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One of the major items in any program of municipal sanitation is the double problem of water supply and waste water disposal. Since streams are the general source of water supply and also the final receptacle for sewage, this dual use of the stream often leads to complications. Sewage and water treatment are considered man-made lines of defense against the passage of bacterial pollution. Bacterial self-purification can be considered a natural barrier. This thesis presents a study of the die-off of certain specific organisms in a stream receiving treated sewage.

The study was accomplished by drawing samples of the receiving stream at specified sampling stations and determining the density of the organisms Escherichia-coli and fecal streptococci, and the total number of viable cells present in the sample. All bacterial counts were made using the membrane filter technique except for the total

counts, which were obtained using the standard pour-plate method. Curves showing bacterial pollution versus river miles were obtained relating the die-off of the bacteria to miles below the sewer outfall.

Conclusions reached from this study were: (1) Both the Escherichia-coli or fecal streptococci counts yielded a good index of stream pollution, however, the E-coli colonies proved to be the easier of the two to count. (2) The membrane filter technique offered reliability and ease in counting for large numbers of samples. (3) Increased temperature caused increased bacterial die off in incubated samples of river water. (4) For both 10°C and 20°C temperatures, the E-coli were noted to die off more rapidly than the fecal streptococci. (5) In Mary's River, during the winter months, 64 percent of the E-coli died-off in a distance of five and a half miles. Within the same distance, 71 percent of the fecal streptococci died-off.

THE FATE OF SPECIFIC ORGANISMS IN A RECEIVING STREAM

by

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FATE OF SPECIFIC ORGANISMS IN A RECEIVING STREAM

One of the major items in any program for municipal sanitation is the double problem of water supply and waste water disposal. When considering the resources of a stream, water supply and sewage disposal are inseparable. Streams, either surface or underground, are the general source of public water supply and also are the final receptacle for sewage or its decomposition products. This dual use of the stream often leads to complications.

This situation of dual use prevails over a great part of the United States, especially in the drainage areas of the Mississippi and its tributaries. The Ohio River is an excellent example of this situation. Here one great city after another discharges its sewage and the waste of its industries into the stream, and each of these cities in turn draws its domestic water supply from the same stream. As a result, each community uses the diluted sewage of all the upper communities and contributes its own wastes to the drinking water of the lower communities.

Wholly apart from the esthetic values involved, the continuous intake of water by man, which has been contaminated by human excreta, leads inevitably to the transfer of pathogenic organisms of infectious diseases, especially those of the intestinal tract, such as typhoid fever, cholera, and the dysenteries.

There are three lines of defense against passage of pathogenic

organisms from sewers of one city to the water supply and eventually the consumer in another. Sewage and water treatment can be classified as man-made lines of defense; and bacterial self-purification as a natural line of defense. The scope of this thesis is concerned only with the natural barriers to passage of possible bacterial pollution.

The discharge into a receiving stream of sewage and other waste waters that are rich in decomposable matter greatly increases the number of microorganisms that are essential to self-purification. The multiplying organisms are derived in part from the waste water, in part from the receiving water. Only after they have come into balance with the food supply under the prevailing environmental conditions does the number decline. Below the points of modal concentration of the bacterial population the numbers decrease at varying rates, depending on the prevailing environmental conditions. It is the purpose of this paper to discuss and evaluate the die-off of certain specific organisms. These organisms, Escherichia coli and fecal streptococci, are the most representative of sewage pollution and in most cases the simplest to obtain for quantitative analysis. Along with these specific indices of fecal contamination, a total count of bacterial density will be considered to indicate the general pollution of the stream.

The discussion will also present significant factors affecting

bacterial self-purification, a short synopsis of the specific organisms, an outline of the study procedure, and results and conclusions.

FUNDAMENTAL CONSIDERATIONS

A brief study of the basic phenomena considered important in bacterial self-purification is necessary for an adequate background. The specific organisms can also play a leading role in the reliability of any results obtained; therefore, a mastery of the microbiology of the organisms is required. Several studies similar to the investigation described herein have been attempted, some with excellent results; others with questionable results. A more general field of view could be obtained if the procedures and results of some of these investigations were explored thoroughly to further strengthen or repudiate observed results.

Factors Affecting Bacterial Self-purification

The examination of any stream of water at various points below a source of contamination, such as a sewer outlet, will invariably show a rapid diminution in the number of bacteria as the distance below the sewer outlet increases. The factors responsible for this so-called "self-purification" are usually listed as sedimentation, the activity of predator organisms, light, temperature, food supply, dilution, and perhaps more obscure conditions such as variations in osmotic pressure and bacteriophage.

The total effect of these factors is well illustrated in the

following data collected along the Ohio and Illinois Rivers.

Figure 1

Bacterial concentration as a percentage of maximum count in a stream reach. *

Illinois Data					Ohio Data		
flow in hours(app.)	% Bacteria				flow in hours(app.)	% Bacteria	
	Total Count		Coliform			Total Count	Coliform
	Summer	Winter	Summer	Winter		Summer	Summer
0	100.0	41.8	100.0	99.0	3	93.5	82.4
2	50.9	56.5	47.2	76.2	12	97.0	100.0
14	38.1	100.0	38.4	100.0	17	100.0	91.8
31	14.4	19.1	15.8	55.6	35	49.1	36.8
41	3.0	35.4	5.6	85.8	83	9.4	11.0
84	1.7	4.7	0.6	10.0	140	1.1	1.2
130	0.7	2.9	0.6	8.3	183	0.7	0.4
183	0.1	1.2	0.0	2.8	498	0.1	0.1

* From Public Health Bulletin No. 143.

It is rather difficult to evaluate the effect of each of the "self-purification" factors alone, since two or more are almost invariably operating simultaneously. However, a general discussion of these factors will follow.

Sedimentation. Jordan, in a study of bacterial changes in the Illinois River where the fall is approximately 30 feet in 225 miles, gave as his opinion that sedimentation was ample to explain all decreases observed in bacterial numbers. He sums up his opinion relative to this factor as follows:

> It is noteworthy that all instances recorded in the literature where a marked bacterial purification has been observed are precisely those where the conditions have been most favorable for sedimentation (10, p. 227).

The settling velocity of a particle, whose size approaches that of a single bacteria, is approximately one foot per 55 hours. The specific gravity of this particle is 2.65 as compared to the specific gravity of a bacterium whose specific gravity is near 1. Due to this very low settling velocity, it may be assumed that subsidence of bacteria cells is due almost entirely to the attachment of bacteria to larger particles.

The following table illustrates the effect of sedimentation in the removal of bacteria.

Figure 2

Removal of bacteria from stored water due to sedimentation from Kansas River flood waters *

Hours Standing	Original Water Plate Count	Supernatant Water		Sediment Resuspended Plate Count
		Plate Count	Percent Removed	
0	75,000	____	____	____
24	_____	7,800	89.6	76,000
48	_____	6,250	91.7	82,000
72	_____	6,150	91.8	69,500

* From Gainey and Lord.

The experiments conducted on the flood waters of the Kansas River showed that under quiescent conditions approximately 90 percent of the bacteria subsided within 24 hours. From these data the investigators concluded:

> In view of the slow settling rate of bacteria, the question naturally arises as to how such marked results were obtained as indicated in the data for Kansas River flood waters. Two facts must be kept in mind in this connection: 1) the samples were taken only just below the surface after subsidence; 2) the water contained a large amount of silt. As the silt settled, it acted more or less as a filter screen and carried down with it smaller particles; as a result, a much more rapid removal of the finer suspended particles, including bacteria, would take place than would occur if the smaller particles alone were present (10, p. 228).

Temperature. The effect of temperature upon the survival of bacteria in water is rather difficult to measure quantitatively. Temperature not only affects the matabolism of the bacteria themselves, but the water in which they live. The viscosity of water is a function of temperature; hence temperature influences subsidence.

An elevation of temperature speeds up the metabolic activities of a given species until an optimum temperature is reached. Above this optimum, activity rapidly decreases and soon ceases altogether. The temperature of most natural waters is well below the optimum for most species of bacteria, meaning that in natural conditions microbial

metabolism increases as the temperature increases. If conditions in the water are otherwise suitable for growth, an increase in temperature results in an increase in bacterial numbers. However, if the environmental conditions are not such as to favor growth and microbial metabolism is speeded up by any factor, the net result is increased death rather than growth. For example, high temperatures will cause the cell to metabolize its own protoplasm if no external food supply is available. Accordingly, it may be safely assumed that bacteria will die-off more rapidly under warmer conditions. The condition just described appears to exist in most waters.

Most microorganisms cannot grow in low temperatures since the water which makes up 80 percent of the cell freezes, preventing further reaction.

Food Supply. It is beyond the scope of this thesis to dwell in detail upon the influence which the food supply of a water course may have upon the numbers of intestinal and soil bacteria. Numerous studies have been made in relation to this particular factor, all of which show, in general, how delicately the bacteria respond to comparatively slight changes in their food supply.

Unless there is adequate food to make growth possible, a gradual decrease in numbers is inevitable. On the other hand, the presence of adequate food does not necessarily imply that bacterial growth will occur. Other conditions may be such as to prohibit reproduction. The

more limited the quantity of food materials, the less likelihood, other things being equal, of the bacterial growth rate exceeding the death rate.

The conditions in water sometimes become especially favorable for a particular type or types of bacteria to multiply, in which case the growth of this particular kind may exceed the death rate of all other types present, resulting in a marked increase in total numbers. This often happens when large quantitites of easily decomposable organic material are present in the stream. A typical illustration of the diverse effects of a food supply upon bacterial numbers is that which may be observed following the treatment of a stored water with copper sulfate to kill algae. The death of large quantities of algae, which may serve as organic food for bacteria, may result in very large increases in the number of bacteria present. Many other illustrations may be made, but all point to the very pronounced effect of the availability of a food supply upon the bacterial population.

Antagonistic Action of Other Organisms. The role of such action is difficult to measure quantitatively. It is well established, however, that many protozoa subsist primarily upon bacteria and finely disintegrated organic materials. It is also known that many such forms are found in waters, and that a marked increase in protozoa may be noted following pollution with sewage. The ingestion of bacteria by protozoa would result in a direct decrease in bacterial

numbers, while the consumption of organic materials by protozoa would result in the removal of bacterial food from water. Since protozoa find certain species of bacteria more acceptable as a food than others, the presence of protozoa may also result in a qualitative alteration of the bacterial population.

The utilization of bacteria as food by protozoa is of considerable importance as a factor influencing the bacterial population of polluted waters. As the bacterial population increases, more food in the form of bacterial cells becomes available for the protozoa. As a result, the protozoa population increases at the expense of the bacterial population until the latter is reduced to the point where insufficient protozoa food is available, after which the protozoa population falls. Thus an inverse quantitative relationship often can be observed between the bacterial and protozoa population. When an equilibrium exists in which there is a preponderance of protozoa, any factor, which largely eliminates the somewhat less resistant protozoa, will result in an enormous increase in the bacterial population.

Under certain conditions the numbers of bacteria have been observed to increase to a marked extent in waters containing relatively large numbers of protozoa. When the bacterial and protozoa populations of a polluted stream have reached an equilibrium and this equilibrium is upset by the entrance of unpolluted water, it might be expected that, following the confluence of the two streams, a decrease

in bacterial content per unit of water would be noted. However, if the bacterial population of the polluted stream is held in check by protozoa, then, following dilution with the unpolluted water, it appears that, in the new environment, bacteria multiply much more rapidly than do the protozoa; and therefore, a marked increase in bacterial numbers may be noted for a limited time. As soon as the protozoa population has had time to increase significantly, a new equilibrium is established.

The possible effect of ultra microscopic viruses, commonly known as bacteriophage, should be mentioned since there has been a growing concern over the influences of similar viruses upon humans. Studies have shown that waters of some rivers are relatively lethal to certain intestinal bacteria. The destruction of bacteria in these cases has been more rapid than can be explained by the usual processes of self-purification(25, p. 13 and 10, p. 203). Workers have attempted to explain this peculiar bacterial change. All have observed that the autolytic process consists of gradual swelling of the cell, followed by cell disintegration (30, p. 423). The nature and origin of these lytic agents are not fully understood and appear to act only on the active organisms at certain phases of growth.

Light. In the laboratory it is easy to demonstrate that light rays of the proper wave length, whether emanating from the sun or from an artificial source, are effective in destroying bacteria. The

effectiveness of the light waves depends primarily upon the extent to which they penetrate the water. In chemically pure water about 46 percent of the light energy incident upon the surface is transmitted through the first meter; 80 percent of that penetrating the first meter passes through the second. At five meters there remains about 29 percent of the original light energy. (31, p. 165-170)

The transmission of light energy in natural waters is influenced by substances in solution and in suspension. In general, natural waters free from suspended material, but having substances in solution, will transmit only five percent of the light energy to a depth of five meters, as compared to 29 percent for chemically pure water (31, p.169).

Many investigations have been made concerning the effect of light on bacteria in flowing water. Results have been confused and for the most part without practical meaning (25, p. 14). However, it is pointed out that climatic conditions play a major role as the cause source. In tropical countries sunlight is an important factor in the self-purification of rivers and is noted to be partly responsible for the rapid disappearance of fecal streptococci and certain intestinal bacteria from water (30, p. 623).

The ordinary rays of sunlight play little part in disinfection of waters. Ultra-violet radiations assisted by heat rays seem to be the main factors. It has been suggested that the bactericidal action is

due to oxidizing substances such as ozone and hydrogen peroxide produced by the rays. There is evidence that the presence of oxygen is necessary for the germicidal activity of ultra-violet rays. (30, p. 623-624)

Osmotic Pressure and Toxic Salts. Bacteria, as a rule are not as easily influenced by variations in osmotic pressure as are higher plant and animal cells. Plasmolysis is a shrinking of the cell due to loss of water by osmotic pressure. Plasmoptysis, in contrast, is a swelling of the cell due to a gain of water (10, p. 55). Both plasmolysis and plasmoptysis are known to occur. However, it is doubtful that the concentration of solutes, aside from inland salt lakes, ever becomes high enough to cause plasmolysis. On the other hand, the molecular concentration in waters of extremely high purity may be low enough to result in the death of some forms of bacteria by plasmoptysis. (10, p. 233)

It is known that the discharge of various trade wastes containing inorganic compounds may, because of toxicity, bring about decreases in the microbial population in the stream. Distinct antiseptic or disinfecting action has been noticed in connection with acid wastes. Carbon dioxide has shown antiseptic effects, although a certain minimum amount is essential for bacterial life (25, p. 15-16). Dissolved mineral salts have disinfecting effects upon bacterial viability. Mineral salts may be obtained from natural soil formations or through

pollution by industry (30, p. 422).

Occasionally decreases in bacterial numbers are encountered which are difficult to explain upon the basis of any known combination of factors; this merely indicates the inadequacy of information on this subject.

Dilution. The dilution of sewage or heavily polluted water with relatively unpolluted water produces, at times, a rather unexpected effect upon bacterial density. In the study of the natural purification of the Ohio River, Frost noticed an increase in the number of bacteria in the water during the first ten to 15 hours of flow below certain points of major pollution; and Streeter observed that the addition of water from a tributary to a more heavily polluted stream frequently results in an increase, instead of a decrease, in the bacterial density of the mixture. An explanation of this phenomenon, which appears to be largely the result of dilution, may perhaps be found in the suggestion that for a given set of conditions a maximum bacterial population can be supported, and, if for any reason the population falls below this level, multiplication of the bacteria follows. Subsequent studies have furnished further evidence that bacterial multiplication is an important factor in the readjustment of the biological balance disturbed by the dilution of a polluted water. Another suggestion states that normally protozoa and other destructive agencies keep the bacterial density of such a water below its limiting level, but dilution so reduces this

density that bacterial concentration, needed by the protozoa present, is no longer available and the bacteria, therefore, are provided with an opportunity to increase their numbers. Since the bacteria can multiply much faster than the protozoa, a marked increase in the bacterial density may result before the biological balance is restored. It is probable that disintegration of solid and bacterial masses is also partly responsible for the larger numbers of bacteria observed in sewage and polluted waters subsequent to their dilution (25, p. 11-13).

After this initial increase in bacterial density has reached its maximum, signifying restoration of the biological balance, a progressive decline in numbers sets in being rather marked for the first few hours followed by a more gradual decrease thereafter until another biological balance is established for the different set conditions.

Indices of Bacterial Pollution

Since the discovery, that intestinal disease-producing organisms can be distributed through the medium of drinking water and that it is very difficult to detect their presence, it has been endeavored to find some other means by which the possible presence of fecal contamination might be indicated. In absence of practical methods for detecting and enumerating pathogenic organisms, microbiologists have discovered specific groups of non pathogenic organisms whose origin is in fecal matter and hence may be considered an indicator of fecal

pollution. They are, in essence, measures of guilt by association. Some qualifications which must be considered for an indicator organism are: 1) they must always be present in sewage, even after treatment so that the presence of sewage potentially contaminated with pathogens could be detected; 2) they should be more numerous than contaminating organisms; 3) they should be easy to isolate and identify; 4) they should be easy to enumerate so that estimates of their numbers can be obtained.

Coliform Group. It was learned early that the intestinal tract of man, as well as other warm-blooded animals, contains large numbers of micro-organisms, principally harmless types. Escherich isolated an organism from feces which he named Bacillus coli and described as the characteristic organism of human feces. Other investigators isolated similar bacteria from soil, plants, and water.

The Bacillus coli, defined as all aerobic or facultative anaerobic, Gram-negative, non spore-forming, rod-shaped bacteria capable of fermenting lactose with gas formation within 48 hours at 35°C (1, p. 494), was later found to consist of not just a single organism, but a group of organisms. This group was given the name coliform group.

The coliform bacteria are members of the family Enterobacteriaceae. They include the genera Escherichia and Aerobacter along with several others which are of lesser importance in sanitary microbiology.

The coliforms were originally believed to be entirely of fecal origin. However, it has since been shown that Aerobacter and certain Escherichia can grow in soil. Efforts have been made to distinguish between fecal coliforms and non-fecal coliform organisms. These differential tests are necessary for positive identification if coliforms are to be used to indicate the presence of fecal contamination. The possibility of a false reaction is one of the major disadvantages of the coliform group as an indicator of pollution, since the completed test is time consuming and many times is completely forgotten.

Results of investigations have proven that the bacteria of the genera Escherichia, more specifically Escherichia coli, is almost entirely of fecal origin. The Aerobacter and the intermediate forms of Escherichia are predominately of soil origin. Although the differentiation of two main types of coliforms is possible to a fairly high degree, the control of water purity is still based on the presence of any coliforms, soil or fecal forms (22, p. 154).

Streptococci. J. M. Sherman (1937) stresses our lack of knowledge of the streptococci in the following words:

> For the most part, the known species of streptococci are those which have brought themselves clearly out to attention as the agents of disease or as more or less dominant organisms in familiar habitats... It should be recognized that the species which are now clearly defined represent only a

> small portion, perhaps a very small fraction, of those which actually exist. (25, p. 207).

Although the significance of the streptococci as sewage organisms is not established with the same definiteness which marks our knowledge of the coliform group, these bacteria have been isolated so frequently from polluted sources and so rarely from normal waters that it seems reasonable to regard their presence as indicative of pollution. Investigations by several persons have proven beyond any reasonable doubt that the presence of streptococci, although usually found fewer in number than coliform organisms, is far more indicative of recent fecal pollution than the coliform group (20, p. R-400).

The enterococci have a very interesting history as indicators of sewage pollution. Originally they were thought to be good indices, then followed a period in which they were totally disregarded, but now again they have regained recognition. This recognition has been due to the development of better methods of isolation, but it is still largely academic in that procedures are still being reported and evaluations and uses are still experimental.

The enterococci were originally reported in 1894 and later in that decade were confirmed to be of fecal origin and therefore an indicator of a possible health hazard. Work in the early 1910's generally agreed that streptococci died out rapidly in sewage contaminated water in marked contrast to coliform organisms. Thus the streptococci

were recognized as a test of minor importance.

During the 30 years that extended between 1920 and 1950, the streptococci were second to the coliform group as an indicator of pollution, but increased investigations into better media for evaluation of both brought forth many difficulties. Many discrepancies had been found in the coliform group test. Many coliform organisms causing positive presumptive tests were confirmed to be different species of the group and were found to be of questionable use as indicators of fecal pollution. The streptococci test, on the other hand, had gained momentum from the discovery of both a presumptive and confirmatory broth which allowed the enumeration of the organisms in a more direct method than before (19, p. 873-874). Also the noticeably greater die-off of the enterococci when compared with the coliform group was used to advantage by enabling the recency of the pollution to be detected with greater accuracy.

Other reasons for the failure of the coliform test have placed even more importance upon the enterococci group. The coliform group has been found in a wide range of natural environments, and their ability to live and multiply outside the animal body has caused many problems.

However, Streptococci have been found to be present in feces, sewage, and contaminated water, and not in potable waters, virgin soils, and sites out of contact with human and animal life. They do

not multiply outside the human body (14).

Total Count. Among the many tests that have been proposed for ascertaining the biological purity of water, the number of bacteria per unit volume of water is by far the oldest. The presence of relatively high numbers of bacteria, although not necessarily dangerous, is fairly indicative of some abnormal condition, possibly of an undesirable nature.

There has been a general hesitancy, on the part of any group appointed for the purpose of recommending standard procedures for bacteriological analysis, to set any arbitrary limit as to the numbers of bacteria that may be safely tolerated in drinking water (10, p. 161).

Although the total count of bacterial numbers in water is of relatively small value, since the adoption of the coliform group as the primary indicator of pollution, it often enables the sanitary engineer to detect the origin of pollution when the origin is questionable.

Formulation of Bacterial Death Rate Curves

Quite extensive observations of the decrease of bacteria in polluted waters indicate that such changes follow a fairly regular course, modified by variation in environmental conditions, but yet having an orderly arrangement of reduction. A simple and direct method for determining the rates of bacterial decrease in streams, if it were practicable, would be to observe the changes occuring in

stored samples of the water under consideration. Unfortunately the decrease in such stored samples does not correspond invariably with the natural rates occuring in the stream.

Since the publication, in 1908, of the studies of Dr. Harriet Chick concerning the rules of disinfection (4, p. 92), it has become generally recognized that, when bacteria die under conditions of unfavorable environment, they appear to be subject to a constant death rate; a given percentage of the remaining population dies during each successive time unit (24, p. 203 and 8, p. 477). The death rate can be formulated by the following:

$$\frac{dN}{dt} = kN$$

$$\ln \frac{N_1}{N_2} = k(t_2 - t_1)$$

Where N_1 is the number of organisms present at time t_1

N_2 is the number of organisms present at time t_2

k is a constant coefficient

A curve of decreasing steepness is traced by bacterial numbers plotted logarithmically against time arithmetically.

There have been various attempts to explain why this particular formula, which is in the form of a monomolecular reaction, should apply. None have been convincing. However, for purposes of analysis,

it has been found quite satisfactory to apply this formula to data in a purely empirical manner. Due to the numerous factors contributing to the removal of bacteria from flowing water, it must be recognized that this law represents only an approximation of bacterial die away (8, p. 507-508). Distortion of the actual from theoretical values may be presumed to be due to the non-homogeneity of the population of bacteria under study (24, p. 204).

The k value mentioned in the formula above, sometimes called the constant lethal influence, seldom holds constant in natural stream conditions. The most marked effects upon the constant lethal influence are due to temperature. For all practical purposes, however, this temperature effect is so masked by other variations in stream conditions that it is hardly distinguishable except in a broad classification, such as summer and winter conditions as shown in figure 3 (24, p. 205).

Frost, in the study of the pollution of the Ohio River, found that the data from a natural stream environment could not be explained with a great deal of accuracy by the use of a single-termed monomolecular reaction. To enable them to fit curves to the data, Chick's law was modified to a multi-termed monomolecular reaction. An example of the formula fitting such a reaction is shown below.

$$B = B_o \times 10^{-kt} + B'_o \times 10^{-k't}$$

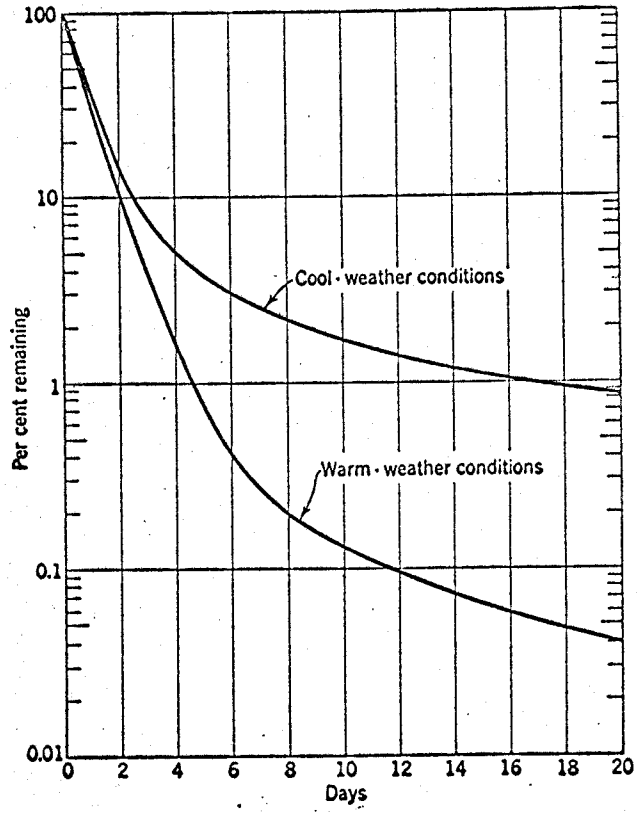

Figure 3
The effect of temperature upon bacterial purification in the Ohio River. B-coli Counts after Phelps

This formula, in a general form, states that the residual number of bacteria at any time is made up of two fractions; one resulting from the application of a death rate constant, k, to the initial population, B_o, and the other from similar application of the rate, k, to the initial population, B_o. As a result of the use of this formula, the function traces out a curve asymptotic to two nearly straight lines when the percentage of bacteria remaining is plotted logarithmically against time plotted arithmetically (9, p. 303-321). The two termed form of the monomolecular reaction was found to fit the curves quite well except for the transition point of the two intersecting straight lines. A typical semi-log plot is shown in figure 4.

Similar Investigations

Many studies have been made which are similar to this study. A summary of these investigations is important since many of the problems may bear a relation to those found in the Mary's River.

Ohio River. The most extensive studies of pollution and self-purification of streams and rivers has been made by the U. S. Public Health Service. In 1913 a group of bacteriologists and engineers set up a series of laboratories along the Ohio River. The central office, the forerunner of the Robert Taft Sanitary Engineering Center, was located in Cincinnati because of central location.

The Cincinnati group, in addition to their extensive studies of

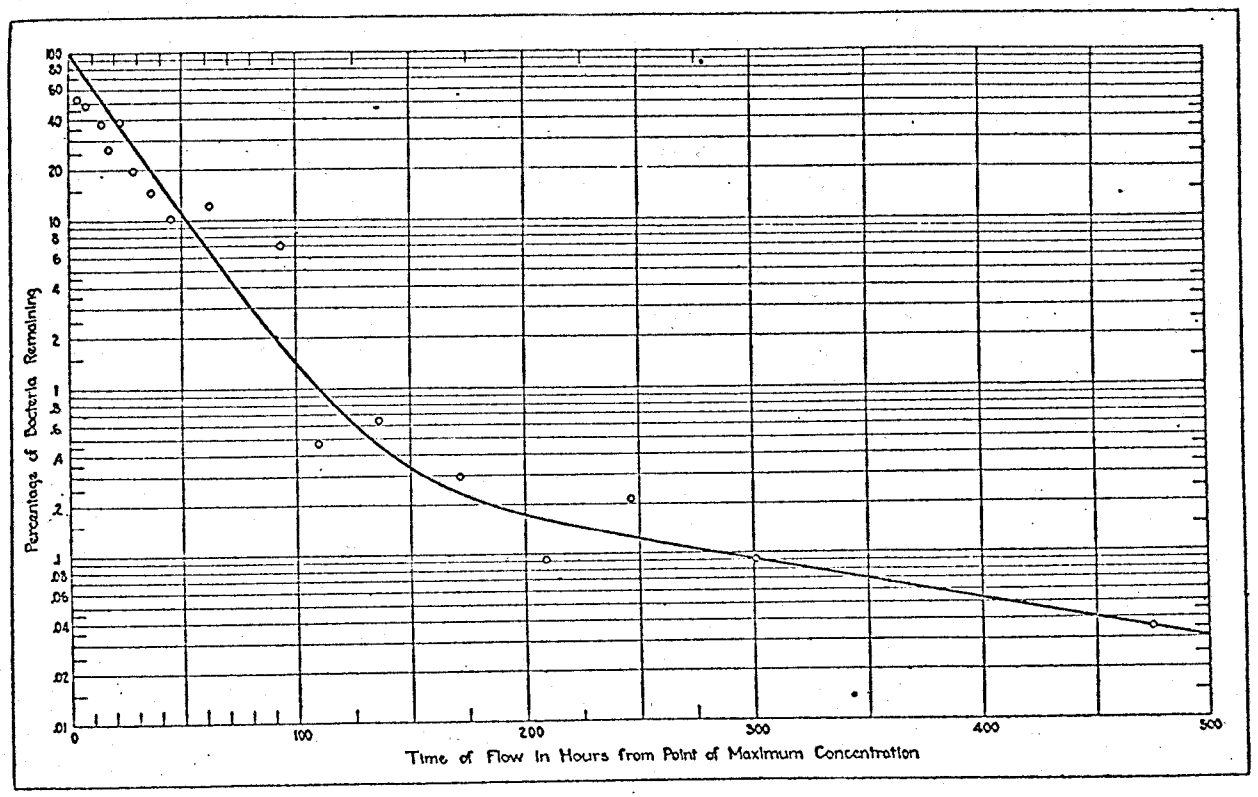

Figure 4

A typical semi-log plot showing bacterial death rates in the Ohio River. B-coli Counts after Frost

stream pollution and natural purification in general, have made very important contributions to our knowledge of bacterial self-purification under natural environmental conditions.

Over a considerable stretch of the Ohio River, observations were made continuously for about three years at a large number of cross-sections. An examination of the samples drawn at these sampling stations was made to determine both chemical and bacterial self-purification characterisitics. Hydrologic and geologic studies were also made of the entire river basin so that stream flow conditions, especially times of passage between sampling stations, were rather definitely known. The wide variation in characteristics of the Ohio River provide a background for comprehensive analysis of the relation of stream conditions to bacterial death rates (9, p. 1-8).

The bacteriological study of the Ohio River consisted of determination of total numbers of bacteria capable of growing on agar, at 37°C and on gelatin at 20°C, and of the n umbers of the sewage organism, Bacillus-coli.

Frost and Streeter found it convenient, in order to avoid the large unwieldy numbers involved, to report the bacteria in terms of a quantity unit, this unit being the number of bacteria at a given point in one day, if the concentration is 1000 organisms per milliliter and the stream flow is one cubic foot per second (9, p. 243).

The degree of bacterial pollution contributed by cities was

separated into two principal cases. The first case was concerned with the intensity of bacterial density that will result in the stream in the zone of highest pollution below the point at which the sewage is discharged. The second consideration was the proportion of such contributed bacteria that will remain in the stream of a known distance or time of flow below the point at which they were added.

In observations of the effect of pollution upon the intensity of bacterial density it was consistently noted that the zone of greatest bacterial density in the receiving stream does not occur immediately below sewer outfalls, but at a point from 15 to 20 hours downstream from the point where such pollution was added. Figures 5 and 6 illustrate this phenomenon. The location of this maximum zone seems to be influenced by seasonal temperatures, being farther downstream during the winter months. Whether an actual multiplication of organisms in the stream takes place until this maximum is reached, or whether the observed increase in bacterial numbers is due to the physical separation of solid matter, has not been definitely determined, though evidence seems to point to a mixture of the assumptions as the most logical explanation (11, p. 3 and 9, p. 297-298).

The observations of the decrease of bacteria in the polluted waters indicated a fairly consistent trend (see figures 7 and 8). From the maximum point the bacterial count decreased rapidly and quite regularly throughout the remainder of the range of observations so

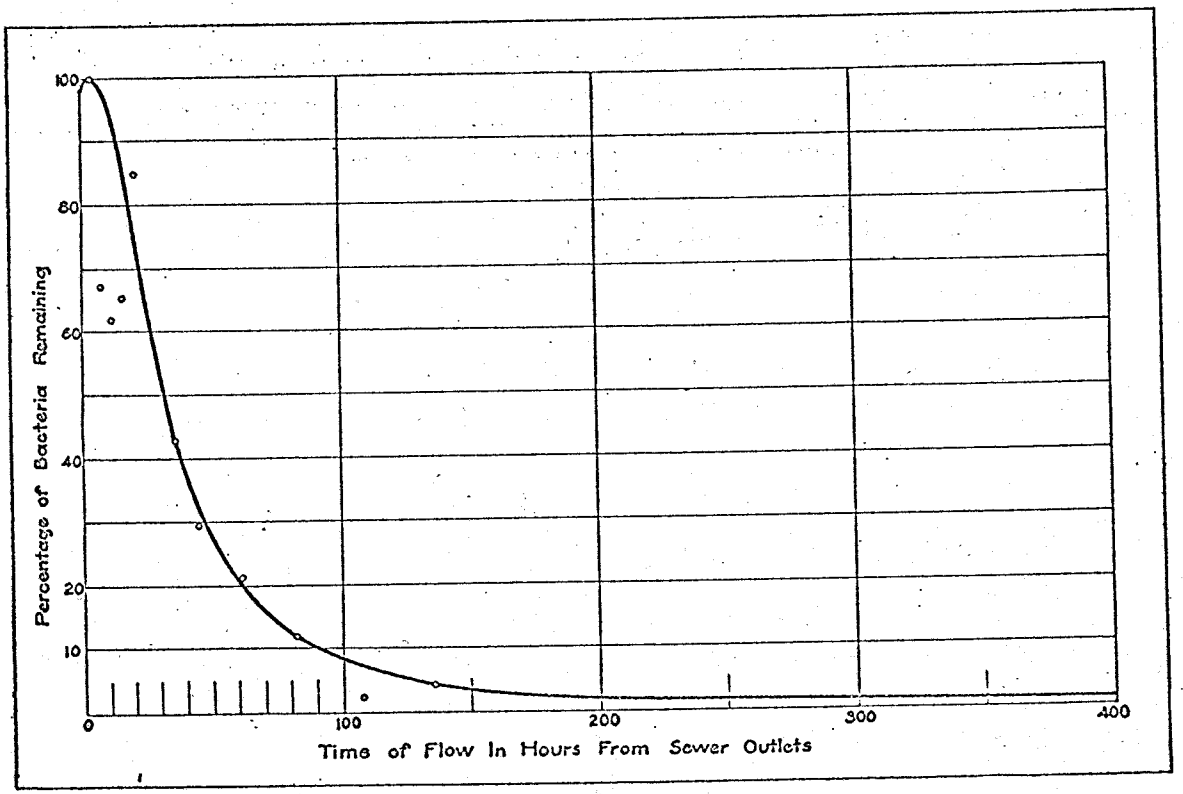

Figure 5
Bacterial purification in the Ohio River between Cincinnati and Louisville in relation to time of flow from sewer outfalls of the Cincinnati metropolitan district. B-coli Counts after Frost

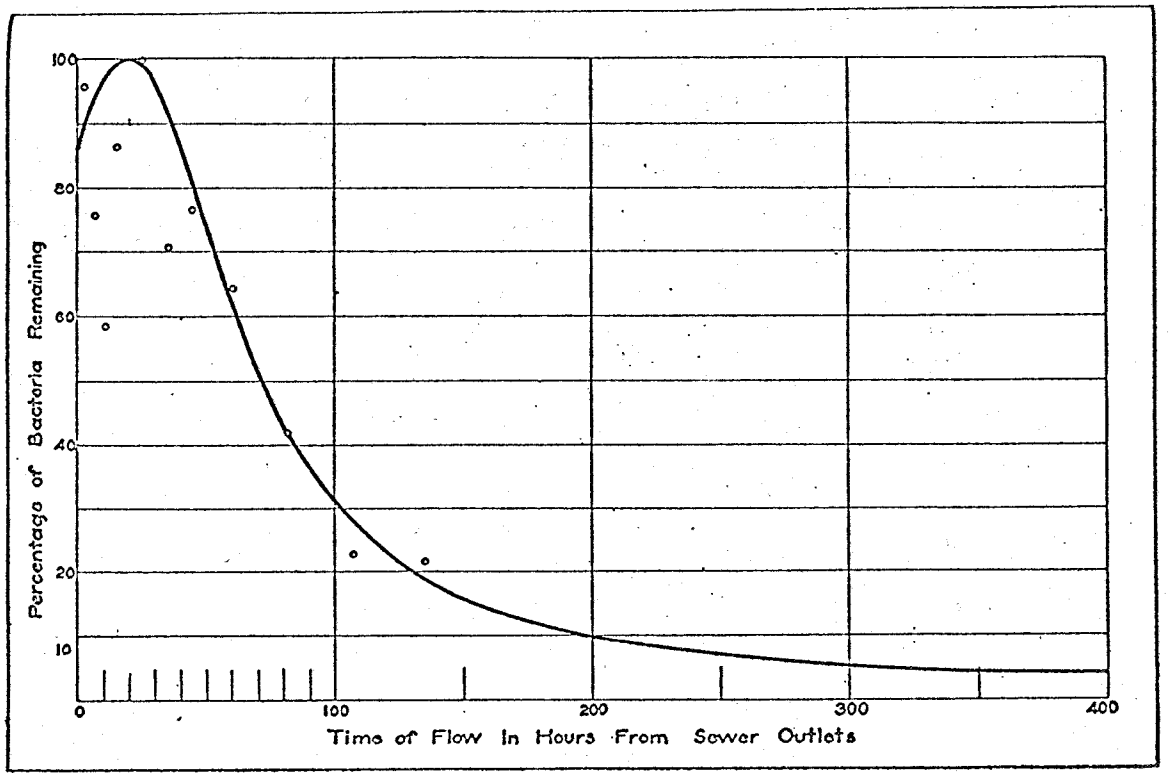

Figure 6
Bacterial purification in the Ohio River between Cincinnati and Louisville in relation to time of flow from the sewer outfalls of the Cincinnati metropolitan district. B-coli Counts after Frost

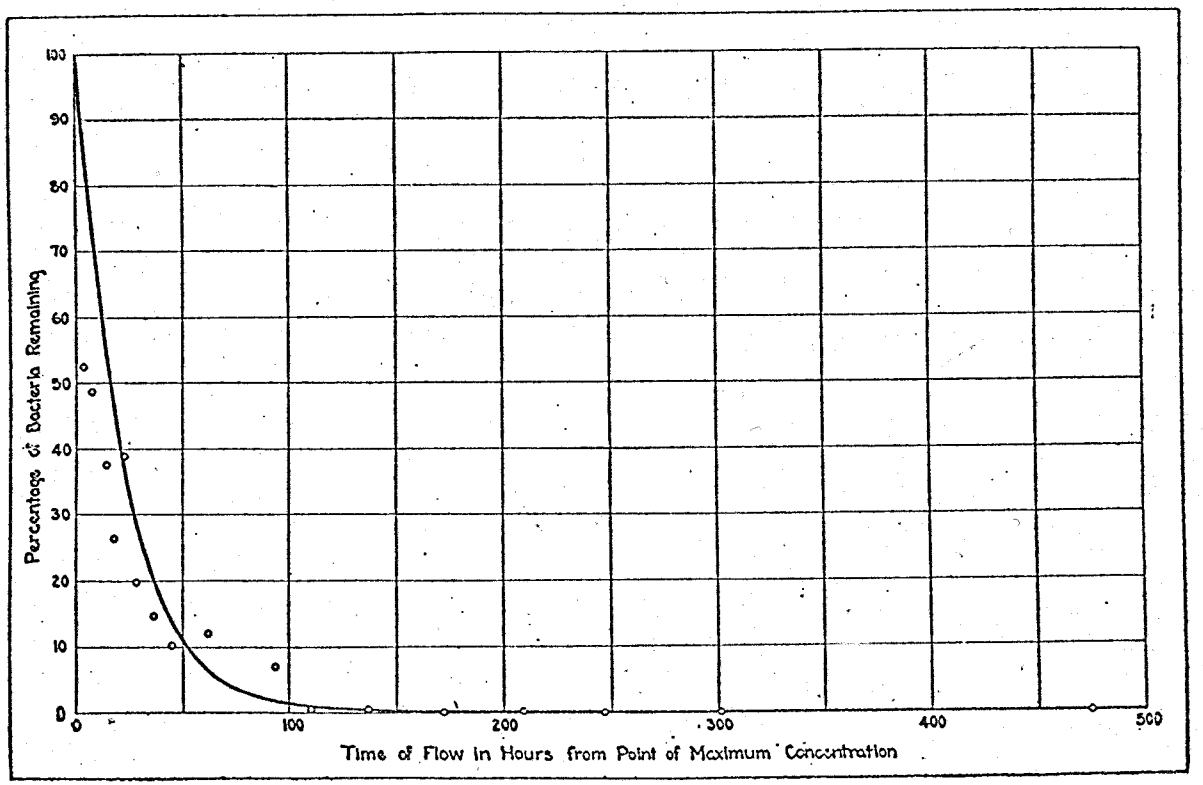

Figure 7
Bacterial purification in the Ohio River between Cincinnati and Louisville in relation to time of flow from zone of maximum pollution. B-coli Counts. after Frost

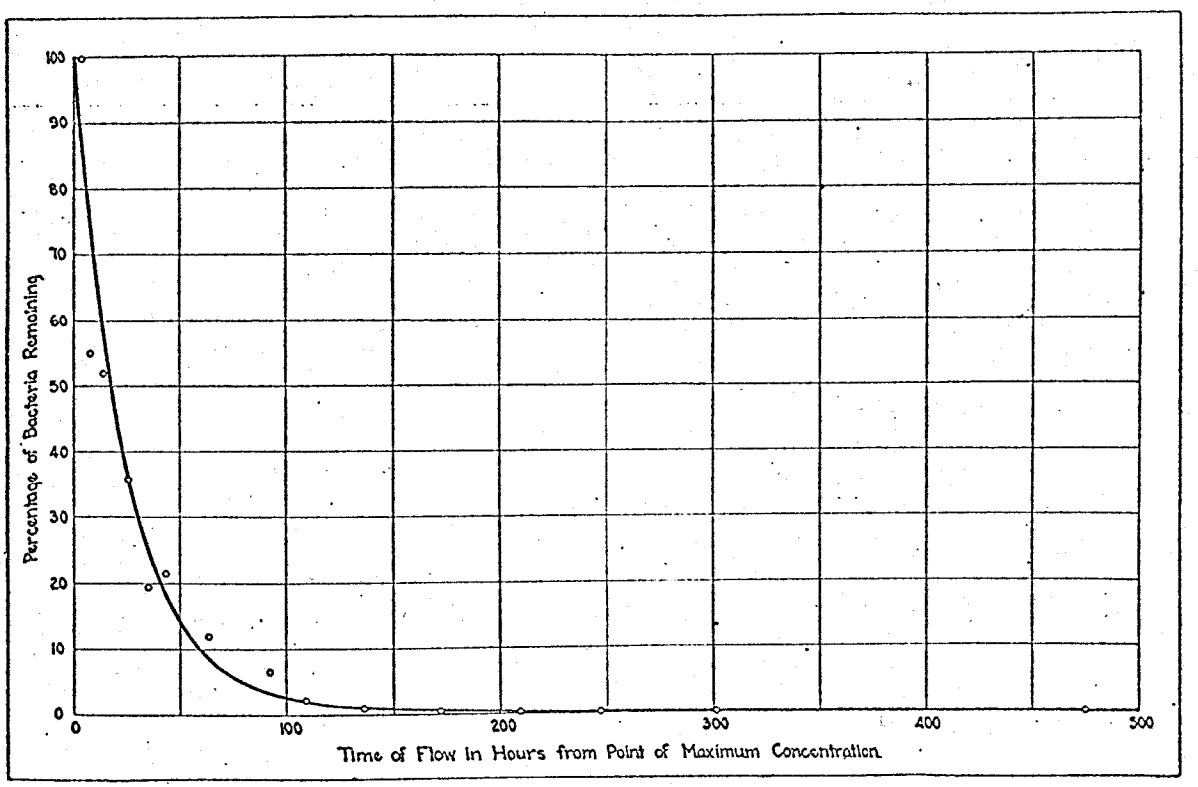

Figure 8
Bacterial purification in the Ohio River between Cincinnati and Louisville in relation to time of flow from zone of maximum pollution. Agar Counts after Frost

that the bacteria remaining after 183 hours were only one percent of the maximum; and after 315 hours were less than one-tenth of one percent (9, p. 288).

When the logarithm of the observed bacterial counts were plotted versus time of flow, they were found to lie along two straight intersecting lines. The formulation of empirical formulae to fit such an arrangement has already been discussed (see figure 4).

Another observation made by Frost in conjunction with the Ohio River study was the fluctuation in bacterial counts added to the stream by sewage pollution from Cincinnati throughout the entire seasonal cycle. During periods of high temperatures, i.e. the summer months, the bacterial numbers seemed to be considerably higher than those during the winter. This observation was suggested as a reason for the apparent multiplication of microorganisms, and may be summarized in the following statement.

> A quite unexpected and hitherto unnoted phenomenon has been shown, namely a great increase in the bacterial evidence of pollution in the warmer months. This effect is shown so consistently in the work of several laboratories, and upon various river, that there can be no doubt of its reality. It is hardly to be believed that there is actually multiplication of the intentinal organisms in the streams themselves, although this possibly cannot, with our present knowledge, be entirely eliminated. It is more probable that the bacterial content of the sewage

> shows a seasonal variation. Whether this be traceable to actual multiplication of intestinal bacteria within the sewers or to a greater per capita discharge of these organisms in the summer months cannot be stated (24, p. 209).

Illinois River. In 1921 the U. S. Public Health Service undertook an investigation of the Illinois River to study pollution and natural purification. Broad objectives of the study were: 1) to develop practical procedures for the measurement of stream pollution and suitable forms for expression of the degree of pollution encountered; 2) to ascertain the probable effects to be anticipated from increasing pollution loads and to determine the power of the streams to recover from such imposed burdens, throughout the operation of natural agencies; and 3) to observe the effects of stream pollution on the public health, as reflected in the quality of water supplies, procurable from polluted sources and as influenced by methods of removal and disposal of domestic sewage and industrial wastes (12, p. XI).

The Illinois River was selected for intensive study for several reasons. The stream, approximately 300 miles long, is excessively polluted at its source by the sewage flow of the City of Chicago. Comparatively small amounts of additional pollution are added throughout its remaining reaches, so that it is well adapted to a study of the natural agencies active in stream purification.

The study procedure followed very closely to that of the Ohio River Study.

Studies of the Illinois River further substantiated the results and conclusions made concerning the Ohio River study. The marked seasonal variation in bacterial counts was observed in both studies.

Due to the sewage pollution of Peoria, the Illinois River was divided into the upper and lower river. With this division in mind, there was a progressive decline in all bacterial counts below the outfalls of Chicago and Peoria. It was possible to represent average rates of purification by smooth curves which most nearly fit actual results. These curves are justified only because they constitute a convenient method of presenting the general rates of purification as defined by the considerable mass of data (12, p. 165).

Others. Many additional studies have been made on rivers throughout the country. The Potomac, Genesee, and Cowseeset Rivers are only a few (31, p. 347-357). All seem to show the rather orderly trend of bacterial decrease, but with environmental factors causing variation in relative numbers.

Comparison of Studies. Studies of natural purification of the Ohio River indicated that changes in the bacterial content between Cincinnati and Louisville were quite orderly and that rates of decrease could be represented in a general way by empirical formulae. Similar observations on the Illinois River have tended to confirm this conclusion and have indicated that such changes may be of rather general occurence, rather than confined to these two streams.

A comparison of the curves defining the rates of bacterial purification in the upper and lower Illinois and of similar curves for the Ohio River shows that, starting at the point of maximum concentration on the two sets of curves, and disregarding the concentration of bacteria, the various rates of bacteria decrease are not comparable (12, p. 193).

The concentrations of bacteria and rates of decrease in the upper Illinois were much higher than those in the lower Illinois or in the Ohio. As may be seen in figure 9, there is considerable divergence in rates of decrease when all curves start at 100 percent of bacteria remaining at the point of maximum bacterial count. However, if the differences in bacterial density are taken into consideration and comparisons made at points of equal bacterial density, a closer correlation can be obtained. Such a comparison has been made in figure 10 (12, p. 194-195).

While such a comparison of these rates appears to indicate the varying effect of initial density upon velocity of decrease in bacteria, the coincidence of the curves thus adjusted is not especially striking. It is possible that the bacterial death rate in streams is a function of the initial density. The degree of bacterial self-purification, therefore, could be generally described by a family of curves, having their maximum points at a common origin with respect to time and at ordinates corresponding to the bacterial density at the maximum. For the

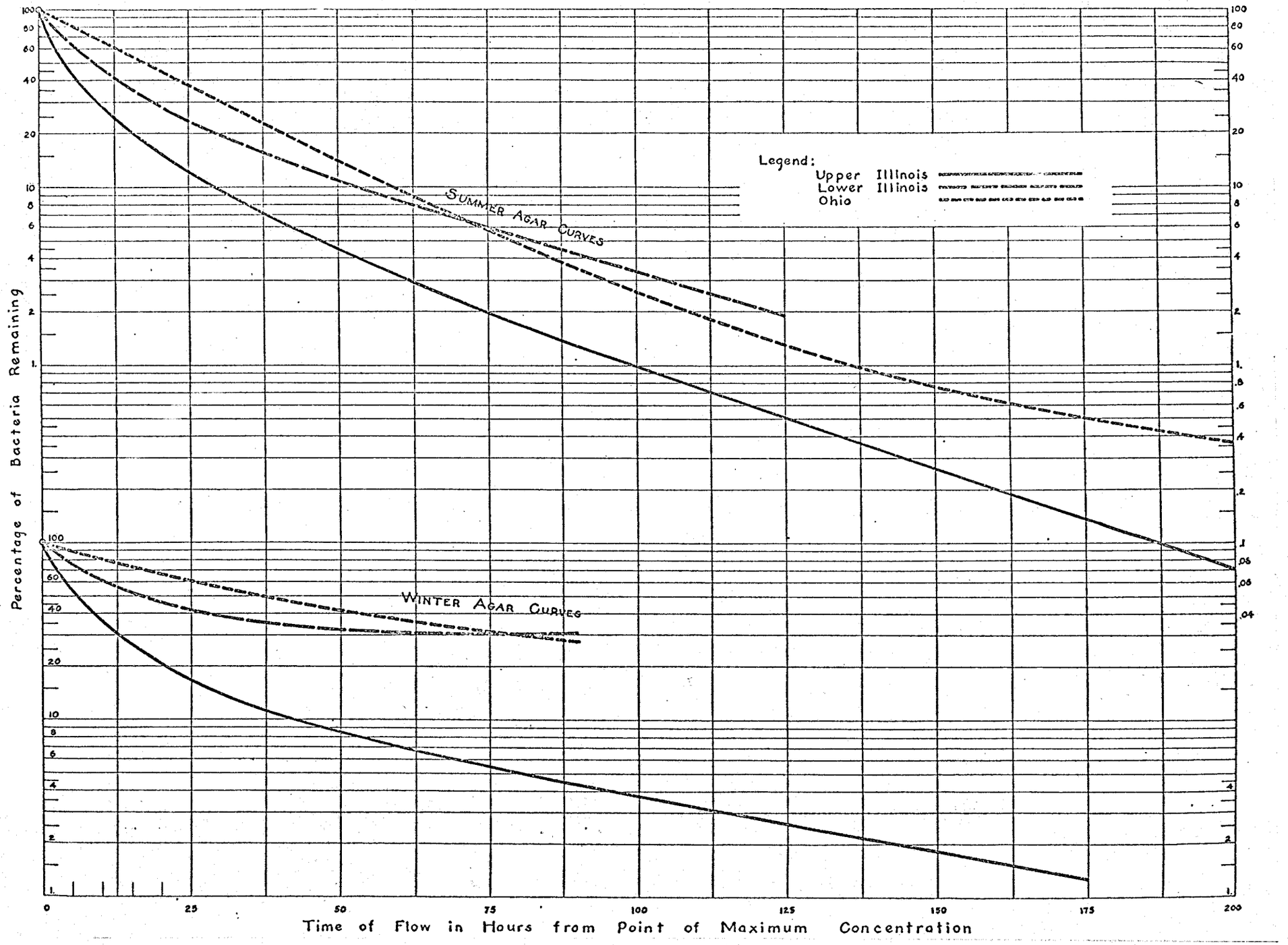

Figure 9
Comparison of rates of bacterial purification in the Ohio, upper Illinois, and lower Illinois Rivers in relation to time of flow from zones of maximum pollution
after Hoskins

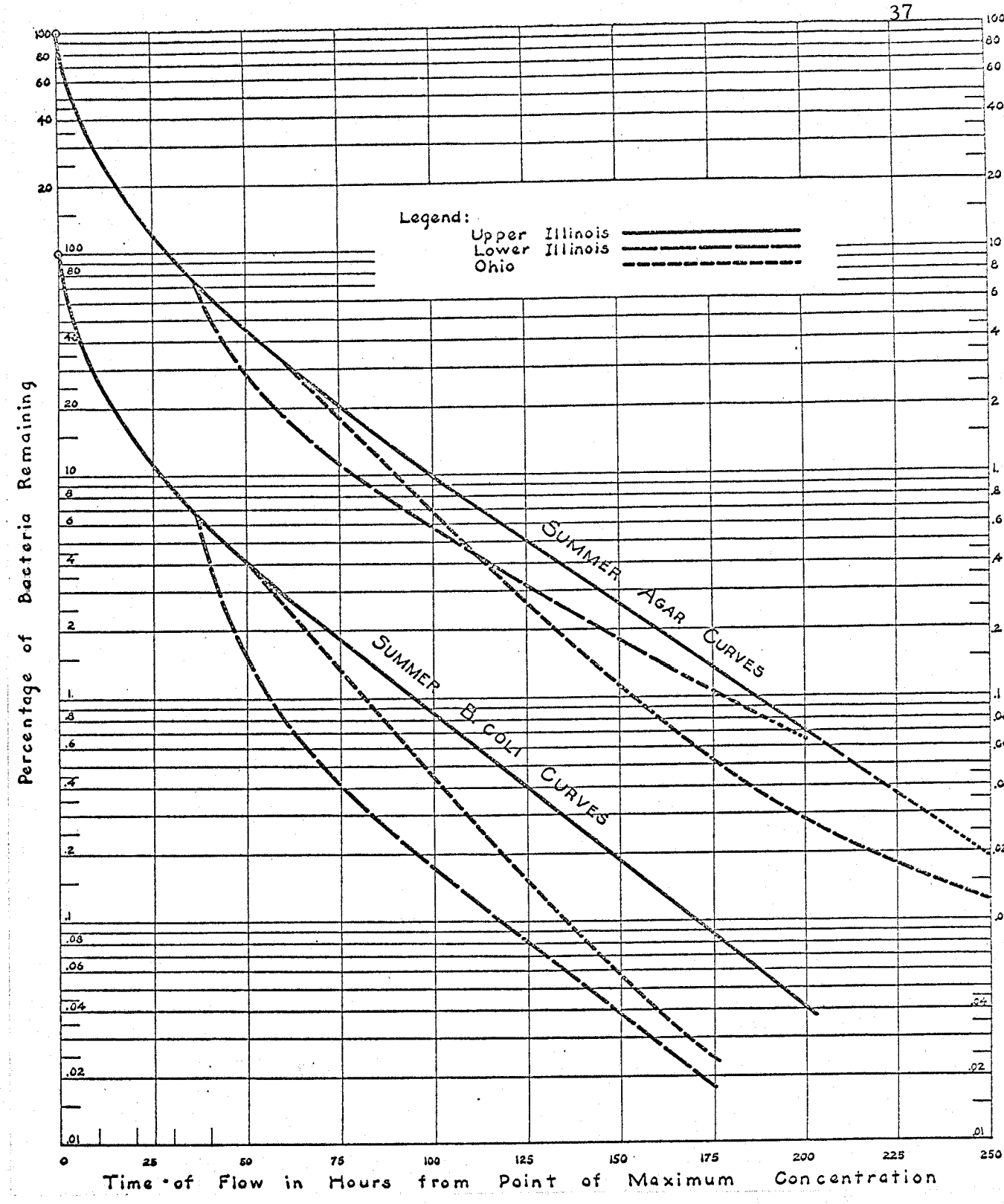

Figure 10

Comparison of rates of bacterial purification in Ohio, upper Illinois, and lower Illinois Rivers in relation to time of flow from zones of equal concentration. after. Hoskins

determination of such a family of curves, observations on a considerable number of streams would be necessary to verify the existence of this type of general law of bacterial purification.

It should be noted that the curve for winter conditions is much flatter than is that of summer, indicating that natural purification proceeds at much slower rates in cold weather. It is entirely possible therefore, regardless of the generally lower numbers of bacteria contributed during the winter season, that the critical or most severe period of bacterial pollution to be expected from a given sewage discharge upstream, may occur during the winter, rather than during the summer. This is, of course, the reverse of what would be expected of the critical oxygen depletion condition resulting from the same sewage pollution.

STUDY PROCEDURE

The study consists of the investigation of the bacterial self-purification capacity of a receiving stream. Mary's River was chosen due to its convenient location and size. The source of bacterial pollution is the effluent from the City of Philomath sewage treatment plant. The study procedure is divided into three parts. These are: 1) the investigation of the receiving stream; 2) sampling procedure; 3) the measurement of bacterial densities. Unless otherwise stated, all tests followed the standard procedures set forth in Standard Methods for the Examination of Water and Wastewater (1).

Investigation of Receiving Stream

Mary's River is located in the east central portion of Benton County. It flows in a south easterly direction from the foothills of the Coast Range approximately 35 miles to where it joins the Willamette River near Corvallis. In its upper reaches the Mary's River is relatively unpolluted, receiving bacterial contamination from surface run-off and septic tank effluents drained into the soil. The City of Philomath discharges its treated sewage into the Mary's River. This seems to be the river's major source of pollution, except for small tributaries near Corvallis.

The portion of the river used in the determination of bacterial

die-away extends from a point one-half mile upstream from the City of Philomath sewage treatment plant outfall to a point eight miles downstream. This portion of the stream is shown in figure 11. Ten sampling points were chosen with due regard for convenience. As can be seen, they are located at bridges or easily accessible locations.

Fortunately there is a river stage gage located near the middle of the reach of the stream desired. To adjust the bacterial counts for dilution, gage levels and stream discharges were obtained from the U. S. Geologic Survey for the days of a test run.

Sampling Procedure

Samples for bacterial examination were collected in 1000 milliliter sample bottles which had been thoroughly cleansed and sterilized. The assumption that the stream provided adequate mixing of the treatment plant effluent was necessary to reduce the number of samples from any cross section to a number that would provide a representative sample, but still reduce the time required for testing to a minimum. Examination of the stream deemed this assumption to be quite logical due to the size and velocity of the stream flow.

Samples were, in all cases, taken midstream from a convenient bridge or by wading into the stream itself. The bottle was held one to two feet below the surface of the water. An ample air space was left in the bottle to facilitate mixing of the sample preparatory to testing.

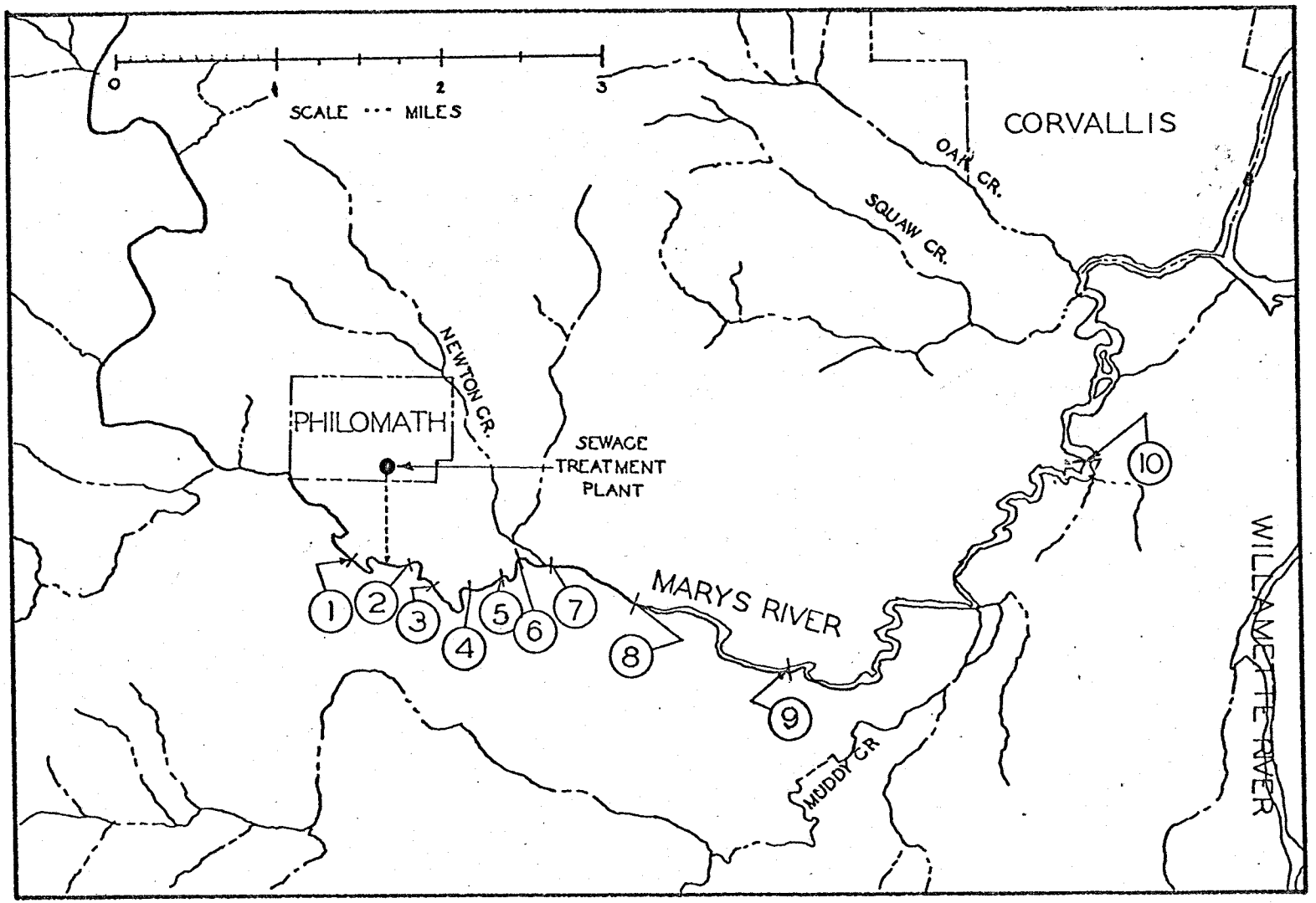

Figure 11
General area map showing location of sampling stations.

Care was taken to assure that no contamination from an outside source could occur prior to examination. All samples were marked and identified at the time of withdrawal from the stream.

Because the samples could not be examined immediately, they were stored in a dark refrigerator at a temperature between 0°C and 5°C to inhibit any growth. Care was taken not to freeze the samples. All samples were examined within 36 hours of the time of collection.

Measurement of Bacterial Densities

Bacterial densities of Escherichia coli and fecal streptococci were measured by means of membrane filters manufactured by the Millipore Filter Corporation (23, p. 6-8). Pre-sterilized disposable plastic petri dishes, membrane filters, and absorbent pads were used. The conventional pour plate method was used in the determination of the total count.

The nutrient media for E-coli density was the modified Endo media (M-Endo) which is specific for coliform organisms (26, p. 47 and 23, p. 6 and 1, p. 488). K F Streptococcus Broth was used in the enterococci determination (6). This media is specific for enterococci, inhibiting all others (15, p. 1553 and 18, p. 873-379). The general purpose media, nutrient agar was used in the total count test (1, p. 486). The above media were prepared by reconstitution of dehydrated media and sterilized according to the recommendations of the manufacturer.

The membrane cultures of the E-coli tests and total plate counts were incubated for 24 ± 2 hours; the enterococci membrane culture for 48 ± 2 hours. All cultures were incubated at 35°C in an inverted position with 100 percent humidity.

The E-coli colonies were red or pink and had a metallic sheen on the surface when studied under reflected light. This sheen either covered the entire colony or appeared only in the center. Non-coliform colonies also ranged from colorless to pink but did not have the characteristic sheen. Most fecal streptococcus colonies were 0.5-2mm in diameter and pale pink to dark red in color. Some streptococcus colonies, however, appeared to be colorless. All colonies grown on the general purpose media for the total count were tallied.

The colonies were counted with the aid of a Quebec Colony Counter and reported as colonies per 100 milliliters.

RESULTS

Bacterial Die-off in Mary's River

To measure the bacterial density of Mary's River at different points, samples were drawn and tested in triplicate for the organisms Escherichia-coli and fecal streptococci, and the total number of viable cells. The averages of these triplicate counts are plotted versus miles downstream from sampling station No. 1, which is located approximately one-half mile upstream from the sewer outfall. The locations of the sampling stations are illustrated in figure 11.

As shown in the first three test runs, figures 12-14, a die-off is indicated between the point immediately below the outfall and a point at river mile two. However, when additional samples were taken at points between these two sampling stations, a definite rise in bacterial density proceded the die-off. This is shown in figures 15 and 16. The peak density was found to be one to two miles downstream from the sewer outfall.

In all curves, except test run No. 1, the same trend was noted. This consisted of a second rise in bacterial density in the vicinity of river mile three. After this second peak, the die-off was distinct and followed a rather definite pattern.

The initial peak could be attributed to either actual bacterial

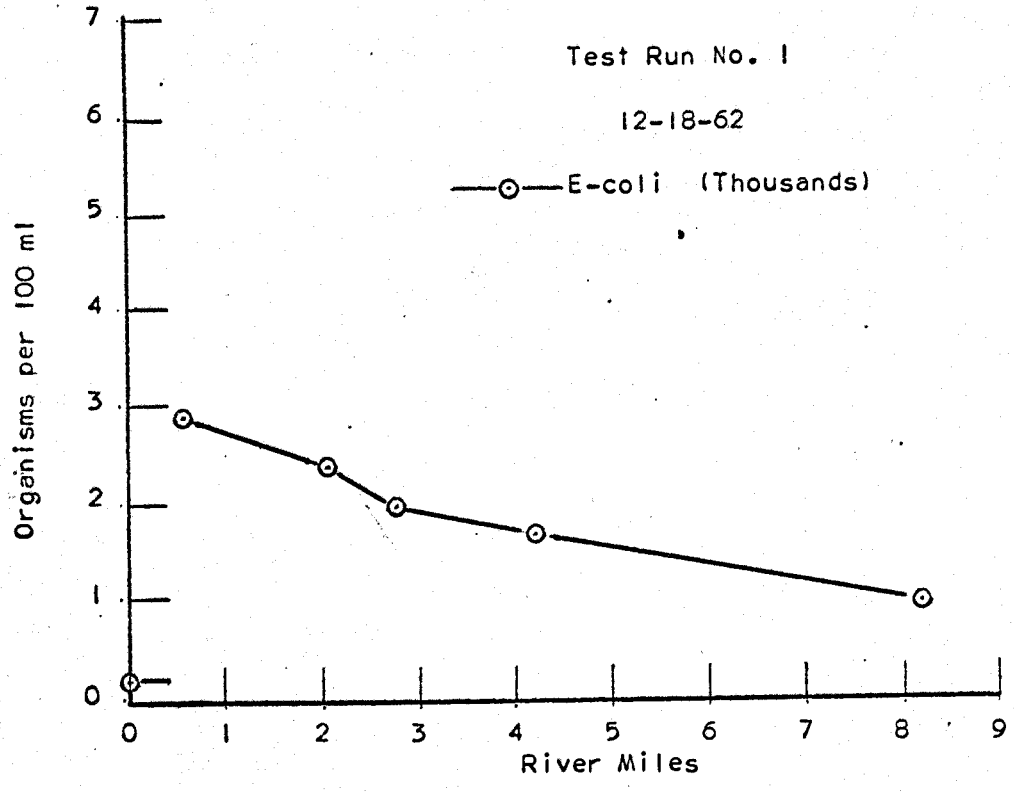

Figure 12

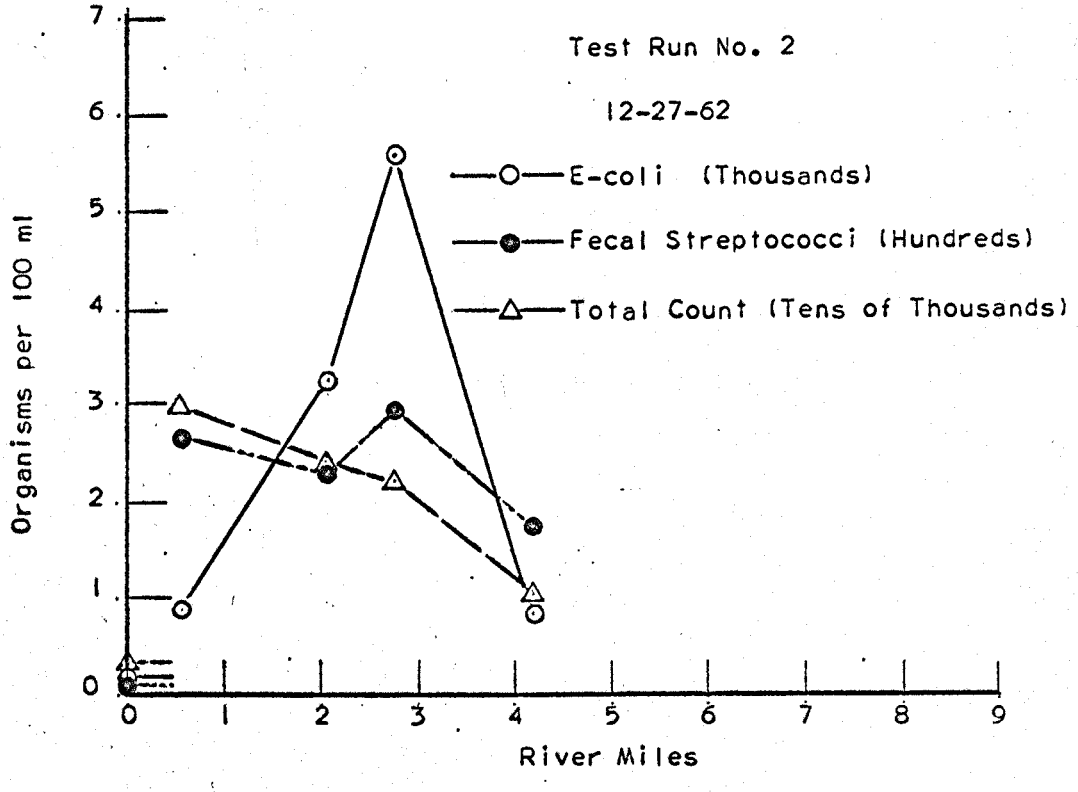

Figure 13

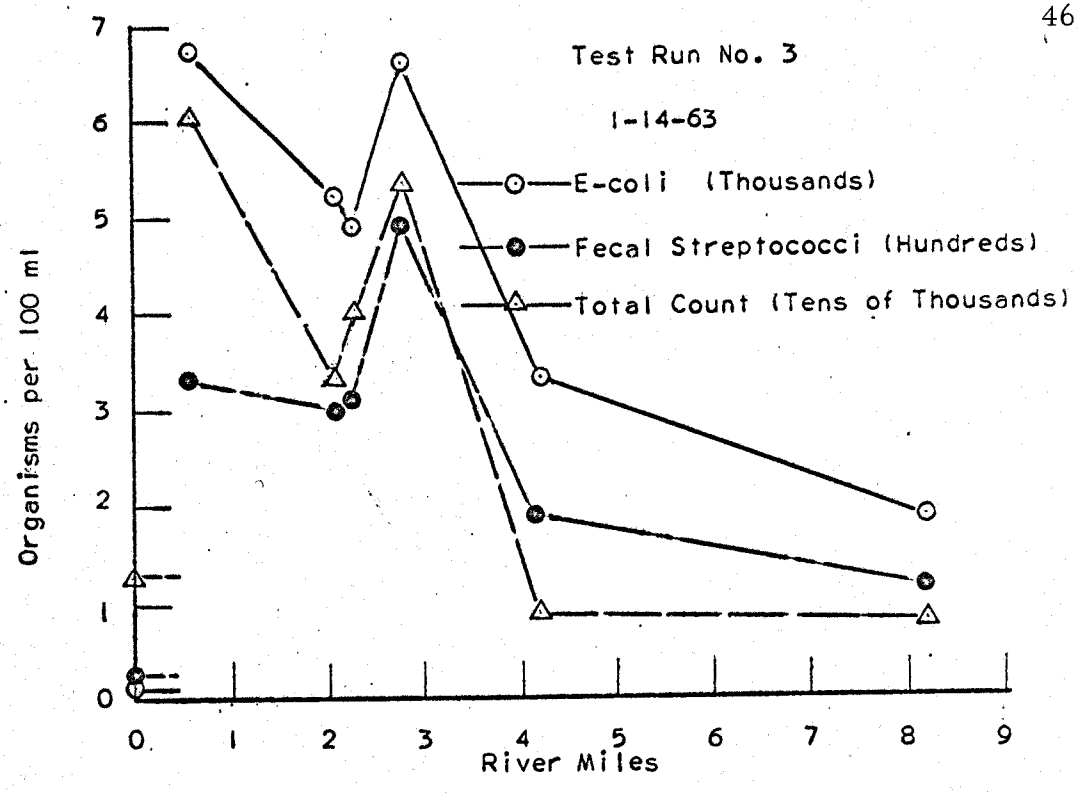

Figure 14

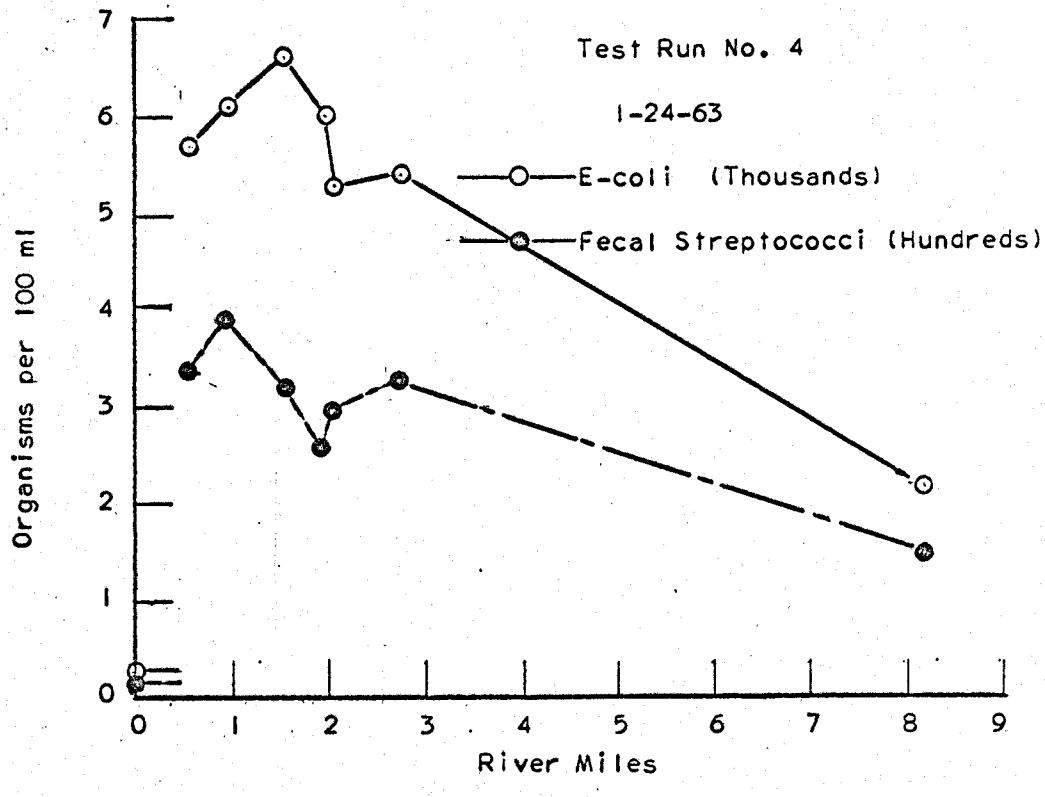

Figure 15

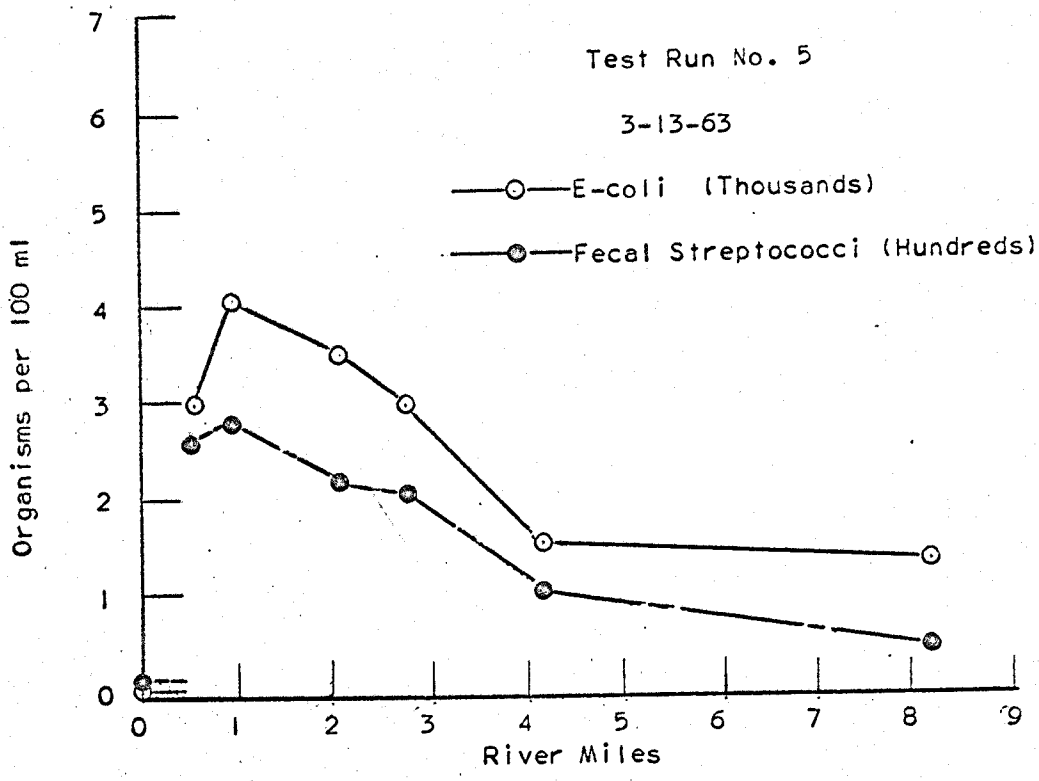
Test Run No. 5
3-13-63
E-coli (Thousands)
Fecal Streptococci (Hundreds)
Organisms per 100 ml
River Miles
0
1
2
3
4
5
6
7
8
9

Figure 16

multiplication, the breaking up of clumps containing bacteria, or to a mixture of both. The second peak could be due to the inflow of Newton Creek, which flows along the outskirts of Philomath. It is situated near many unsewered dwellings which discharge their wastes into septic tanks.

In order to adjust the bacterial counts for different river discharges each bacterial count was multiplied by the corresponding river discharge in hundreds of cubic feet per second. This gave a relative measure of the number of organisms passing the sampling station at the time of sampling. These values were then averaged for all five test runs. Figure 17 is a plot of these averaged values and describes the average conditions in Mary's River during the winter months. This curve also exhibits two peaks, one following the initial pollution at the sewer outfall, and the other downstream from the confluence of Newton Creek and Mary's River.

Results obtained from the E-coli, fecal streptococci, and total counts are in good agreement. During the first test run the results for the fecal streptococci and total count were invalidated due to errors in rehydrating the media. However, test runs two and three brought results that agreed in a qualitative manner. Changes in the bacterial count of one of the indicator organisms was accompanied by corresponding changes in the others. After test run three the total count was discontinued. In test runs four and five the E-coli and fecal

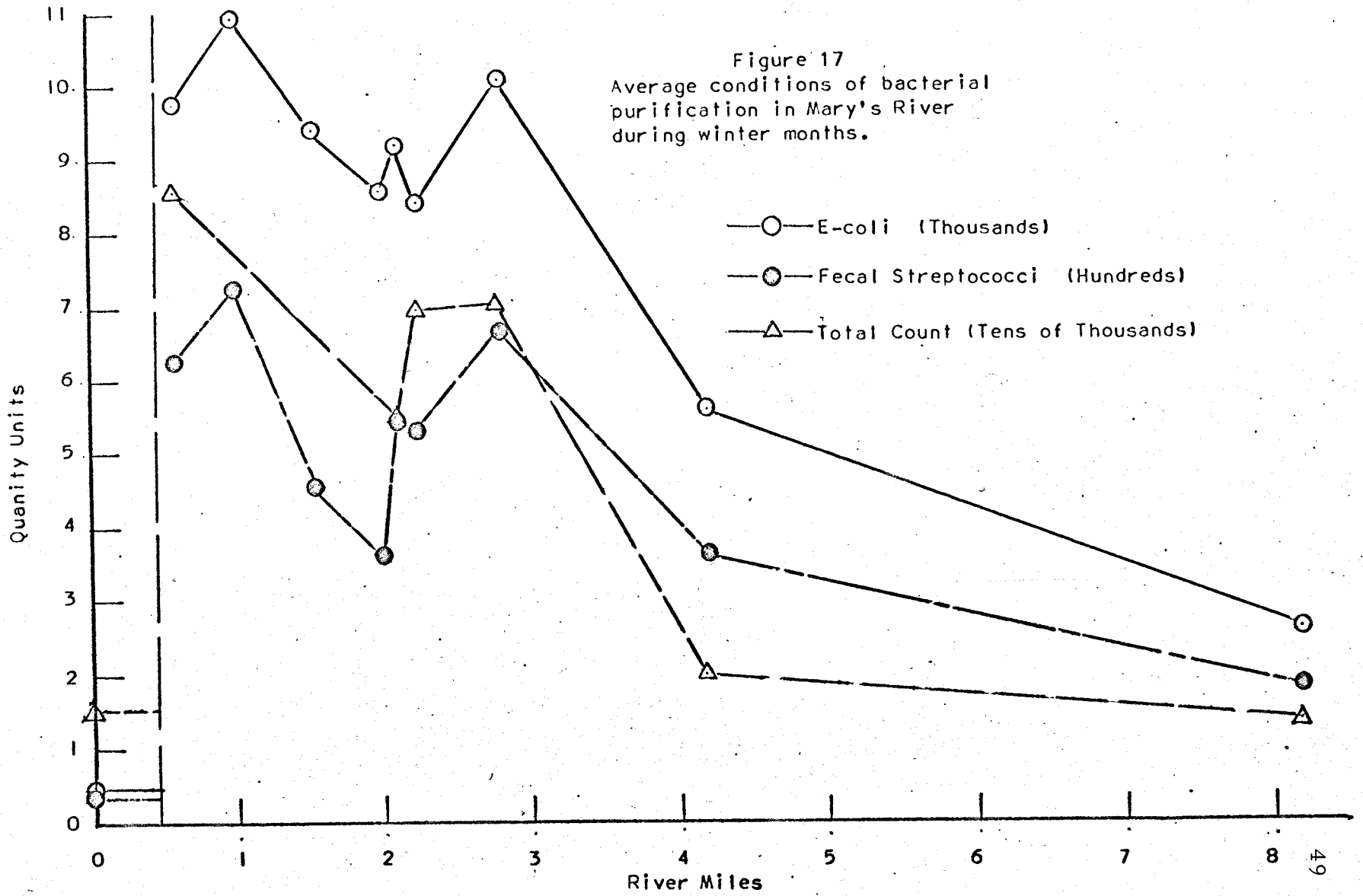

Figure 17
Average conditions of bacterial purification in Mary's River during winter months.

streptococci counts showed the same trends throughout the entire reach of the river.

A study was made to investigate the effects of temperature upon the die-off of bacteria. Two samples, taken from Mary's River just below the outfall, were incubated in dark refrigerator-incubators at 10°C and 20°C. Bacterial counts of E-coli and fecal streptococci were made over a period of 193 hours to determine the die-off. Figures 18 and 19 show that at the higher temperature the rate of bacterial die-off was greater for both E-coli and fecal streptococci. When these two figures are superimposed upon each other it can easily be seen that the E-coli die off at a greater rate than do the fecal streptococci.

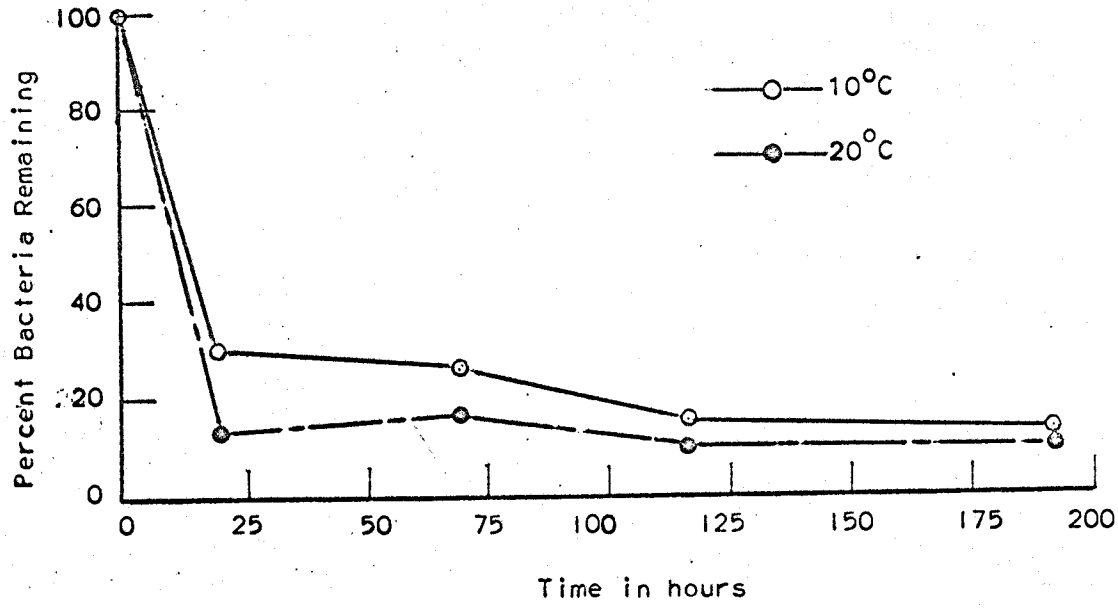

Figure 18

Die-off of E-coli in samples of
Mary's River water incubated at 10°C and 20°C

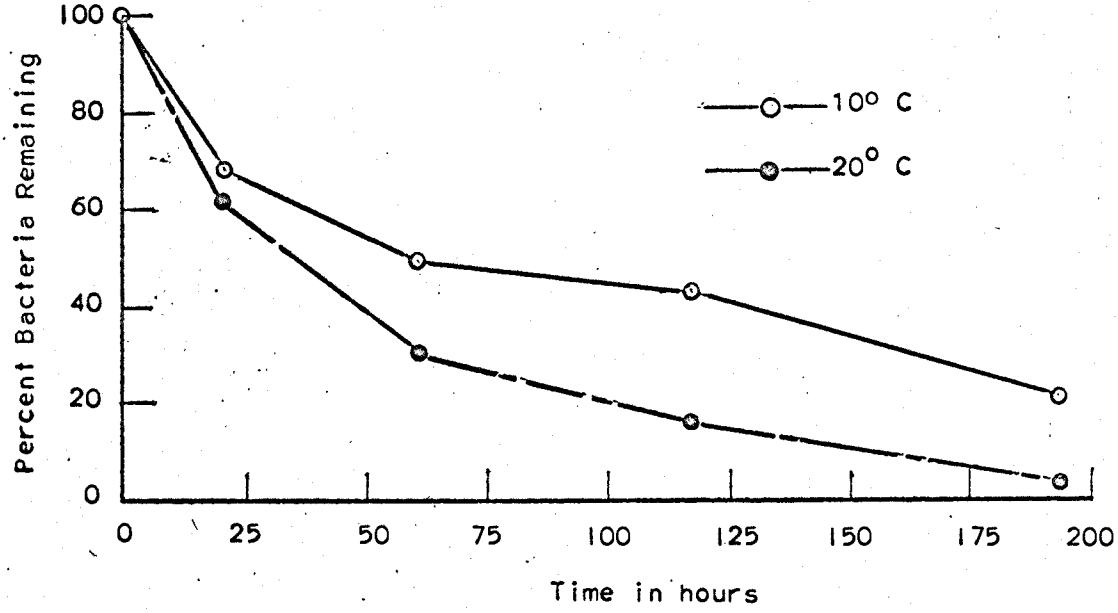

Figure 19

Die-off of fecal streptococci in
samples of Mary's River water incubated at 10°C and 20°C

PRECISION OF MEMBRANE FILTER TECHNIQUE

During one of the test runs, a statistical interpretation of the membrane filter data was made. Following the same procedure used for all tests a single sample was tested using ten separate filtrations for the Escherichia-coli count. Due to an experimental error only nine of the ten plates proved to be of value. From these nine the standard error was calculated and found to be 84 organisms per 100 milliliters. However, when divided by the dilution, the standard error was found to represent only four colonies on the petri dishes. In terms of percent of the mean count, the standard error was 11 percent.

Ten separate filtrations were made from another sample for the fecal streptococci counts. The standard error of these ten plates was found to be two organisms per 100 milliliters, representing only two colonies on the petri dishes. In terms of percent of the mean count, the standard error was eight percent.

Many microbiologists feel reproducibility up to ten percent to be excellent, considering the errors which are inherent of the procedure itself.

To check for positiveness of the E-coli colonies, 20 fermentation tubes containing brilliant green bile were inoculated with bacteria from 20 different colonies showing the typical metallic sheen of the E-coli colonies. Also ten tubes were inoculated with bacteria from

atypical colonies present on the petri dishes. Of the 20 tubes inoculated with bacteria from the typical colonies, 19 produced gas within 48 hours. Of the ten inoculated with bacteria from the atypical colonies, none produced gas.

DISCUSSION OF RESULTS

In view of the data obtained from this study, the E-coli and fecal streptococci counts offered a good index of stream pollution. The bacterial counts show satisfactory relative agreement when compared. During the laboratory work, it was found that the E-coli colonies were the easier of the two to count. The colonies grown on the M-Endo media are dark and easily counted, due to the contrast in color. The fecal streptococci colonies, on the other hand, were from white to pale pink in color. This made them difficult to count, since the background color of the media itself was light pink. However, comparison of the two statistically showed that the fecal streptococci test offered better quantitative results. If some means of increasing the color contrast between the colonies and the media could be found, it would improve the ease in counting of the fecal streptococci colonies, and would probably improve the reliability. The membrane filter technique is, by far, the easiest method of determining the bacterial density when large numbers of samples are to be processed.

Although it is hard to estimate the exact importance of each factor affecting the bacterial purification capacity of a stream, the general phenomena is readily noticeable. It is no single agent which brings about self-purification, but a complex of little understood conditions which is called environment. If any one factor is of prime

importance, it is probably food supply, for nearly all bacteria are unable to multiply in the presence of small amounts of organic matter in ordinary potable water. Temperature, also, is probably a major factor, since it affects not only metabolic rates but the physical properties of the water itself. Of lesser importance are sedimentation and predatory action since conditions for these factors to be of importance exist only in deep sluggish streams with a relatively high degree of pollution.

It is obvious that the bacterial self-purification of streams follows a general trend of die-away. If the observations are representative of some general biological law, they could be of some practical value for estimating the increased burden placed on a stream receiving the sewage of a community, and consequently the added loads that water purification plants must be prepared to handle where such polluted water courses are used as sources of water supply. Before estimates of such general trends can be expected with complete confidence, it is necessary that observations upon a considerable number of streams of different physical characteristics be made and used to determine the influence of physical factors.

In developing a water-supply, this procedure might be employed to obtain a profile of bacterial count, either of E-coli or fecal streptococci. In this manner points of low bacterial count could be found and incorporated into the selection of the location of a water supply intake.

This may, of course, be in conflict with the other design factors of the intake, but a reasonable compromise could be made.

CONCLUSIONS

1. Both the Escherichia-coli and fecal streptococci counts yielded a good index of stream pollution, however, the E-coli colonies proved to be the easier of the two to count.

2. The membrane filter technique offered reliability and ease in counting for large numbers of samples.

3. Increased temperature caused increased bacterial die-off in incubated samples of Mary's River water.

4. For both 10°C and 20°C temperatures, the E-coli were noted to die-off more rapidly than the fecal streptococci.

5. In Mary's River, during the winter months, 64 percent of the E-coli died-off in a distance of five and a half miles. Within the same distance 71 percent of the fecal streptococci died-off.

RECOMMENDATIONS FOR FURTHER STUDY

The author recommends the following areas for further study:

1. The investigation of bacterial die-off during periods of low river discharge and summer temperatures.

2. The effects of chlorination of the sewage treatment to plant effluent upon bacterial die-off in Mary's River.

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APPENDIX

TABLE 1

Summary of bacterial counts of river test runs

TEST RUN NO. 1

Sampling Station	River Miles	Replications 1	2	3	Average
Escherichia-coli Counts*					
1	0.0	190	230	240	220
2	0.6	3300	4000	4400	3900
6	2.1	1700	3000	2600	2400
8	2.8	2000	1400	2500	2000
9	4.2	1300	1900	2000	1700
10	8.2	700	1200	1000	1000
Effluent		$15x10^5$	$14x10^5$	$20x10^5$	$16x10^5$
Control		0			

TEST RUN NO. 2

Sampling Station	River Miles	Replications 1	2	3	Average
Escherichia-coli Counts*					
1	0.0	220	220	200	210
2	0.6	860	1020	1000	950
6	2.1	2600	3500	3700	3300
8	2.8	5900	6700	4200	5600
9	4.2	810	970	900	980
Effluent		$27x10^5$	$31x10^5$	$26x10^5$	$28x10^5$
Control					
Fecal Streptococci Counts*					
1	0.0	5	7	4	5
2	0.6	240	350	220	270
6	2.1	290	180	230	230
8	2.8	320	370	200	300
9	4.2	170	180	180	180
Effluent		$18x10^4$	$22x10^4$	$17x10^4$	$19x10^4$

* All counts are in organisms per 100 milliliters.

TABLE 1

Summary of bacterial counts of river test runs.

Sampling Station	River Miles	Replication 1	2	3	Average
Total Counts*					
1	0.0	3500	4200	3500	3700
2	0.6	28000	31000	30000	30000
6	2.1	28000	15000	29000	24000
8	2.8	22000	19000	25000	22000
9	4.2	14000	16000	3000	11000
Effluent		47×10^5	57×10^5	20×10^5	41×10^5
Control		0			

TEST RUN NO. 3

Sampling Station	River Miles	Replication 1	2	3	Average
Escherichia-coli Counts*					
1	0.0	150	120	150	140
2	0.6	5500	8100	6400	6700
6	2.1	6200	4500	4900	5200
7	2.3	5400	4600	4800	4900
8	2.8	6200	7000	6700	6600
9	4.2	4600	4100	4200	4300
10	8.2	2000	1600	2100	1900
Effluent		23×10^5	22×10^5	----	23×10^5
Control		0			
Fecal Streptococci Counts*					
1	0.0	23	22	31	25
2	0.6	---	360	320	330
6	2.1	490	200	200	300
7	2.3	390	220	310	310
8	2.8	550	470	440	490
9	4.2	200	170	200	190
10	8.2	100	150	100	120
Effluent		23×10^4	27×10^4	21×10^4	24×10^4
Control		0			

* All counts are in organisms per 100 milliliters.

TABLE 1

Summary of bacterial counts of river test runs.

Sampling Station	River Miles	Replications 1	2	3	Average
Total Counts*					
1	0.0	17000	12000	10000	13000
2	0.6	74000	48000	57000	60000
6	2.1	36000	28000	35000	33000
7	2.3	29000	37000	54000	40000
8	2.8	59000	49000	50000	53000
9	4.2	8600	8900	8400	8600
10	8.2	7900	8500	7600	8000
Effluent		$27x10^5$	$35x10^5$	$26x10^5$	$29x10^5$
Control		0			

TEST RUN NO. 4

Sampling Station	River Miles	Replications 1	2	3	Average
Escherichia-coli Counts*					
1	0.0	240	320	230	260
2	0.6	6000	5100	5900	5700
3	1.0	----	5900	6200	6100
4	1.5	7600	5900	6400	6600
5	2.0	5900	5300	6800	6000
6	2.1	5400	5300	5300	5300
8	2.8	5200	6300	4800	5400
10	8.2	1600	2000	3100	2200
Effluent		$28x10^5$	$29x10^5$	$21x10^5$	$26x10^5$
Control		0			
Fecal Streptococci Counts*					
1	0.0	25	17	22	21
2	0.6	340	360	310	340
3	1.0	440	390	330	390
4	1.5	330	270	360	320

* All counts are in organisms per 100 milliliters.

TABLE 1

Summary of bacterial counts of river test runs.

Sampling Station	River Miles	Replications 1	2	3	Average
Fecal Streptococci Counts* (Cont'd)					
5	2.0	230	300	250	260
6	2.1	300	270	320	300
8	2.8	310	400	280	330
10	8.2	120	170	160	150
Effluent		31×10^4	24×10^4	26×10^4	27×10^4
Control		0			

TEST RUN NO. 5

Sampling Station	River Miles	Replications 1	2	3	Average
Escherichia-coli Counts*					
1	0.0	60	100	50	70
2	0.6	2900	3200	3000	3000
3	1.0	4600	3900	4000	4100
6	2.1	3600	3500	3500	3500
8	2.8	2900	----	3100	3000
9	4.2	1200	1900	1700	1600
10	8.2	1000	1700	1400	1400
Effluent		25×10^5	12×10^5	15×10^5	16×10^5
Control		0			
Fecal Streptococci Counts*					
1	0.0	17	21	14	17
2	0.6	300	250	240	260
3	1.0	300	250	280	280
6	2.1	200	300	170	220
8	2.8	210	200	180	210
10	8.2	53	47	50	50
Effluent		24×10^4	18×10^4	12×10^4	18×10^4
Control		0			

* All counts are in organisms per 100 milliliters.

TABLE 2

Summary of average bacterial counts.

Sampling Station	River Miles	Test Run No. 1	2	3	4	5
Escherichia-coli Counts*						
1	0.0	220	210	140	140	70
2	0.6	3900	950	6700	5700	4050
3	1.0	----	----	----	6100	4100
4	1.5	----	----	----	6600	----
5	2.0	----	----	----	6000	----
6	2.1	2400	3300	5200	5300	3500
7	2.3	----	----	4900	----	----
8	2.8	2000	5600	6600	5400	3000
9	4.2	1700	980	4300	----	1600
10	8.2	1000	----	1900	2200	1400
Effluent		16×10^5	28×10^5	23×10^5	26×10^5	16×10^5
Fecal Streptococci Counts*						
1	0.0	----	5	25	21	17
2	0.6	----	270	330	340	260
3	1.0	----	----	----	390	280
4	1.5	----	----	----	320	----
5	2.0	----	----	----	260	----
6	2.1	----	230	300	300	220
7	2.3	----	----	310	----	----
8	2.8	----	300	490	330	210
9	4.2	----	180	190	----	110
10	8.2	----	----	120	150	50
Effluent		----	19×10^4	24×10^4	27×10^4	18×10^4
Total Count*						
1	0.0	----	3700	13000	----	----
2	0.6	----	30000	60000	----	----
3	1.0	----	----	----	----	----
4	1.5	----	----	----	----	----
5	2.0	----	----	----	----	----
6	2.1	----	24000	33000	----	----
7	2.3	----	----	40000	----	----
8	2.8	----	22000	53000	----	----
9	4.2	----	11000	8600	----	----
10	8.2	----	----	8000	----	----
Effluent		----	41×10^5	29×10^5	----	----

* All counts are in organisms per 100 milliliters.

TABLE 3

Summary of adjusted bacterial counts of river test runs.

Sampling Station	River Miles	Test Run No. 1	2	3	4	5	Adjusted Average
Escherichia-coli Counts*							
1	0.0	972	475	239	360	224	454
2	0.6	17200	2150	11450	8100	9600	9700
3	1.0	----	----	----	8650	13100	10900
4	1.5	----	----	----	9350	----	9350
5	2.0	----	----	----	8520	----	8520
6	2.1	10600	7460	8900	7520	11200	9140
7	2.3	----	----	8380	----	----	8380
8	2.8	8840	12650	11300	7670	9600	10010
9	4.2	7500	2220	7350	----	5120	5550
10	8.2	4420	----	3250	2020	4510	3550
Fecal Streptococci Counts*							
1	0.0	----	11	43	30	54	35
2	0.6	----	610	564	483	831	622
3	1.0	----	----	----	554	895	725
4	1.5	----	----	----	454	----	454
5	2.0	----	----	----	359	----	359
6	2.1	----	520	513	426	705	541
7	2.3	----	----	530	----	----	530
8	2.8	----	678	838	469	672	664
9	4.2	----	407	325	----	352	361
10	8.2	----	----	205	213	160	193
Total Counts*							
1	0.0	----	8400	22000	----	----	15200
2	0.6	----	67800	102500	----	----	85150
3	1.0	----	----	----	----	----	----
4	1.5	----	----	----	----	----	----
5	2.0	----	----	----	----	----	----
6	2.1	----	54300	56400	----	----	55350
7	2.3	----	----	68400	----	----	68400
8	2.8	----	49700	90600	----	----	70150
9	4.2	----	24900	14700	----	----	19800
10	8.2	----	----	13700	----	----	13700

* All counts are in quantity units.

TABLE 4

Summary of data for river and effluent.

	Test Run No.				
	1	2	3	4	5
Date	12/18/62	12/27/62	1/14/63	1/24/63	3/13/63
River Temperature	9°C	2°C	0°C	0°C	6°C
Effluent Discharge in mgd	0.305	0.124	0.126	0.109	0.117
Gage Height in feet	6.000	4.75	4.44	4.00	5.35
River Discharge in cfs	442	226	171	142	320
Computed bacterial count immediately downstream from the outfall sewer in organisms per 100 ml					
Escherichia-coli	1700	2620	2780	3340	980
Fecal Streptococci	--	170	300	341	120
Total Count	--	7200	15300	--	--

TABLE 5

Summary of Bacterial Counts in Incubated Samples.

Time	Replications 1	2	3	Average	Per cent Bacterial Remaining
Fecal Streptococci counts					
10°C					
0	390	300	320	336	100.0
20	210	200	280	230	68.5
70	152	178	170	166	49.5
117	151	144	135	143	42.5
193	67	66	72	68	20.3
20°C					
0	340	360	300	353	100.0
20	330	140	150	206	62.0
70	94	86	122	102	30.6
117	See Statistical Interpretation			52	15.7
193	8	8	5	7	2.1
Escherichia-coli counts					
10°C					
0	5100	4900	4700	4900	100.0
20	1400	1700	1500	1530	31.8
70	1380	1440	1020	1280	26.1
117	See Statistical Interpretation			762	15.6
193	720	640	590	650	13.3
20°C					
0	6700	6000	7100	6600	100.0
20	500	700	1500	900	13.6
70	960	1220	1060	1110	16.8
117	695	610	705	670	9.9
193	660	720	650	670	9.8

TABLE 6

Statistical interpretation of membrane filter data.

Replications	Escherichia-coli Counts*	Fecal Streptococci Counts*
1	680	40
2	790	24
3	770	24
4	680	24
5	780	25
6	920	21
7	740	23
8	840	29
9	---**	34
10	660	18
Mean Count	762	26
SS	56778	370
s^2	7097	4
Standard Error, s	84	2
Percent of Mean Count	11%	8%

* All counts are in organisms per 100 milliliters

** Missing observation due to experimental error.