RELATIONSHIP BETWEEN *ESCHERICHIA COLI*, TYPE I, COLIFORM AND ENTEROCOCCI IN WATER

By N. Bruce Hanes,* G. A. Delaney** and C. J. O'Leary***

(Presented at a meeting of the Sanitary Section, B.S.C.E., on March 3, 1965.)

Introduction

Various methods have been used to evaluate the bacteriological condition of a stream. Total plate counts on gelatin or nutrient agar media will indicate a change in condition in the stream, but do not determine whether this change is a result of fecal contamination. Further, the total plate count does not differentiate between harmless and potentially dangerous bacteria. Another method of evaluation which could be utilized would be to test for pathogens. This would be the most direct method, but suffers from serious drawbacks. The number of different analyses required to test for various pathogens would be sizeable and the test difficult. Secondly, it is still possible that some pathogens may be missed even with a large number of tests. In addition, it is extremely dangerous for relatively untrained personnel to work with pathogenic bacteria.

The most satisfactory method for bacterial evaluation of a water, to date, is through the use of an indicator organism. Ideally, indicator bacteria should be harmless to man, simple to identify, not present in natural water, found only in fecal material, and have a greater survival time in water than enteric pathogens. According to Mallman and Litsky (1), 1951, only two groups of bacteria found in fecal material are suitable as indicators, namely the coliform and the enterococci groups.

At the present time, the coliform group of microorganisms is used as the standard indicator of fecal contamination in this country. In particular, this group has proved satisfactory in the evaluation of potable water. While the coliform group possesses most of the advantages of an "ideal" indicator, it suffers from two serious dis-

* Assistant Professor, Department of Civil Engineering, Tufts University, Medford, Mass.
** Graduate Student, Tufts University, Medford, Mass.
*** Engineer, Massachusetts State Department of Health.

advantages which influence its use in stream pollution work. Members of the coliform group are ubiquitous in nature and may be found naturally on plants and may also be found in the soil. As a result, the presence of the coliform group in water does not necessarily indicate fecal contamination. Secondly, under certain conditions, members of the coliform group may multiply in water.

For many years the enterococci group has been periodically investigated as an indicator of fecal contamination, but until recently, was not thought to be of importance because of difficulty in testing, and their comparatively small numbers in sewage. Recent work, using sodium azide as an inhibitor, has developed a simple test for the enterococci. The results of these studies indicate the group is present in sewage in numbers approaching the coliform organisms. It has also been demonstrated recently that the enterococci do not multiply in water and have a more predictable death rate than the coliform group. At the 61st Annual Meeting of the Society of American Bacteriologists, Heukelekian and Rosenberg (2) stated:

"Until a more specific analysis for *E. coli* is perfected, the enterococcus count appears to be the most easily performed and reliable method for determination of fecal pollution."

Recently, a practical method was developed for rapid determination of the number of *E. coli*, Type I, bacteria present in water (3). As a result of this finding, attention has been focused on the use of *E. coli* as an indicator of fecal contamination.

The purpose of this paper is twofold. First, to establish if a definite relationship exists between these indicator bacteria, and secondly, if these relationships can be used to determine if the source of indicator bacteria is human fecal material.

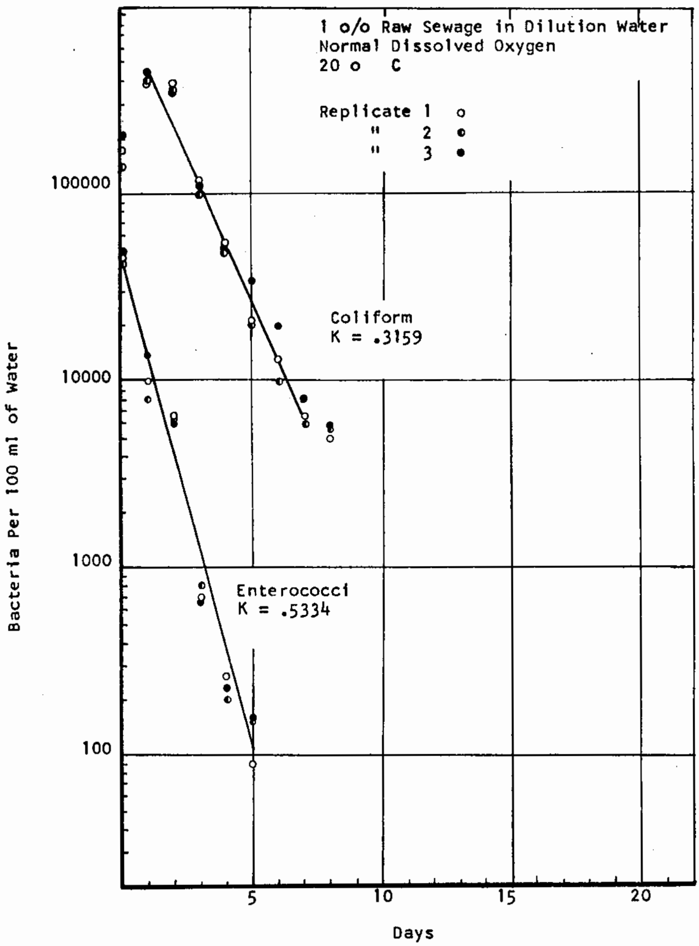
Relationship Between Indicator Bacteria

In the past, several attempts have been made to establish a relationship between the number of *Escherichia coli* in water and the number of enterococci. Of these studies, only two went into the problem with any depth. The first was by Lattanzi and Mood in 1951 (4) where they reported that a straight line relationship exists between *E. coli* and enterococci on coordinate paper. The samples were collected from ten stations in the New Haven, Connecticut, Harbor. A positive correlation of +.70 between these data and the straight line was reported.

The second study was by Litsky, et al., 1955 (5), and reported that a straight line relationship existed between *E. coli* and enterococci on log-log paper. His data showed a positive correlation of +.84. These samples were collected from fourteen stations on the Connecticut River over a period of two years. As a result of these two reports, the question arises which relationship, coordinate or log-log, provides the greater degree of accuracy.

If a point source of indicator bacteria is assumed in a river, which

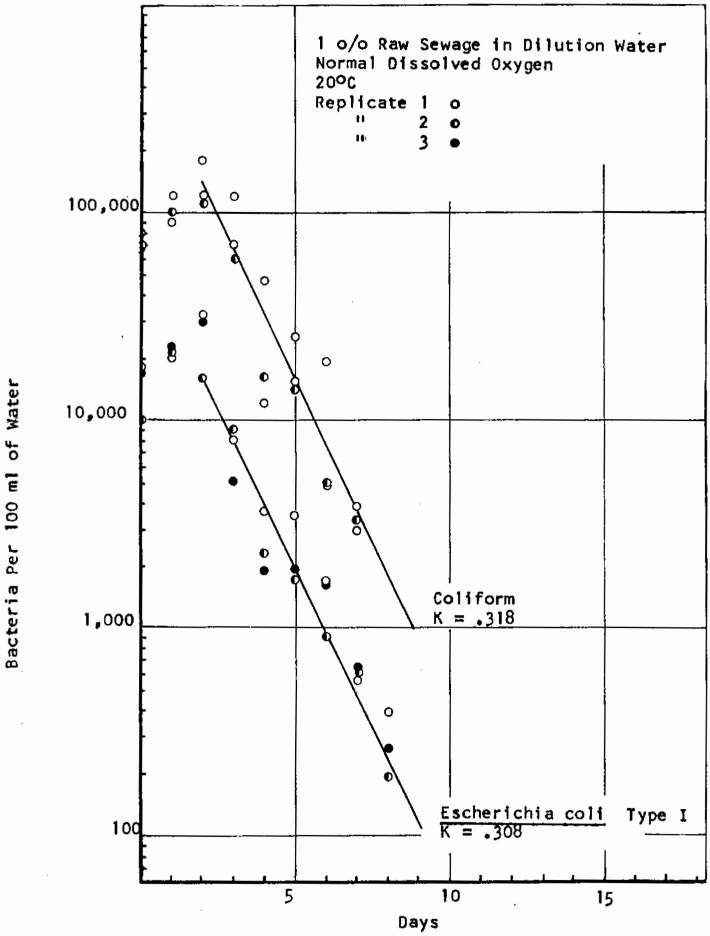
FIGURE I



could be the case, a laboratory study of survival of these bacteria in water would show which relationship would be the most likely to be correct.

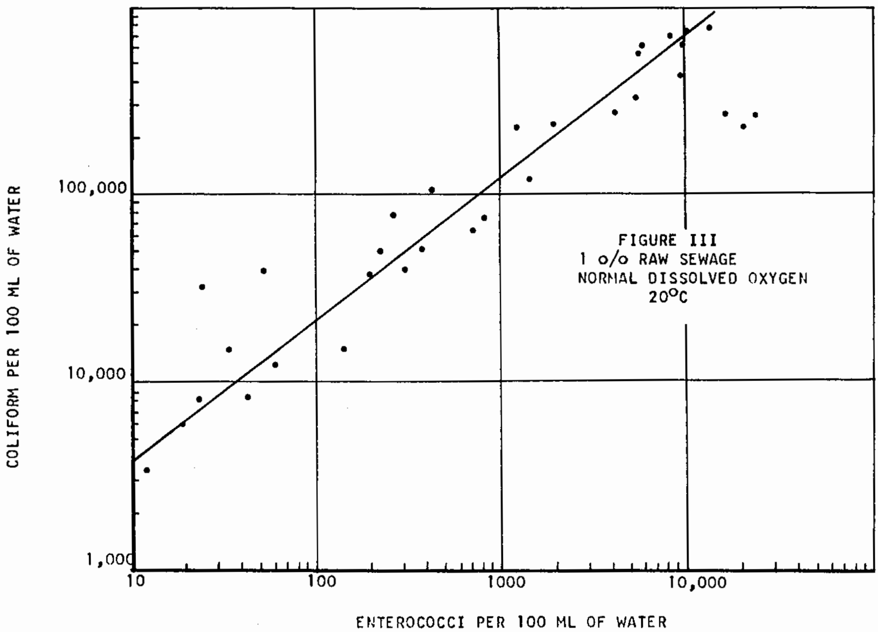
The results of a study of this type by Hanes et al., 1964 (6) indicated that under laboratory conditions the coliform group experiences a period of growth before they go into a death phase while no such period of growth is evident for the enterococci. Also, a well defined logarithmic death phase was demonstrated for both groups of

FIGURE II



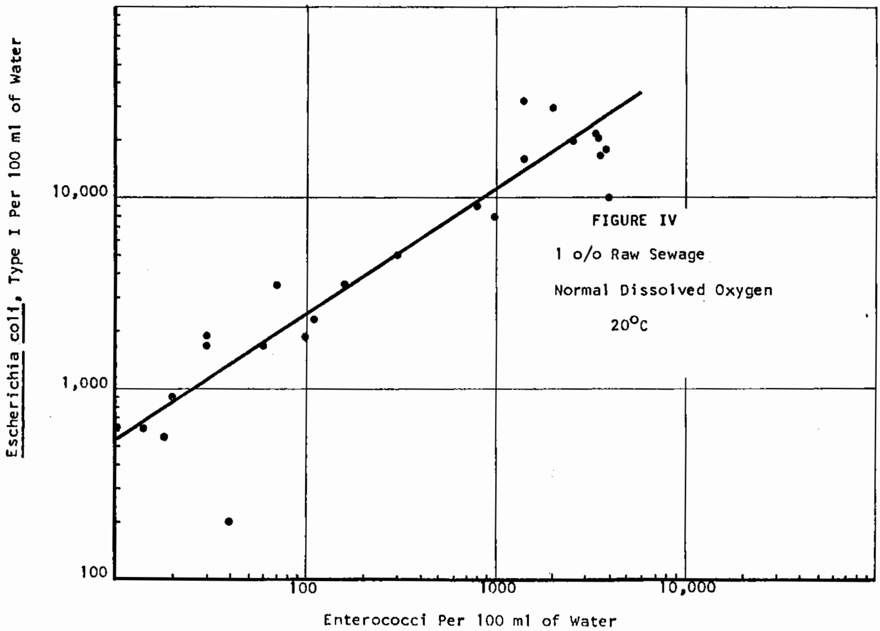
indicator bacteria (Fig. 1). It has since been demonstrated by O'Leary, 1965 (7), that *Escherichia coli*, Type I, demonstrates a survival curve in water that is very similar to that of the coliform group (Fig. 2).

It can be shown, theoretically, that if both the enterococci and the coliform or *E. coli*, Type I, exercise only logarithmic death, that is, their numbers decrease as a straight line on semilog paper, then



when either coliform or *E. coli*, Type I, are plotted against the enterococci on log-log paper, the mathematical relationship would always be lineal. This would not be true when the data is plotted on coordinate paper except for one special case which occurs when the slope of the log-log plot is unity. The data presented in Figs. 1 and 2 have been replotted on log-log paper (Figs. 3, 4) and it can be seen that straight line relationships do exist between the coliform and the enterococci and the *E. coli*, Type I, and the enterococci except during the short period of growth by the coliform and the *E. coli*, Type I. A positive correlation of +.95 was established for the coliform and +.94 for the *E. coli*, Type I, relative to the enterococci.

When Lattanzi and Mood's (4) data is replotted on log-log paper, a straight line may be fitted, under these conditions, by the method of least squares. The correlation coefficient was computed for this plot and it was found to be +.76. This represents a 20 per cent improvement of the estimation over the coordinate plot. This improvement indicates that Lattanzi and Mood's data best fits a straight line on a log-log plot.



It is noted that the positive correlation of the data for both field studies was less than that obtained in the laboratory when plotted on log-log paper. The reason is that under the field conditions studied it is doubtful if a point source of bacteria was present in either study but rather bacteria were entering the river from several sources. As a result, the bacteria from each source were at different points on the survival curves, some being in growth and some in death, giving a greater scatter to the points.

Determination if Source of Indicator Bacteria is Human Feces

Many authors have concluded that the presence of either enterococci or *E. coli*, Type I in water indicates fecal contamination. This may be true, but it must be pointed out that both enterococci and *E. coli*, Type I are present in the feces of many animals whose natural habitats are the drainage basins of our streams. In other words, the mere presence of these indicator bacteria will not tell if a dangerous condition exists from a public health standpoint. For example, Delaney (8) collected some unusual bacteriological samples from Hubbard Brook (Table I). This stream, which drains into the Pemigewasset River, is located near Woodstock, New Hampshire. Stations 1 through 5 are located on the upper reaches of the stream. The watershed in this area is thickly forested and no homes, farms, or summer camps are permitted. A sanitary survey has been made of this area and there are no possible sources of human fecal contamination. If these data for stations 1 through 5, however, are examined in light of the present Massachusetts Bacteriological Standards, stations 1 and 2 could be questioned as a satisfactory bathing area (coliform counts greater than 2400 per 100 ml) and all stations would be questioned as being satisfactory for the taking of market shellfish (coliform counts greater than 70 per 100 ml). Also, in all cases, both enterococci and *E. coli*, Type I were present. From these data it is apparent that counts of these three indicator bacteria alone are not adequate to evaluate a water from a public health standpoint.

Goldreich et al., 1964, (9) reported that a difference in the ratio between fecal coliform and fecal streptococci occurred, depending on the source of the fecal material. He further indicated that when ratios are greater than 2:1, usually this indicates that the pollution was primarily derived from domestic sewage, whereas ratios less than 1:1 indicate the major quantity of pollution was primarily derived from wastes from warm blooded animals other than man, such as stockyard and dairy animals. This study was concerned with bacterial densities in various sewages including a prison dairy.

It is realized that in stream water the ratio between *E. coli*, Type I and enterococci may not remain constant at all times because of the possibility of different death rates for these indicator bacteria. For this reason, it was felt that a more general approach than a ratio was needed to differentiate between human fecal contamination and fecal contamination from other warm blooded animals in water. It seemed reasonable to assume if a water was contaminated with sewage or other fecal material with different *E. coli*, Type I to enterococci ratios, then the linear relationship on log-log paper between these indicator bacteria from the different sources should also be markedly different. This was evaluated by comparing log-log plots of *E. coli*, Type I and enterococci in two streams, one containing no human wastes (Hub-

TABLE I
HUBBARD BROOK BACTERIOLOGICAL DATA
SUMMER 1964

Station	August 12, 1964			August 17, 1964		
	Coliform per 100 ml	Enterococci per 100 ml	*E. coli,* Type I per 100 ml	Coliform per 100 ml	Enterococci per 100 ml	*E. coli,* Type I per 100 ml
1	3,480	1,830	56	290	100	5
2	710	3,360	150	307	110	17
3	1,000	1,530	100	157	57	30
4	2,810	1,130	110	287	173	23
5				347	103	10
6				747	557	316
7	170	450	6	150	30	4

NOTE: The August 12, 1964 samples were collected during a period of increasing stream level following a long drought.

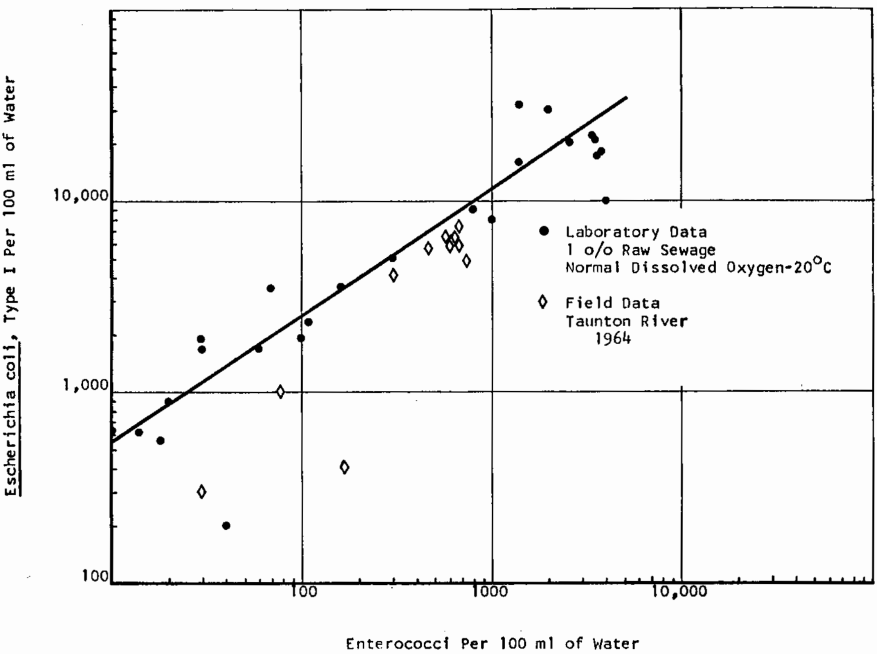
bard Brook) and the other which contained a large amount of domestic sewage (Taunton River). The Taunton River is located in the southeastern part of Massachusetts. At the time the samples were collected (Table II) the Taunton River was receiving a large amount of untreated sewage from the city of Brockton, Massachusetts.

TABLE II
TAUNTON RIVER BACTERIOLOGICAL DATA
February 8, 1964

Station	Coliform per 100 ml	Enterococci per 100 ml	*E. coli,* Type I per 100 ml
1	1,100	30	300
2	2,000	60	
3	1,000	170	400
3a	48,000	750	4,900
4	56,000	650	6,400
5	45,000	690	5,800
6	39,000	680	7,400
7	48,000	580	6,400
7a	4,000	80	1,000
8	36,000	600	6,200
9	36,000	470	5,700
10	28,000	310	4,100

A check was made to determine if the log-log relationship between *E. coli,* Type I and enterococci in the Taunton River represented human fecal contamination. This was done by comparing this relationship to one obtained in the laboratory when biochemical oxygen demand (B.O.D.) dilution water was seeded with domestic sewage. A one per cent dilution was used and bacteriological samples were obtained daily for eight days. It can be seen from Fig. 5 that the Taunton River field data compared closely to the laboratory data, indicating that the indicator bacteria present in the Taunton River were primarily of human origin. A graphical comparison of the relationship between *E. coli,* Type I and enterococci for Hubbard Brook and the Taunton River is presented in Fig. 6. The line labeled "no domestic sewage" passes through the data points from Hubbard Brook. The line labeled "domestic sewage" passes through the data from the Taunton River. Several data points have been plotted between these lines. These points represent waters that receive fecal contamination from both man and other warm blooded animals. Most of them were collected on the Beverly-Salem, Massachusetts Watershed which received a small amount of treated septic tank effluent and one was collected below a boys' camp which has a septic tank on lower Hubbard Brook. It can be seen from Fig. 6, depending on the lineal relationship, that it may be possible to determine if indi-

FIGURE V



cator bacteria found in streams are of human origin and hence whether the water is potentially dangerous to public health or if the indicator bacteria are the result of the natural habitat of the drainage basin. Further studies are underway to confirm these initial findings.

Conclusions

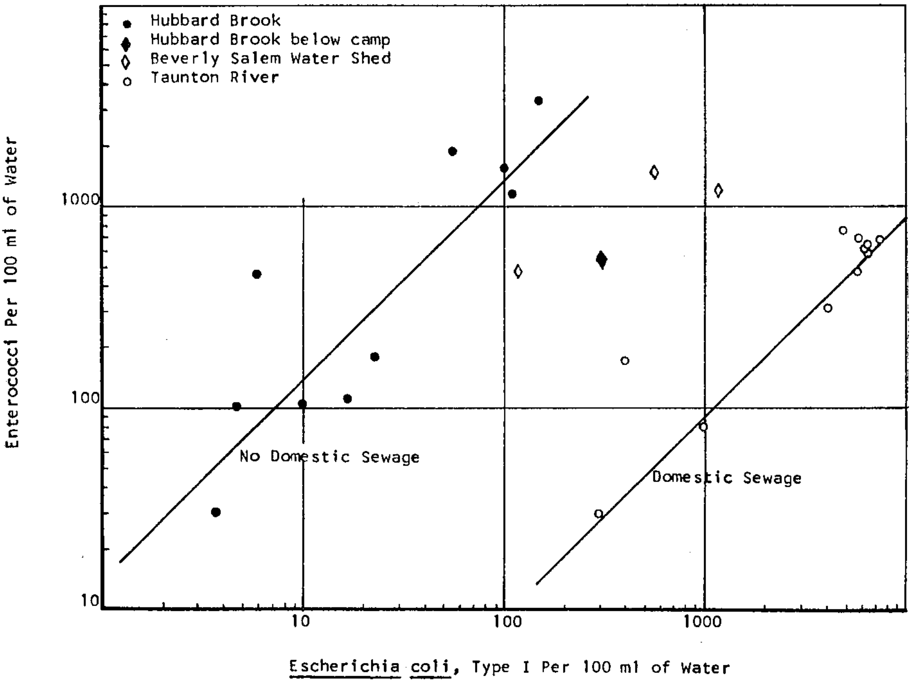
1. The best relationship between enterococci and the coliform group or *E. coli*, Type I will be developed on a log-log plot.

2. A definite equation cannot be developed for the relationship

between enterococci and the coliform group or *E. coli,* Type I because the relationship will vary with the source of contamination.

3. A graphical relationship, such as Fig. 6, between *E. coli,* Type I and enterococci may be used to differentiate between human fecal contamination and fecal contamination from other warm blooded animals in stream water.

FIGURE VI



References

1. Mallman, W. L., and Litsky, W., "Survival of Selected Enteric Organisms in Various Types of Soil," American Jour. Public Health, **41**, 38-44 (1951).
2. Heukelekian, H., and Rosenberg, F. A., "Coliforms and Enterococci as Indicators of Household Well Water Pollution," Abst. Bacteriological Proceedings of the 61st Annual Meeting of the Soc. of American Bacteriologists, 61 (1961).
3. Delaney, J. E., McCarthy, J. A., and Grasso, R. J., "Measurement of *E. coli,* Type I by the Membrane Filter," Paper presented before the Laboratory Section, Annual Meeting, American Public Health Assoc., Detroit, Michigan, November 14, 1961.

4. Lattanzi, W. E., and Mood, E. W., "A Comparison of Enterococci and *E. coli* as Indices of Water Pollution," Sew. and Ind. Wastes, **23**, 1154-1160, 1951.
5. Litsky, W., Mallman, W. L., and Fifield, C. W., "Comparison of the MPN of *E. coli* and Enterococci in River Waters," American Jour. Public Health, **45**, 1049, 1955.
6. Hanes, N. B., Sarles, W. B., and Rohlich, G. A., "Dissolved Oxygen and Survival of Coliform Organisms and Enterococci," Jour. American Water Works Assoc. **56**, 441-446, 1964.
7. O'Leary, C. J., "Survival of Coliform *Escherichia coli,* Type I and Enterococci in Water," Unpublished Master's Thesis, Tufts University, Medford, Massachusetts, 1965.
8. Delaney, G. A., Unpublished Data, Tufts University, Medford, Massachusetts, 1965.
9. Goldreich, E. E., Clark, H. F., and Haft, C. B., "A Study of Pollution Indicators in a Waste Stabilization Pond," Jour. Water Pollution Control Federation, **36**, 1372-1379 (1964).

This study was supported in part by Public Health Service Training Grant No. 1T1-WP40-01, from the Division of Water Supply and Pollution Control.