about 15 feet and in those with a heavy algal bloom there are often few weeds beyond a depth of 4 feet. The production of a heavy plankton crop by fertilization has been found to be an effective way to control weed growth in fish ponds (Smith and Swingle 1941). Conversely, plankton production may be limited by robust growth of the larger aquatic plants. Observations along this line have been made by Kofoid (1903) and more recently by Bennett (1943) who recommends the elimination of larger aquatic plants from fish ponds because "they take up nutrient materials and light that would otherwise produce algae."

A few general observations have been made on algal control and the growth of larger aquatic plants. Huff (1923) notes that concurrent with algal control in Lake Vadnais there was "cutting and removal of great quantities of submerged vegetation each year." Domogalla (1935) states that during 11 years of treatment of Lake Monona "weeds have grown luxuriantly in 18 feet of water" and flourished despite the use of a weed cutting machine.

It appears that control of plankton algae with copper sulphate can be expected to have little or no detrimental effect on larger aquatic plants and may even increase their growth.

Acquired Tolerance of Algae to Copper Sulphate

Hale (1942) and Whipple (1927) are both definitely of the opinion that algae do not acquire a tolerance to copper. However, acclimatization of other microorganisms to copper and similar heavy metals is fairly well known (Heilbrun 1937, p. 294).

In the Fairmont lakes, *Aphanizomenon* seems to have acquired an increased tolerance to copper as a result of 26 years of treatment. These lakes were first treated in 1921 by Huff (1922). He carefully noted the kinds of algae present, the concentrations of copper sulphate used and the results. The concentrations used for successful control in the 4 lakes at that time were: Amber, .15 ppm; Hall, .14 ppm; Budd, .20 ppm; and Sisseton, .24 ppm. In the period 1943-46 much higher concentrations were necessary to obtain the same results; the average of treatments in these years being Amber, .35 ppm; Hall, .73 ppm; Budd, .80 ppm; and Sisseton, .50 ppm. Two to five times as much copper sulphate must now be used as was necessary in 1921.

The high concentrations used in later years on the Fairmont lakes were necessary and not due to calculation or judgment errors of the Fairmont Water Department. Moyle and Wilson (1946) supervised treatment of Hall Lake with .5 ppm and achieved only partial success in eliminating *Aphanizomenon*—a reduction of 1,200,000,000 to 21,800,000 filaments per 100 liters 5 days after treatment. This alga made a rapid recovery and it was soon necessary to treat the lake again. In both 1921 and 1946 the boat and burlap bag method of application was used.

Two other hard-water Minnesota lakes with *Aphanizomenon* blooms were treated for the first time in the summer of 1947. No filaments were found following treatments with .3 ppm in Little Elk Lake, Sherburne County, and no return of the bloom for a month was noted in Spring Lake, Scott County, which was treated with .11 ppm.

Summary

Although much work has been done on the toxicity of copper sulphate to different aquatic plants and animals, little consideration has been given

to the effect of its continued use as an algicide on the productivity of waters. In Minnesota, 4 lakes have been regularly treated with copper sulphate for 26 years. The average rough fish yield from these lakes for the last 24 years is slightly greater than that of 5 adjacent untreated lakes. In the treated lakes, the blue-green alga, *Aphanizomenon*, seems to have acquired an increased tolerance to copper as a result of the 26 years of treatment. From theoretical considerations and the few data available, it appears that algal control with copper sulphate is not detrimental and may even favor the growth of aquatic seed plants. It is also likely that ordinary algal control has little effect on the production of plankton animals. Whether or not copper accumulates in the bottom muds to the extent of being toxic to the bottom fauna is at present uncertain.

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