# Coliforms, Enterococci, Thermodurics, Thermophiles, and Psychrophiles in Untreated Farm Pond Waters

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## Abstract

Malaney, G. W. (Ohio State University, Columbus), H. H. Weiser, R. O. Turner, and Marilyn Van Horn. Coliforms, enterococci, thermodurics, thermophiles, and psychrophiles in untreated farm pond waters. Appl. Microbiol. **10**:44–51. 1962.—Untreated waters from ten farm ponds located in central, north central, southeastern, and southwestern Ohio were examined for numbers of coliforms, enterococci, thermodurics, thermophiles, and psychrophiles. The median population densities per 100 ml water for all ponds were: coliforms, 23; enterococci, 3.6; thermodurics, 6,000; thermophiles, 450; psychrophiles, 1,000. The results indicate that these farm pond waters were only lightly polluted and suggest that farm ponds, properly maintained, are a source of raw water of high bacteriological quality, requiring a minimum of treatment to be made suitable for domestic and livestock purposes.

In many rural areas, well water is insufficient in quantity or is of such poor quality as to be unsuitable for domestic or livestock use. This situation has been reported for Ohio, Missouri, Iowa, Indiana, and Oklahoma, even during periods of normal rainfall. One solution to this problem is hauling water from more fortunate areas, at some cost to the farmer. Another solution is the construction of farm ponds for the impoundment of surface or runoff waters. By 1956, more than 300 Ohio farmers were using ponds as the source of water for the home or the milk room of the dairy farm. The rate of farm pond construction has been even greater in other states.

Very little information is available on the microbiology of farm pond water. By their nature, such waters would appear to be highly susceptible to contamination by way of surface washings, septic tank or cesspool effluents, animal droppings, etc. This suggests a potential hazard to the health of humans and livestock. Also, such waters may be a source of nuisance organisms in milk production or a source of spoilage organisms in the water used for domestic purposes in the home or in food processing plants.

In order that state health departments be in a position to make recommendations concerning the use and treatment of farm pond waters, it is essential that information on the microbiological condition of untreated pond waters be available. With this end in view, a survey of farm pond waters in Ohio was made at ten installations in seven counties during the period April, 1959, to April, 1961. The specific purpose of the study was an estimation of population densities in untreated pond water of bacterial groups of public health significance (coliforms, enterococci), as well as those of economic importance (thermodurics, thermophiles, and psychrophiles).

## Materials and Methods

The ten ponds surveyed were divided into four groups according to geographical location (Hill and Schwab, 1959). Pond 90 was located in north central Ohio in Crawford County; four in central Ohio in Delaware County (ponds 1, 5, 6, and 8); three in southeastern Ohio in Washington (pond 23), Jackson (pond 25), and Vinton (pond 26) Counties; and two in the southwestern part of the state in Highland (pond 62) and Brown (pond 60) Counties. These ponds were were also selected so that each of the three major soil regions in Ohio was represented: glacial limestone (ponds 1, 60, and 62), glacial sandstone and shale (ponds 5, 6, 8, and 90), and residual sandstone and shale (ponds 23, 25, and 26). A complete description of these ponds has been reported by Hill and Schwab (1959).

Samples were taken one foot above the bottom (the "bottom" samples) and one foot below the surface (the "top" samples), both at the deepest point in the pond. Samples were placed on ice in a portable chest immediately upon collection and remained under refrigeration until analyzed. The maximal time elapsing between collection and examination of the sample was 12 hr.

The total bacterial population was estimated by the standard plate count (SPC) technique as outlined in *Standard Methods for the Examination of Dairy Products* (APHA, 1953), with incubation at 35 C. The density of thermoduric bacteria was estimated by the *Standard Methods* laboratory pasteurization test. The water sample was heated at 143 F for 30 min in a David Bradley home milk pasteurizer and the surviving

bacterial population determined by the SPC technique. The thermophilic and psychrophilic densities were estimated by the SPC technique with incubation at 55 C and 0 to 10 C, respectively.

The conventional multiple-tube most probable number (MPN) method, using Winter-Sandholzer (W-S) media and three tubes per dilution, was employed for estimation of the enterococci population. Although the W-S enterococcus method has been reported previously as yielding low results, parallel tests for enterococci in farm pond waters by the present authors using several methods including the W-S method, the membrane filter method, and the azide dextrose-ethyl violet azide method failed to reveal any over-all trend by the W-S test toward low results as compared with values obtained with the other methods. It is possible that variations in the nature of surface waters or seasonal fluctuations in microflora cause differences in the relative response to the various test methods. The coliform density was estimated by the MPN method recommended in *Standard Methods for the Examination of Water and Wastewater* (APHA, 1960). The specific procedure was initial inoculation into lactose, three tubes per dilution, with confirmation in brilliant green lactose bile broth (BGB). All positive BGB tubes were streaked on eosin methylene blue (EMB) agar, followed by transfer of colonies to secondary lactose broth and agar slant (Malaney *et al.*, 1961).

## Experimental Results

The estimated population densities of the various bacterial groups in the ten ponds sampled are given in Tables 1 to 10. Results for corresponding top and bottom samples are listed in adjacent columns. It should be noted that all counts are given in terms of 100 ml of sample.

At the bottom of each column is given the range of the counts listed in that column, as well as the median value. The median rather than the arithmetic mean was used as the measure of central tendency to eliminate the effects of extreme values. Also, indeterminate values made it difficult to calculate a meaningful arithmetic average. Where indeterminate values occurred in the middle of a set of results so as to interfere with the

TABLE 1. *Population of enterococci, coliforms, thermodurics, thermophiles, and psychrophiles in pond no. 1 (glacial limestone)*

| Date | Counts per 100 ml pond water | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | SPC* | | Thermodurics | | Thermophiles | | Psychrophiles | | Enterococci | | Coliforms | |
| | T† | B† | T | B | T | B | T | B | T | B | T | B |
| *1959:* | | | | | | | | | | | | |
| 4-26 | 6,000 | 28,000 | 3,900 | 4,200 | — | — | — | — | <30 | <30 | — | — |
| 7-7 | 35,000 | 54,000 | — | — | 1,500 | 21,000 | 32,000 | 36,000 | <30 | <30 | — | — |
| 7-14 | 35,000 | 64,000 | — | — | 5,000 | 4,600 | 6,300 | 2,000 | <30 | <30 | — | — |
| 7-28 | — | — | 2,600 | 6,300 | 1,900 | 1,300 | 3,500 | 3,100 | <30 | <30 | — | — |
| 8-18 | 68,000 | 20,000 | — | — | 800 | 2,000 | 800 | 1,400 | <30 | <30 | — | — |
| *1960:* | | | | | | | | | | | | |
| 1-12 | 420,000 | — | — | — | — | — | — | — | 9.1 | — | — | — |
| 1-12 | 580,000 | — | — | — | — | — | — | — | <3.0 | — | — | — |
| 1-21 | 2,900,000 | — | — | — | 50,000 | — | 100,000 | — | — | — | — | — |
| 4-21 | 13,000 | 12,000 | 16,000 | 33,000 | <100 | <100 | 9,000 | 2,000 | <3.0 | <3.0 | — | — |
| 5-6 | >300,000 | 10,000 | 410,000 | 7,000 | 100 | — | 1,000 | <1,000 | — | — | 3.6 | 23 |
| 5-20 | 11,000 | — | — | — | <100 | 1,500 | 1,000 | 7,000 | — | — | 23 | 9.1 |
| 5-27 | 35,000 | 49,000 | 19,000 | 5,000 | 100 | <100 | 800 | 200 | — | — | 9.1 | 43 |
| 6-2 | — | 82,000 | 65,000 | 8,000 | <100 | <100 | — | — | 23 | 3.6 | 43 | 15 |
| 8-2 | 5,000 | 96,000 | 8,000 | 11,000 | 100 | 100 | 600 | 6,500 | <3.0 | 3.6 | 43 | 460 |
| 8-9 | 67,000 | 100,000 | — | — | <100 | 1,700 | 4,800 | 36,000 | 23 | 9.1 | 43 | 15 |
| 9-27 | 50,000 | 35,000 | 11,000 | — | <100 | 200 | 7,100 | 2,700 | <3.0 | <3.0 | 1,100 | 290 |
| 10-14 | 68,000 | 72,000 | 3,600 | 4,000 | 100 | <100 | 1,500 | 500 | 3.6 | <3.0 | 93 | 43 |
| 10-28 | 40,000 | 43,000 | 2,000 | 2,200 | 800 | 2,500 | 500 | 800 | <3.0 | 3.6 | 460 | 23 |
| 12-6 | 20,000 | 41,000 | 1,500 | 1,200 | 300 | 1,000 | 4,300 | 5,000 | <3.0 | 3.6 | 43 | 93 |
| *1961:* | | | | | | | | | | | | |
| 1-12 | 25,000 | 3,000 | — | — | 200 | 400 | 5,000 | 6,200 | <3.0 | <3.0 | 3.6 | 240 |
| 2-9 | 1,000,000 | 1,100,000 | 1,800 | 2,100 | 200 | <100 | 210,000 | 230,000 | 3.6 | <3.0 | 15 | <3.0 |
| 3-9 | 100,000 | 36,000 | 3,800 | 3,900 | 500 | 100 | 220,000 | 140,000 | 3.6 | <3.0 | 9.1 | 43 |
| Median..... | 45,000 | 43,000 | 3,900 | 4,600 | 200 | 400 | 4,600 | 3,100 | 3.6 | 3.6 | 43 | 430 |
| Max value..... | 2,900,000 | 1,100,000 | 410,000 | 33,000 | 50,000 | 21,000 | 220,000 | 230,000 | 23 | 9.1 | 1,100 | 460 |
| Min value..... | 5,000 | 3,000 | 1,500 | 1,200 | <100 | <100 | 500 | 200 | <3.0 | <3.0 | 3.6 | <3.0 |

* SPC = standard plate count.

† T = top samples; B = bottom samples.

calculation of the median, these indeterminate values were eliminated from the set. Unfortunately, because of laboratory accidents or equipment difficulties, it was not always possible to obtain counts of all bacterial groups on a given sample. This reduces somewhat the reliability of any comparison of median values.

## Discussion

To compare the populations of the various groups of bacteria in the ten ponds and thus obtain a general picture of the bacteriological condition of the ponds, the median values for the top samples only are listed in Table 11. The ponds are grouped according to soil region. It is noted that these pond waters show a very low bacterial population. They must be classed as only slightly polluted waters. The densities of thermodurics, thermophiles, psychrophiles, and total bacteria, when converted to counts per ml, are quite low. The median of the medians, given at the bottom of each column in Table 11, represents in a general way the bacterial status of farm ponds in Ohio. Of course, in any interpretation of the data, it must be kept in

TABLE 2. *Population of enterococci, coliforms, thermodurics, thermophiles, and psychrophiles in pond no. 5 (glacial sandstone and shale)*

| Date | Counts per 100 ml pond water | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | SPC* | | Thermodurics | | Thermophiles | | Psychrophiles | | Enterococci | | Coliforms | |
| | T† | B† | T | B | T | B | T | B | T | B | T | B |
| *1959:* | | | | | | | | | | | | |
| 5-7 | 20,000 | 5,600 | 13,000 | 140,000 | 400 | 200 | <100 | <100 | <30 | <30 | — | — |
| 7-7 | 23,000 | 140,000 | — | — | 2,600 | 37,000 | 28,000 | 8,200 | <30 | <30 | — | — |
| 7-14 | 110,000 | 96,000 | — | — | 7,200 | 7,000 | 3,300 | 9,500 | <30 | <30 | — | — |
| 7-28 | 30,000 | >30,000 | 33,000 | >30,000 | 1,700 | — | 1,000 | 1,500 | <30 | 230 | — | — |
| 8-18 | 680,000 | 280,000 | — | — | 5,000 | 30,000 | 600 | 1,500 | 36 | 73 | — | — |
| *1960:* | | | | | | | | | | | | |
| 3-3 | 130,000 | 210,000 | <10,000 | <10,000 | 5,000 | 10,000 | 5,000 | 5,000 | <3.0 | <3.0 | — | — |
| 4-21 | 92,000 | 31,000 | 7,000 | 44,000 | 800 | <100 | <1,000 | <1,000 | <3.0 | <3.0 | — | — |
| 5-6 | >300,000 | 12,000 | 14,000 | 5,000 | 200 | 200 | <1,000 | 1,000 | — | — | 15 | 43 |
| 5-27 | 23,000 | — | 6,000 | 30,000 | 500 | 300 | 400 | 500 | — | — | 19 | 150 |
| 7-25 | 68,000 | 95,000 | 18,000 | 40,000 | <100 | <100 | 100 | 14,000 | 23 | 240 | 240 | 1,500 |
| 8-2 | 94,000 | 65,000 | 7,000 | 11,000 | 900 | 400 | 1,000 | 14,000 | 75 | 460 | 150 | 460 |
| 8-9 | 24,000 | 200,000 | — | 61,000 | 300 | 3,900 | 600 | 20,000 | 150 | 43 | 23 | 750 |
| 11-15 | 45,000 | 46,000 | 7,500 | 8,500 | 6,100 | 9,500 | 4,000 | 4,500 | 3.6 | 9.1 | 23 | 150 |
| Median..... | 83,000 | 80,000 | 10,000 | 30,000 | 900 | 2,200 | 1,000 | 4,500 | <30 | <30 | 23 | 300 |
| Max value..... | 680,000 | 280,000 | 33,000 | 140,000 | 7,200 | 37,000 | 28,000 | 20,000 | 150 | 460 | 240 | 1,500 |
| Min value..... | 20,000 | 5,600 | 6,000 | 5,000 | <100 | <100 | <100 | <100 | <3.0 | <3.0 | 15 | 43 |

* SPC = standard plate count.
† T = top samples; B = bottom samples.

TABLE 3. *Population of enterococci, coliforms, thermodurics, thermophiles, and psychrophiles in pond no. 6 (glacial sandstone and shale)*

| Date | Counts per 100 ml pond water | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | SPC* | | Thermodurics | | Thermophiles | | Psychrophiles | | Enterococci | | Coliforms | |
| | T† | B† | T | B | T | B | T | B | T | B | T | B |
| *1959:* | | | | | | | | | | | | |
| 4-30 | 42,000 | 49,000 | 8,400 | 23,000 | 200 | 600 | <100 | <100 | <30 | <30 | — | — |
| 7-14 | 250,000 | — | — | — | 2,500 | — | 3,600 | — | <30 | — | — | — |
| 7-28 | 21,000 | — | 8,000 | 7,000 | 1,500 | 2,600 | 900 | 9,900 | <30 | 36 | — | — |
| 8-18 | 74,000 | 67,000 | — | — | 1,700 | 5,800 | 700 | 1,400 | <30 | <30 | — | — |
| *1960:* | | | | | | | | | | | | |
| 7-25 | — | — | — | 17,000 | — | — | 700 | 10,000 | <3.0 | 14 | 23 | 240 |
| 8-9 | 44,000 | — | 69,000 | 32,000 | 100 | — | 300 | — | <3.0 | <3.0 | 15 | 20 |
| Median..... | 44,000 | 58,000 | 8,400 | 20,000 | 1,700 | 2,600 | 700 | 5,700 | <30 | <30 | 19 | 130 |
| Max value... | 250,000 | 67,000 | 69,000 | 32,000 | 2,500 | 5,800 | 3,600 | 10,000 | — | 36 | 23 | 240 |
| Min value... | 21,000 | 49,000 | 8,000 | 7,000 | 100 | 600 | <100 | <100 | <3.0 | <3.0 | 15 | 20 |

* SPC = standard plate count.
† T = top sample; B = bottom sample.

mind that the results may be biased as a consequence of restricted sampling, limited number of ponds sampled, selection of ponds, and other unrecognized sampling errors.

Although the SPC is of no great significance, this parameter was measured to give some indication of the heterogeneous bacterial population. Even though the pond water might consist principally of runoff from

TABLE 4. *Population of enterococci, coliforms, thermodurics, thermophiles, and psychrophiles in pond no. 8 (glacial sandstone and shale)*

| Date | Counts per 100 ml pond water | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | SPC* | | Thermodurics | | Thermophiles | | Psychrophiles | | Enterococci | | Coliforms | |
| | T† | B† | T | B | T | B | T | B | T | B | T | B |
| *1959:* | | | | | | | | | | | | |
| 5-10 | 44,000 | 71,000 | 4,600 | 44,000 | — | 200 | <100 | <100 | <30 | <30 | — | — |
| 7-7 | 31,000 | 12,000 | — | — | 6,800 | 26,000 | 33,000 | 13,000 | <30 | <30 | — | — |
| 7-21 | 89,000 | 76,000 | 3,200 | 3,600 | 1,200 | 5,800 | 500 | 600 | <30 | <30 | — | — |
| 7-28 | 120,000 | — | 2,900 | — | 600 | 1,700 | 1,700 | 4,800 | <30 | <30 | — | — |
| *1960:* | | | | | | | | | | | | |
| 3-9 | 550,000 | — | 490,000 | 3,000,000 | <10,000 | 10,000 | 10,000 | 20,000 | <3.0 | <3.0 | — | — |
| 4-8 | 80,000 | — | — | — | 200 | — | <100 | — | — | — | — | — |
| 4-21 | 6,000 | 50,000 | 19,000 | 25,000 | <100 | 400 | 1,000 | 2,000 | <3.0 | <3.0 | — | — |
| 5-6 | — | 61,000 | 11,000 | 48,000 | 100 | 3,600 | <1,000 | <1,000 | — | — | 9.1 | 93 |
| 5-27 | 15,000 | — | 9,000 | 15,000 | <100 | <100 | 100 | <100 | — | — | 14 | 15 |
| 6-2 | 49,000 | — | 4,000 | 17,000 | <100 | 400 | — | — | 9.1 | 23 | 460 | 93 |
| 7-18 | 83,000 | 74,000 | 110,000 | 130,000 | <100 | <100 | 3,600 | 67,000 | <3.0 | <3.0 | 93 | 4,600 |
| 7-25 | 97,000 | 94,000 | 7,000 | — | <100 | <100 | 2,800 | 40,000 | <3.0 | 3.6 | 240 | 1,500 |
| 8-2 | 110,000 | 98,000 | 8,000 | 9,000 | 400 | 1,000 | 2,800 | 24,000 | <3.0 | <3.0 | 240 | 120 |
| 8-9 | 190,000 | 54,000 | 51,000 | 9,000 | 100 | 900 | 1,500 | 2,100 | 9.1 | 3.6 | 21 | 36 |
| 9-27 | 22,000 | 60,000 | — | 2,800 | 100 | 200 | 200 | 1,400 | <3.0 | <3.0 | 75 | 290 |
| 10-14 | 200,000 | 110,000 | 4,200 | 6,400 | <100 | <100 | 300 | 5,900 | 3.6 | 3.6 | 93 | 240 |
| 10-28 | 5,000 | 10,000 | 200 | 300 | <100 | 100 | 2,600 | 5,000 | 3.6 | 3.6 | 23 | 150 |
| 11-15 | 55,000 | 45,000 | 2,100 | 3,800 | 800 | 900 | 2,400 | 2,800 | <3.0 | <3.0 | 23 | 23 |
| 12-6 | 17,000 | 45,000 | 2,500 | 2,700 | 1,000 | 500 | 3,500 | 4,000 | 3.6 | <3.0 | 23 | 43 |
| *1961:* | | | | | | | | | | | | |
| 1-12 | 11,000 | 40,000 | — | — | 300 | 800 | 3,800 | 4,700 | <3.0 | 3.0 | 7.3 | 3.6 |
| 3-9 | 110,000 | 120,000 | 7,500 | 8,500 | 400 | 200 | 190,000 | 210,000 | 21 | 240 | 1,100 | 23 |
| Median..... | 68,000 | 61,000 | 7,000 | 9,000 | 150 | 450 | 2,600 | 4,000 | 3.6 | 3.6 | 49 | 93 |
| Max value.. | 550,000 | 120,000 | 490,000 | 3,000,000 | 6,800 | 26,000 | 190,000 | 210,000 | 21 | 240 | 1,100 | 4,600 |
| Min value.. | 5,000 | 10,000 | 200 | 300 | <100 | <100 | <100 | <100 | <3.0 | <3.0 | 7.3 | 3.6 |

* SPC = standard plate count.
† T = top samples; B = bottom samples.

TABLE 5. *Population of enterococci, coliforms, thermodurics, thermophiles, and psychrophiles in pond no. 23 (residual sandstone and shale)*

| Date | Counts per 100 ml pond water | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | SPC* | | Thermodurics | | Thermophiles | | Psychrophiles | | Enterococci | | Coliforms | |
| | T† | B† | T | B | T | B | T | B | T | B | T | B |
| *1959:* | | | | | | | | | | | | |
| 8-11 | 38,000 | 47,000 | 1,600 | 4,800 | 200 | 700 | 600 | 700 | — | — | — | — |
| *1960:* | | | | | | | | | | | | |
| 7-5 | 240,000 | 500,000 | 66,000 | 110,000 | 100 | 600 | 100 | 800 | 23 | 240 | 93 | 21 |
| *1961:* | | | | | | | | | | | | |
| 1-27 | 95,000 | 100,000 | 5,000 | 5,000 | — | — | 150,000 | 110,000 | 93 | 15 | 23 | 210 |
| 3-2 | 57,000 | 65,000 | 13,000 | 12,000 | 600 | 500 | 48,000 | 90,000 | <3.0 | <3.0 | <3.0 | <3.0 |
| Median..... | 76,000 | 83,000 | 9,000 | 8,500 | 200 | 600 | 24,000 | 45,000 | 23 | 15 | 23 | 21 |
| Max value..... | 240,000 | 500,000 | 66,000 | 110,000 | 600 | 700 | 150,000 | 110,000 | 93 | 240 | 93 | 210 |
| Min value..... | 38,000 | 47,000 | 1,600 | 4,800 | 100 | 500 | 100 | 700 | <3.0 | <3.0 | <3.0 | <3.0 |

* SPC = standard plate count.
† T = top samples; B = bottom samples.

well-grassed meadow land, it would still be expected to contain large numbers of soil bacteria. When compared with plate counts of various types of natural waters, the SPC values indicate that farm ponds have a relatively low microbial population.

The significance of the coliform group of bacteria in the domestic drinking water supply as an indicator of fecal pollution is too well known to require comment. The experimental results indicate that, using coliform density as the criterion, pond water is a very lightly polluted raw water. It will be recalled that the coliform index has been used to determine the minimal treatment required of various types of raw water to produce a finished water meeting the U. S. Public Health Service Drinking Water Standards. According to Streeter (1939), raw water averaging not more than 50 coliform organisms per 100 ml may be treated by simple chlorination or its equivalent. This would be considered a good water supply. Apparently, farm pond waters fall into this category.

The concentration of coliform organisms in any milk or milk derivative reflects the care taken in the production and processing of the product. In some areas the coliform count is replacing the SPC in the evaluation of the quality of raw milk in storage tanks prior to pasteurization. Therefore, careful control of all sources of coliforms, including the water supply, is essential to the production of acceptable milk products.

TABLE 6. *Population of enterococci, coliforms, thermodurics, thermophiles, and psychrophiles in pond no. 25 (residual sandstone and shale)*

| Date | Counts per 100 ml pond water | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | SPC* | | Thermodurics | | Thermophiles | | Psychrophiles | | Enterococci | | Coliforms | |
| | T† | B† | T | B | T | B | T | B | T | B | T | B |
| *1959:* | | | | | | | | | | | | |
| 8-11 | 73,000 | 38,000 | 2,500 | 7,000 | 600 | 500 | 200 | 300 | — | — | — | — |
| *1960:* | | | | | | | | | | | | |
| 7-5 | 85,000 | — | 24,000 | 39,000 | 200 | 100 | 100 | 500 | 93 | 460 | 15 | 20 |
| 10-7 | 38,000 | 25,000 | 6,000 | 2,200 | 1,000 | 2,700 | 300 | 2,500 | <3.0 | <3.0 | 21 | 20 |
| 11-4 | 40,000 | 35,000 | 2,500 | 3,900 | 900 | 2,000 | 6,600 | 4,400 | <3.0 | <3.0 | 2,400 | 240 |
| *1961:* | | | | | | | | | | | | |
| 1-27 | 70,000 | 25,000 | 3,500 | 1,000 | — | — | 180,000 | 160,000 | <3.0 | 3.6 | 23 | 43 |
| Median | 70,000 | 30,000 | 3,500 | 3,900 | 800 | 1,600 | 300 | 2,500 | <3.0 | <3.3 | 22 | 32 |
| Max value | 85,000 | 38,000 | 24,000 | 39,000 | 1,000 | 2,700 | 180,000 | 160,000 | 93 | 460 | 2,400 | 240 |
| Min value | 38,000 | 25,000 | 2,500 | 1,000 | 200 | 100 | 100 | 300 | <3.0 | <3.0 | 15 | 20 |

* SPC = standard plate count.
† T = top samples; B = bottom samples.

TABLE 7. *Population of enterococci, coliforms, thermodurics, thermophiles, and psychrophiles in pond no. 26 (residual sandstone and shale)*

| Date | Counts per 100 ml pond water | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | SPC* | | Thermodurics | | Thermophiles | | Psychrophiles | | Enterococci | | Coliforms | |
| | T† | B† | T | B | T | B | T | B | T | B | T | B |
| *1959:* | | | | | | | | | | | | |
| 5-19 | 41,000 | 18,000 | 800 | 1,500 | 200 | 100 | <100 | <100 | <30 | <30 | — | — |
| 8-11 | 100,000 | 130,000 | 4,400 | 10,000 | 100 | 200 | 500 | <100 | — | — | — | — |
| *1960:* | | | | | | | | | | | | |
| 7-5 | 110,000 | 100,000 | 91,000 | 93,000 | 100 | <100 | 100 | <100 | <3.0 | 3.0 | 15 | 93 |
| 10-7 | 22,000 | 46,000 | 2,300 | 3,000 | 500 | 300 | 100 | 4,000 | <3.0 | <3.0 | 460 | 93 |
| 11-4 | 95,000 | 35,000 | 3,000 | 2,100 | 500 | 100 | 5,700 | 6,100 | <3.0 | <3.0 | 93 | 43 |
| *1961:* | | | | | | | | | | | | |
| 3-2 | 85,000 | 2,400 | 13,000 | 8,500 | 500 | 300 | 4,500 | 7,000 | <3.0 | <3.0 | 9.1 | 15 |
| Median | 90,000 | 41,000 | 3,700 | 5,800 | 350 | 150 | 300 | 2,100 | <3.0 | <3.0 | 54 | 68 |
| Max value | 110,000 | 130,000 | 91,000 | 93,000 | 500 | 300 | 5,700 | 7,000 | — | — | 460 | 93 |
| Min value | 22,000 | 2,400 | 800 | 1,500 | 100 | <100 | <100 | <100 | <3.0 | <3.0 | 9.1 | 15 |

* SPC = standard plate count.
† T = top samples; B = bottom samples.

Although the fecal streptococci, or enterococci, have not been widely used in the United States as an indicator of water pollution, they have received increased attention in recent years. It has been suggested that this index might eliminate certain of the drawbacks of the conventional coliform determination. The enterococci MPN data show these pond waters to be very lightly contaminated. The enterococcus counts, therefore, corroborate the results of the coliform analyses.

A great amount of effort is expended by the market milk industry to produce pasteurized milk with a low bacterial content. The thermoduric bacteria are especially troublesome in this regard. It has been shown that a major factor in high total counts in pasteurized milk is the presence of excessive numbers of thermoduric bacteria in raw milk from the dairy farm. Since, by definition, the thermodurics are able to survive conventional pasteurization exposures, excessive numbers in the raw milk make it difficult to meet bacterial standards for pasteurized milk.

Although high thermoduric counts are frequently found in milk from farms where cooling is inadequate, the primary cause of the excessive counts in such instances is poor utensil sanitation. In the water used for washing utensils and equipment, the presence of thermoduric bacteria, even though in small numbers as in the case of pond waters, would serve as an additional source of these nuisance organisms and would aggravate the situation. Treatment of the water prior to use should reduce the thermoduric count to an insignificant level. However, this consideration might complicate the use in farm pond water treatment of the continuous-flow pasteurizer designed by Goldstein, McCabe, and Woodward (1960).

Thermophilic bacteria, as defined by the dairy in-

TABLE 8. *Population of enterococci, coliforms, thermodurics, thermophiles, and psychrophiles in pond no. 60 (glacial limestone)*

| Date | Counts per 100 ml pond water | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | SPC* | | Thermodurics | | Thermophiles | | Psychrophiles | | Enterococci | | Coliforms | |
| | T† | B† | T | B | T | B | T | B | T | B | T | B |
| *1959:* | | | | | | | | | | | | |
| 7-23 | 86,000 | — | 500 | — | 2,700 | 3,500 | 1,400 | 2,100 | <30 | <30 | — | — |
| *1960:* | | | | | | | | | | | | |
| 3-5 | 470,000 | 200,000 | 210,000 | 220,000 | <10,000 | <10,000 | <10,000 | <10,000 | <3.0 | <3.0 | — | — |
| 3-22 | 1,200,000 | 1,800,000 | 64,000 | 82,000 | <10,000 | <10,000 | <10,000 | <10,000 | <3.0 | <3.0 | — | — |
| 4-5 | 16,000 | — | <1,000 | — | <1,000 | — | <1,000 | — | <3.0 | — | — | — |
| 4-12 | 26,000 | — | 15,000 | — | <1,000 | — | <1,000 | — | <3.0 | — | — | — |
| 4-19 | 20,000 | 19,000 | 16,000 | 11,000 | <1,000 | <1,000 | 1,000 | 3,000 | <3.0 | <3.0 | — | — |
| 4-24 | 8,000 | 26,000 | 280,000 | 840,000 | <100 | <100 | <100 | <100 | — | — | 43 | 9.1 |
| 5-3 | 4,000 | 19,000 | 5,000 | 17,000 | <100 | 100 | <1,000 | <1,000 | — | — | <3.0 | <3.0 |
| 5-10 | 96,000 | — | 1,000,000 | — | <100 | — | 1,000 | — | — | — | 460 | — |
| 5-17 | 60,000 | 51,000 | — | 6,000 | 200 | — | <1,000 | 2,000 | — | — | 23 | <3.0 |
| 5-24 | 32,000 | 870,000 | <1,000 | 6,000 | <1,000 | 300 | 200 | 2,500 | — | — | 93 | 1,100 |
| 7-24 | 89,000 | 30,000 | 50,000 | 170,000 | <100 | 1,500 | 1,000 | 2,100 | — | — | 3.6 | <3.0 |
| 8-11 | 300,000 | 33,000 | 20,000 | 58,000 | 600 | 900 | 10,000 | 24,000 | 43 | 9.1 | 1,500 | 750 |
| 9-28 | 26,000 | 46,000 | 5,200 | 6,000 | <100 | 500 | <100 | 1,800 | <3.0 | 3.6 | 23 | 23 |
| 10-5 | 54,000 | 26,000 | 14,000 | — | 100 | <100 | <100 | <100 | <3.0 | 3.6 | 23 | <3.0 |
| 10-12 | 170,000 | 39,000 | 1,600 | 3,800 | <100 | 300 | <100 | 3,300 | 3.6 | 3.6 | 21 | 15 |
| 10-18 | 32,000 | 42,000 | 4,500 | 7,800 | 900 | 6,000 | 3,100 | 10,000 | <3.0 | 3.6 | 23 | 460 |
| 10-26 | 27,000 | 28,000 | 2,000 | 2,500 | <100 | <100 | 500 | 1,500 | <3.0 | 3.6 | 23 | 93 |
| 11-1 | 32,000 | 40,000 | 2,400 | 4,000 | 1,800 | 1,200 | 3,500 | 4,100 | 3.6 | 9.1 | 460 | 460 |
| 11-10 | 80,000 | 75,000 | 3,300 | 5,000 | 1,700 | 2,000 | 30,000 | 22,000 | <3.0 | <3.0 | 460 | 210 |
| 11-23 | 14,000 | 16,000 | 1,700 | 1,900 | 500 | 1,500 | 500 | 1,200 | <3.0 | <3.0 | 43 | 23 |
| 11-29 | 30,000 | 25,000 | 1,200 | 1,600 | 1,800 | 5,000 | 3,000 | 3,800 | <3.0 | <3.0 | 460 | 93 |
| 12-8 | 20,000 | 22,000 | 1,800 | 2,100 | 1,000 | 2,200 | 2,400 | 4,000 | <3.0 | <3.0 | 29 | 21 |
| 12-14 | 7,000 | — | 900 | — | 1,000 | — | 1,200 | — | <3.0 | — | 21 | — |
| *1961:* | | | | | | | | | | | | |
| 1-1 | 16,000 | 25,000 | — | — | 200 | 500 | 7,000 | 13,000 | <3.0 | <3.0 | 23 | 43 |
| 2-21 | 23,000 | 20,000 | 2,200 | 3,000 | 400 | 400 | 110,000 | 45,000 | <3.0 | <3.0 | 43 | 21 |
| 3-8 | 54,000 | 55,000 | 7,500 | 7,800 | 400 | 800 | 130,000 | 85,000 | 43 | 43 | 3.6 | 15 |
| Median..... | 32,000 | 32,000 | 4,500 | 6,000 | 550 | 850 | 1,000 | 3,000 | <3.0 | <3.3 | 23 | 22 |
| Max value... | 1,200,000 | 1,800,000 | 1,000,000 | 840,000 | 2,700 | 6,000 | 130,000 | 85,000 | 43 | 43 | 1,500 | 1,100 |
| Min value... | 4,000 | 16,000 | 500 | 1,600 | <100 | <100 | <100 | <100 | <3.0 | <3.0 | <3.0 | <3.0 |

* SPC = standard plate count.

† T = top samples; B = bottom samples.

dustry, are those organisms able to grow at 55 C. However, the method used enumerates only the aerobic members of this group. Home canned foods are subject to spoilage by thermophilic bacteria, facultative with respect to oxygen. Inadequately treated pond water used in home packing operations may serve as a source of such spoilage organisms.

The thermophilic problem in the dairy industry centers about the growth of bacteria during the pasteurization of milk. As in the case of the thermoduric bacteria, excessive numbers of these organisms may grow and be counted in the mesophilic SPC. This makes it difficult to meet the standards for pasteurized milk. In addition, these organisms are objectionable in milk because their growth may cause off-flavors, high acidity, and a tendency for the milk to curdle upon heating.

The term psychrophilic as used in the dairy industry refers to those bacterial species which are capable of growth at temperatures in the range of 2 to 10 C. Since storage at low temperatures is a common method of preserving milk and milk products from deterioration

TABLE 9. *Population of enterococci, coliforms, thermodurics, thermophiles, and psychrophiles in pond no. 62 (glacial limestone)*

| Date | Counts per 100 ml pond water | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | SPC* | | Thermodurics | | Thermophiles | | Psychrophiles | | Enterococci | | Coliforms | |
| | T† | B† | T | B | T | B | T | B | T | B | T | B |
| *1959:* | | | | | | | | | | | | |
| 5-25 | 16,000 | 14,000 | 24,000 | 11,000 | <100 | 900 | <100 | <100 | <30 | <30 | — | — |
| 7-23 | 43,000 | 65,000 | 2,200 | 2,300 | 3,300 | 5,700 | 2,100 | 1,700 | <30 | <30 | — | — |
| *1960:* | | | | | | | | | | | | |
| 5-17 | 11,000 | 25,000 | 14,000 | 19,000 | <100 | <100 | <1,000 | 2,000 | — | — | — | — |
| 5-24 | 29,000 | 88,000 | 4,000 | 12,000 | 100 | — | <100 | 700 | — | — | — | — |
| 6-23 | — | 36,000 | 55,000 | 5,000 | 300 | 700 | 300 | <100 | — | — | 3.6 | 75 |
| 7-27 | 170,000 | 57,000 | — | — | 300 | 100 | 100 | 100 | 9.1 | <3.0 | — | — |
| 8-1 | 44,000 | 49,000 | 31,000 | 130,000 | <100 | 200 | — | — | <3.0 | 39 | 460 | 93 |
| 8-11 | 41,000 | 46,000 | 15,000 | 20,000 | <100 | 400 | 600 | 14,000 | <3.0 | 23 | 150 | 240 |
| 10-5 | 33,000 | 32,000 | 28,000 | 16,000 | 100 | 100 | <100 | 4,400 | 3.6 | 9.1 | 36 | 240 |
| 10-26 | 90,000 | 9,200 | 2,300 | 4,000 | 100 | 100 | 5,300 | 4,500 | 3.6 | <3.0 | 23 | 93 |
| *1961:* | | | | | | | | | | | | |
| 2-21 | 14,000 | 40,000 | 2,300 | 3,500 | 800 | 1,000 | 58,000 | 85,000 | 3.6 | 9.1 | 21 | 23 |
| 3-8 | 160,000 | 55,000 | 6,500 | 7,500 | 200 | <100 | 110,000 | 85,000 | 15 | 11 | <3.0 | 7.3 |
| Median | 41,000 | 43,000 | 14,000 | 11,000 | 100 | 200 | 600 | 2,000 | 3.6 | 11 | 23 | 93 |
| Max value | 170,000 | 88,000 | 55,000 | 130,000 | 3,300 | 5,700 | 110,000 | 85,000 | 15 | 39 | 460 | 240 |
| Min value | 11,000 | 9,200 | 2,200 | 2,300 | <100 | <100 | <100 | <100 | <3.0 | <3.0 | <3.0 | 7.3 |

* SPC = standard plate count.
† T = top sample; B = bottom sample.

TABLE 10. *Population of enterococci, coliforms, thermodurics, thermophiles, and psychrophiles in pond no. 90 (glacial sandstone and shale)*

| Date | Counts per 100 ml pond water | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | SPC* | | Thermodurics | | Thermophiles | | Psychrophiles | | Enterococci | | Coliforms | |
| | T† | B† | T | B | T | B | T | B | T | B | T | B |
| *1959:* | | | | | | | | | | | | |
| 7-21 | — | 140,000 | — | 2,700 | 700 | 1,000 | 800 | 1,500 | <30 | 91 | — | — |
| *1960:* | | | | | | | | | | | | |
| 12-2 | 70,000 | 64,000 | 4,000 | 3,000 | 1,400 | 1,900 | 11,000 | 9,500 | <3.0 | <3.0 | 93 | 150 |
| *1961:* | | | | | | | | | | | | |
| 1-23 | 77,000 | 62,000 | 6,000 | 4,500 | 700 | 1,100 | 13,000 | 12,000 | — | — | 23 | 23 |
| Median | 74,000 | 64,000 | 5,000 | 3,000 | 1,100 | 1,100 | 11,000 | 9,500 | <17 | <47 | 58 | 87 |
| Max value | 77,000 | 140,000 | 6,000 | 4,500 | 1,400 | 1,900 | 13,000 | 12,000 | — | 91 | 93 | 150 |
| Min value | 70,000 | 62,000 | 4,000 | 2,700 | 700 | 1,000 | 800 | 1,500 | <3.0 | <3.0 | 23 | 23 |

* SPC = standard plate count.
† T = top sample; B = bottom sample.

by bacterial action, the presence of bacterial types able to grow rather well at low temperatures has been a matter of concern in the transportation, processing, and keeping quality of fluid milk products. Since certain members of this group of organisms are capable of producing flavor and aroma defects in raw or pasteurized milk, initial contamination must be kept at a minimum. Pond waters with their low content of psychrophiles do not appear to be a serious hazard in this regard, since treatment of the pond water should reduce the count to an insignificant level.

There has been some discussion regarding the optimal location in depth of the raw water intake unit. Earlier installations often employed the buried pipe, the submerged barrel filled with sand and gravel, or the submerged, sand-filled trench type of inlet. Current thought favors an intake near the surface of the pond, preferably using a floating inlet. Obviously, the intake should be located at such a point as to utilize the water of highest chemical and bacteriological purity.

TABLE 11. *Relative densities of coliforms, enterococci, thermodurics, thermophiles, and psychrophiles in farm pond waters, as indicated by median values of top samples*

| Pond no. | Counts per 100 ml pond water | | | | | |
|---|---|---|---|---|---|---|
| | SPC* | Thermodurics | Thermophiles | Psychrophiles | Enterococci | Coliforms |
| 1 | 45,000 | 3,900 | 200 | 4,600 | 3.6 | 43 |
| 60 | 32,000 | 4,500 | 550 | 1,000 | <3.0 | 23 |
| 62 | 41,000 | 14,000 | 100 | 600 | 3.6 | 23 |
| 5 | 83,000 | 10,000 | 900 | 1,000 | <30 | 23 |
| 6 | 44,000 | 8,400 | 1,700 | 700 | <30 | 19 |
| 8 | 68,000 | 7,000 | 150 | 2,600 | 3.6 | 49 |
| 90 | 74,000 | 5,000 | 1,100 | 11,000 | <17 | 58 |
| 23 | 76,000 | 9,000 | 200 | 24,000 | 23 | 23 |
| 25 | 70,000 | 3,500 | 800 | 300 | <3.0 | 22 |
| 26 | 90,000 | 3,700 | 350 | 300 | <3.0 | 54 |
| Median of medians. | 69,000 | 6,000 | 450 | 1,000 | 3.6 | 23 |

* Standard plate count, incubation at 35 C.

TABLE 12. *Differences in vertical dispersion of bacteria in farm ponds as shown by median values of top and bottom samples*

| | SPC* | Thermodurics | Thermophiles | Psychrophiles | Enterococci | Coliforms |
|---|---|---|---|---|---|---|
| Ponds with top count higher than bottom count | 6 | 3 | 1 | 2 | 1 | 2 |
| Ponds with top count same as bottom count | — | — | 1 | — | 7 | — |
| Ponds with top count lower than bottom count | 4 | 7 | 8 | 8 | 2 | 8 |

* Standard plate count.

The individual results in Tables 1 through 10 show that either top or bottom count might be higher in a given pond on a given day. However, when median values for corresponding top and bottom samples are compared (Table 12), a definite trend for the bottom sample to show a higher count is revealed, except in the case of the SPC and the enterococci count. In the latter case, the medians for top and bottom were usually the same, suggesting a roughly uniform enterococci population throughout the pond.

These data confirm the conclusions of Daniel (1953) and Hill and Schwab (1959) as to the desirability of surface water over the bottom water of ponds. However, caution is necessary in the interpretation of these results; in collecting bottom samples, it is inevitable that occasionally the sampler will strike the bottom sediment, with resultant agitation of the mud. Such accidents would probably increase the bacterial counts of such bottom samples.

No definitive conclusions were made with regard to the effect of soil region upon bacterial densities. A superficial analysis seems to indicate higher bacterial quality in glacial limestone (ponds 1, 60, and 62); however, there are a number of variables which make evaluation of the data difficult. For example, ponds 60 and 62 are partially spring fed and spring water would be expected to have a lower bacterial content than surface runoff. Also, the care taken in the maintenance of the individual watershed and pond environs will influence water quality.

An analysis of the results was made to determine a possible relationship between bacterial density and season, but no significant correlation was observed.

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## LITERATURE CITED

American Public Health Association. 1953. Standard methods methods for the examination of dairy products, 10th ed. New York. 345 p.

American Public Health Association. 1960. Standard methods for the examination of water and wastewater. 11th ed. New York. 626 p.

Daniel, E. R. 1953. Treating farm pond water for domestic use. Oklahoma Agr. Expt. Sta. Bull. No. **B-408**.

Hill, R. D., and G. O. Schwab. 1959. A study of selected farmstead surface water supply installations. Report to U. S. Dept. Agr., Agr. Research Serv., Beltsville, Md.

Goldstein, M., L. J. McCabe, and R. L. Woodward. 1960. Continuous-flow water pasteurizer for small supplies. J. Am. Water Works Assoc. **52**:247–254.

Malaney, G. W., H. H. Weiser, R. M. Gerhold, and F. A. Garver. 1961. Evaluation of methods for coliform counts in farm pond waters. J. Am. Water Works Assoc. **53**:43–48.

Streeter, H. W. 1939. Standards of raw and treated water quality. J. Am. Water Works Assoc. **31**:1479–1488.