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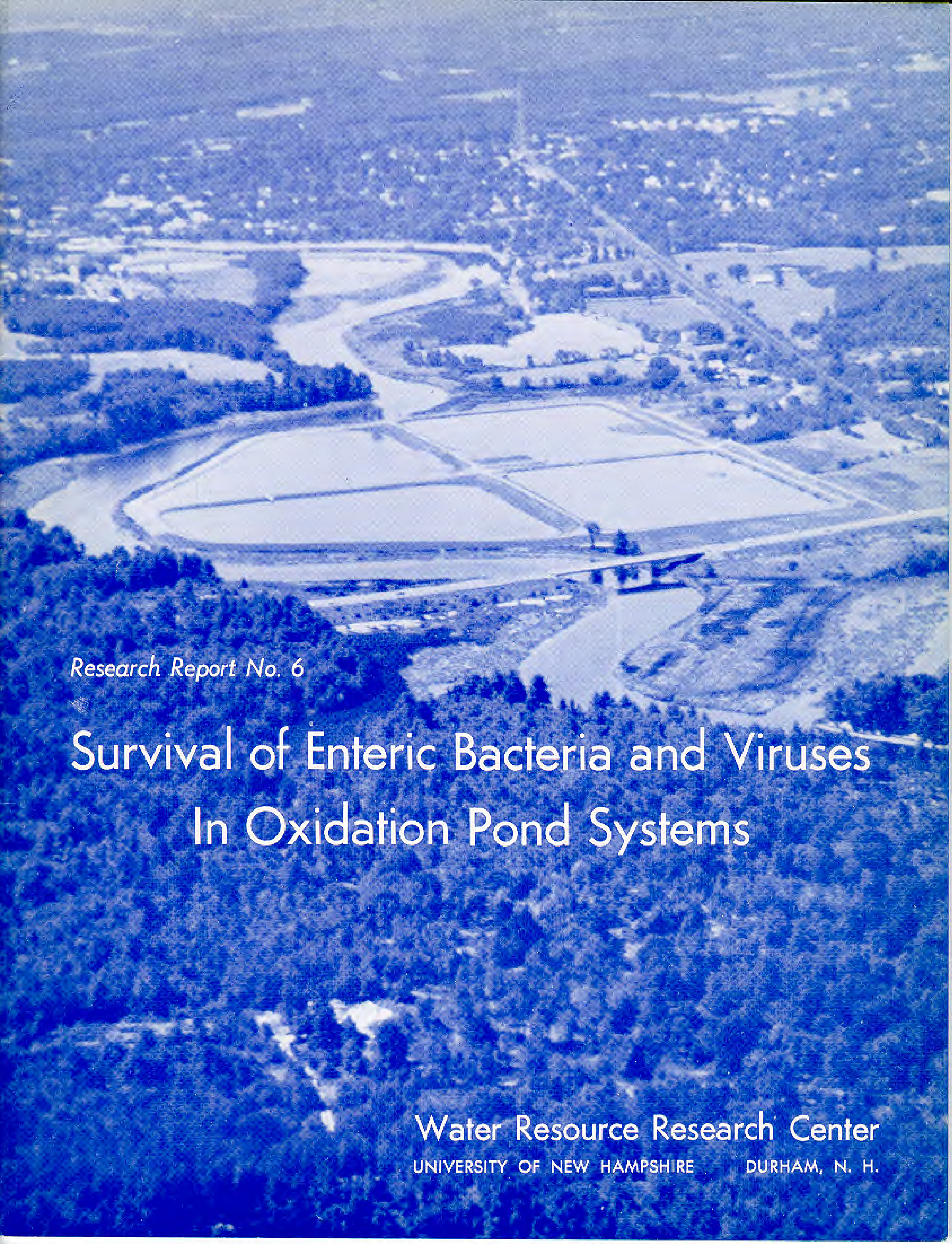

Research Report No. 6

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Water Resource Research Center
UNIVERSITY OF NEW HAMPSHIRE DURHAM, N. H.

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ABSTRACT

Survival of coliforms, fecal coliforms, fecal streptococci, salmonellae and enteric viruses was determined in oxidation pond systems used by three different communities in New Hampshire for the disposal of domestic wastes. When operated at one or two ponds in a series at temperatures of 17° to 26° C, the die-off rate of coliform, fecal coliform and fecal streptococci was from 95 to 99 percent. During the winter when temperatures were in the 1° to 10° range, the die-off rate was much lower. Salmonellae and enteric viruses were isolated from a majority of the samples of effluent from these ponds during all seasons. When the ponds were operated in a series of three or four and at temperatures of 10° to 26° C, very few viable indicator bacteria remained in the effluent from the last pond. However, enteric viruses were isolated from a number of these effluent samples. Indicator bacteria and enteric pathogen isolations were more frequent during the winter.

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Survival of Enteric Bacteria and Viruses in Oxidation Pond Systems

There is limited information in the literature on the fate or survival of enteric bacteria and viruses in oxidation pond systems and on the presence and numbers of these organisms in effluents from such ponds. The majority of the reports published to date have been concerned chiefly with coliform reduction or die-off. Okum (1) reported 99.99 per cent reduction of coliforms in each of five experimental ponds when loaded with up to 100 pounds of BOD per acre per day. Drew (2) found maturation ponds to reduce *Escherichia coli* 99.6 per cent in summer and 96.8 per cent in winter. Coetzee and Fourie (3) report a 99.98 per cent reduction of *E. coli* in stabilization ponds and because of the apparent ease with which it is eliminated, they consider this organism may not be an infallible indicator of pathogenic organisms in such pond effluents. This point of view was also expressed by Malcow-Moller, *et al* (4) who considered *Streptococcus faecalis* and *Clostridium welchii* more reliable indicators of pathogens than *E. coli* in the systems they studied. Gann, *et al* (5) found coliform reduction closely associated with BOD removal indicating that the coliforms are removed because of their inability to compete successively for nutrients. Geldreich, *et al* (6), in a study of raw sewage and effluent from a waste stabilization pond located at a state prison dairy farm, reported a reduction in coliform bacterial density from a low of 85.9 per cent in the winter to a high of 94.4 per cent in the autumn. Fecal coliform reductions were greater than 87.9 per cent while reductions of fecal streptococci were 97.4 per cent or more.

There have been few reports in the literature on the die-off of pathogens in oxidation ponds. Coetzee and Fourie (3) found the total reduction of *Salmonella typhi* in the effluent from two stabilization ponds operated in a series with detention periods of 20 days to be 99.5 per cent. The reduction of *E. coli* was 99.98 per cent during this same period. In heavily polluted water they found *S. typhi* more resistant to die-off than *E. coli*.

Using *Staphylococcus aureus* and *Serratia marcescens* as test organisms in dialysis tubing immersed in the aerobic zone of a lagoon with a detention time of 6 to 7 weeks, Conley, *et al* (7) found an almost complete die-off of these organisms in 36 to 48 hr. This was not the case in the deeper anaerobic zone.

In regard to the elimination of viruses, Malherbe, *et al* (8) using a model system of four ponds in series, concluded that the biological processes which improve the effluent chemically and bacteriologically were unlikely to alter the virus content so that virus removal depended on their adsorption to static surfaces, exposure to rays of the sun, or a retention beyond the normal survival time of the virus. Christie (9) found that field lagoons reduced the titre levels of polio virus from 10^5 to less than 10^3 units per ml.

With the increased use of lagoons or oxidation ponds as an economical method of domestic waste disposal in this country, further information is needed regarding the potential pollutant hazards such pond effluents may have on receiving waters. In the present study, the survival of coliforms, fecal coliforms, fecal streptococci, salmonellae, and enteric viruses was determined in oxidation pond systems used by three different communities in New Hampshire for the disposal of domestic wastes. Particular attention was given to the effects of seasonal temperatures and the operation of the ponds in series on the survival of these microorganisms.

Materials and Methods

Oxidation ponds. The oxidation ponds used for these studies included a one-pond system for Community A, 8 acres in area serving a population of 600 families, a three-pond system for Community B, each pond 5 acres in area treating about two million gallons of domestic waste per week, and a four-pond system for Community C, each pond approximately 10 acres in area treating eight to ten million gallons of domestic waste per week. All ponds were 5 feet in depth. The ponds for Communities B and C could be operated in series. The final effluent from the Community A system was discharged into a fresh water river and from Community C into an estuary which drained into shellfish growing waters. An aerial photograph of the Community C oxidation ponds with the estuary to the left of Ponds 3 and 4 is shown in Figure A.

Figure A. Aerial photograph of Community C oxidation ponds.

Collection and processing of samples. Samples of raw sewage and pond effluents were collected in sterilized disposable quart containers attached to a sterilizable handle. All samples were transported to the laboratory in an iced container and processed immediately.

The numbers of coliforms and fecal coliforms in the samples were established using standard membrane filter procedures or the confirmed MPN method using 5 replicates in 3 significant dilutions (10). Standard membrane filter procedures (10) or a plating technique were employed for the enumeration of fecal streptococci using m-enterococcus agar (BBL).

Procedures similar to those employed by Slanetz, et al (11) were used for the detection of salmonellae. These organisms were concentrated by filtering 1 liter of the sample through a coarse cotton pad covered with about 0.2 grams of diatomaceous earth (Celite - Johns-Manville product) held in a 100 ml diameter Buchner funnel. After filtration of the sample, the pad was cut in half and one section placed into a 250 ml widemouth bottle containing 100 ml cystine selenite broth (BBL) plus 60 micrograms of albamycin per ml to inhibit Proteus organisms, and a layer of mineral oil over the surface to inhibit pseudomonads with incubation at 43 C. The other half of the pad was placed in a similar bottle containing 100 ml of tetrathionate broth (Difco) plus the albamycin and mineral oil with incubation at 35 C. At 24 hr intervals up to 7 days, 0.1-0.2 ml amounts of these cultures were smeared on bismuth sulfite agar (Difco) and brilliant green agar (Difco). Following incubation at 35 C, colonies characteristic for salmonellae were identified by the procedures recommended by Sanders, et al (12). This involved transferring growth from typical colonies into Sander's broth, incubating cultures at 35 C, and subjecting those cultures producing a permenant blue or purple color without pellicle to IMViC and fermentation tests. Finally, cultures giving positive reactions for salmonellae were serologically typed using Poly OA-1 Salmonella antiserum (Difco).

Virus isolations from water were accomplished using gauze pads suspended at the collection site for periods of 3 to 7 days. Fluids and fine particlate matter expressed from pads at pH 8.0 to 8.5 were clarified by light centrifugation and treated overnight with an equal volume of ethyl ether. The subnatant aqueous layer was recovered, clarified by low speed centrifugation, and following loss of ether by evaporation at room temperature, inoculated into cell cultures. Three types of cell cultures were used for each examination: primary monkey kidney (African Green), HEp-2 and a stable monkey kidney cell line, $LLCMK_2$. Cell culture monolayers in plastic flasks were overlain with inocula, a 2 hr adsorption period at room temperature observed, followed by rinsing of monolayers and addition of maintenance media. The cultures were incubated at 36.5 C and observed daily for evidence of cytopathogenic effects. All samples were passed twice in each cell culture type before they were considered negative for virus.

Photographs of sample collection and testing procedures are shown in Figures B, C, D, and E.

Figure B. Collection of samples from ponds.

Figure C. Membrane filter tests for coliforms.

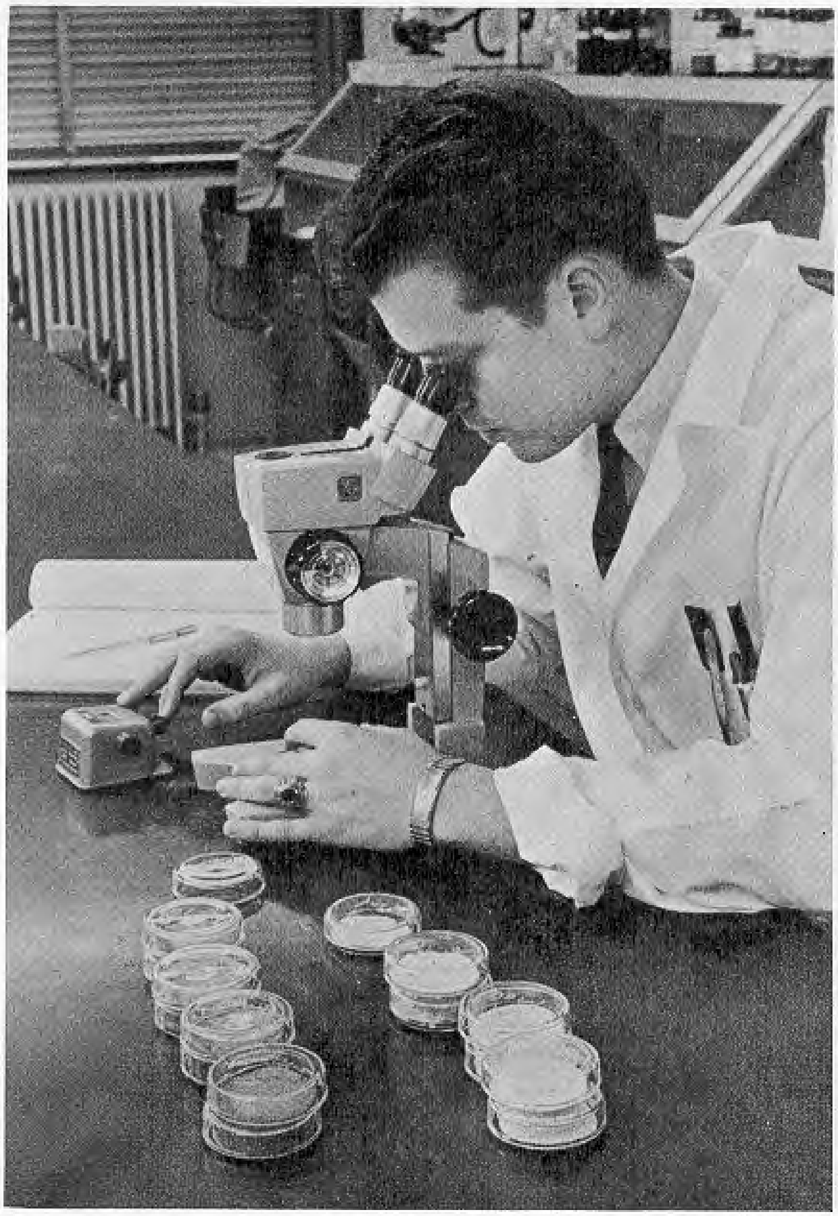

Figure D. Counting colonies of coliforms on membrane filters.

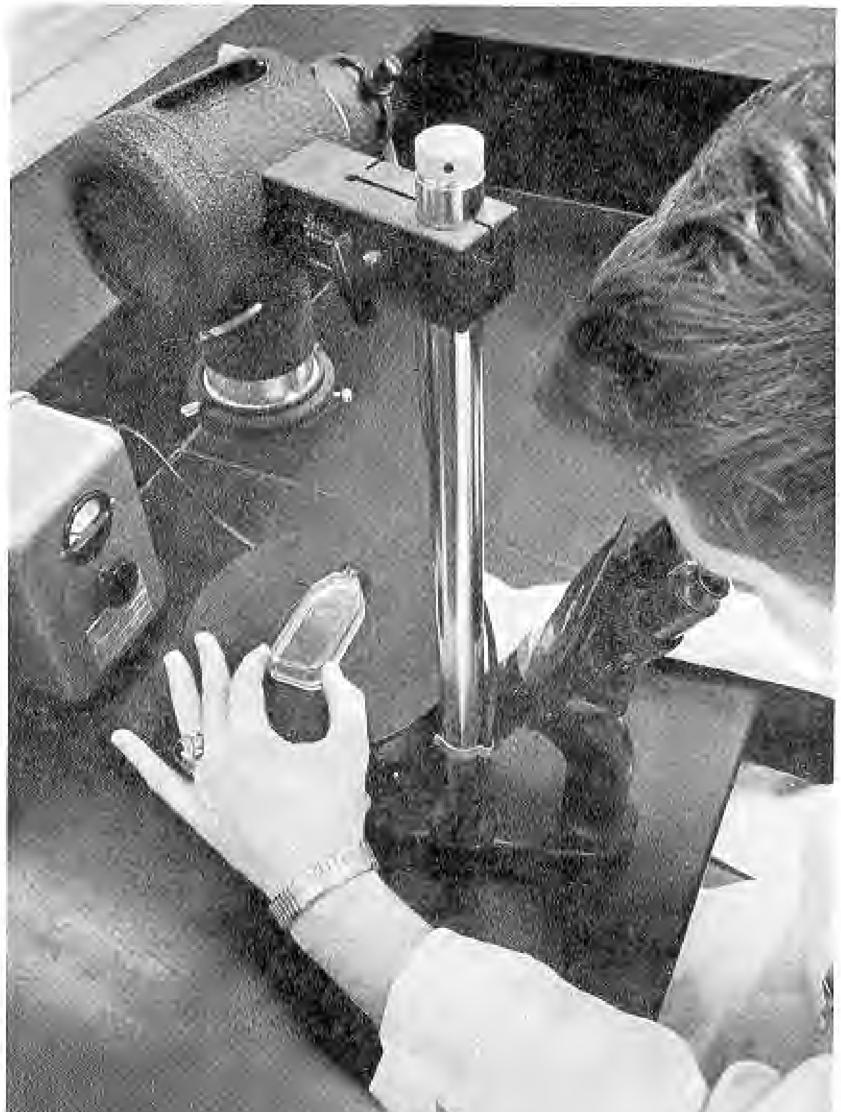

Figure E. Examining cell culture monolayers for viruses.

Results

The numbers of indicator bacteria in raw sewage and the numbers or per cent surviving in effluents from oxidation ponds used to dispose of domestic wastes from three communities are presented in Figures 1-6 and Tables 1-6. The temperatures of the sewage or effluents at the time of collection are noted in the figures and tables, and the salmonellae and enteric virus isolations are indicated in the tables.

The survival rates of coliforms, fecal coliforms, and fecal streptococci in the one-pond system used by Community A ranged from 1.5 to 5, 1.6 to 3, and 0.5 to 10 per cent respectively based on the numbers of these bacteria detected in the effluents as compared to the numbers in the raw sewage discharged into this pond (Figure 1 and Table 1). The numbers of coliforms in the effluents from this pond which was discharged directly into a small river, ranged from 1,400 to 6,000 per ml. The highest per cent survival of the fecal coliforms and fecal streptococci occurred during the low temperature season, September through December. Salmonellae were isolated from 2 out of 5 of the effluent samples and enteric viruses from 2 out of 2 effluent samples tested from this pond.

The results of survival studies of indicator organisms, salmonellae, and enteric viruses in the oxidation pond systems used by Community B are tabulated in Figures 2 and 3 and Tables 2 and 3. The first tests were made when two ponds were being

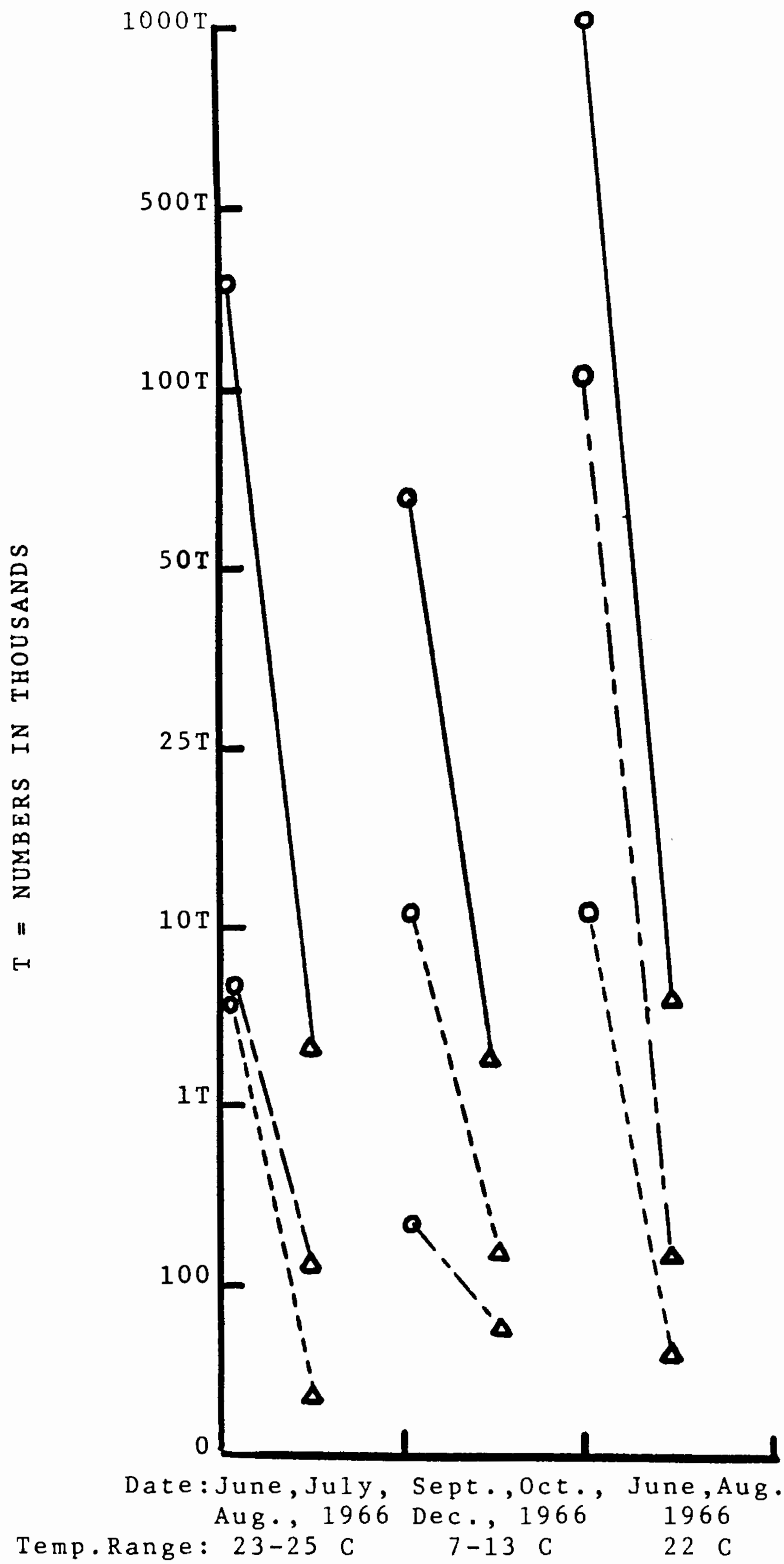

FIGURE 1.--Numbers of indicator bacteria per ml in sewage and pond effluents based on geometric mean counts of weekly samples during test periods. Community A - One pond. ———, coliforms; — — — —, fecal coliforms; -------, fecal streptococci; O, sewage; Δ, Pond 1 effluent.

Table 1

Survival of Indicator Bacteria, Salmonellae, and Enteric Viruses in a One Oxidation Pond System - Community A

Date	Temperature Range	Coliforms	Fecal Coliforms	Fecal Streptococci	Isolation of: Salmonallae	Virus
6/66 - 8/66	23-25 C	1.5*	2.0*	0.5*	1/4**	2/2**
9/66 - 12/66	7-13 C	2.6	3.0	10.0	3/3	2/2
6/67 - 8/67	22-25 C	5.0	1.6	0.6	1/1	-t

* Per cent survival of these bacteria in pond effluents based on numbers originally present in the raw sewage. Percentages represent geometric mean of membrane filter counts for the total number of weekly samples collected during the test periods.

** Number of times isolated from samples tested.

t No test.

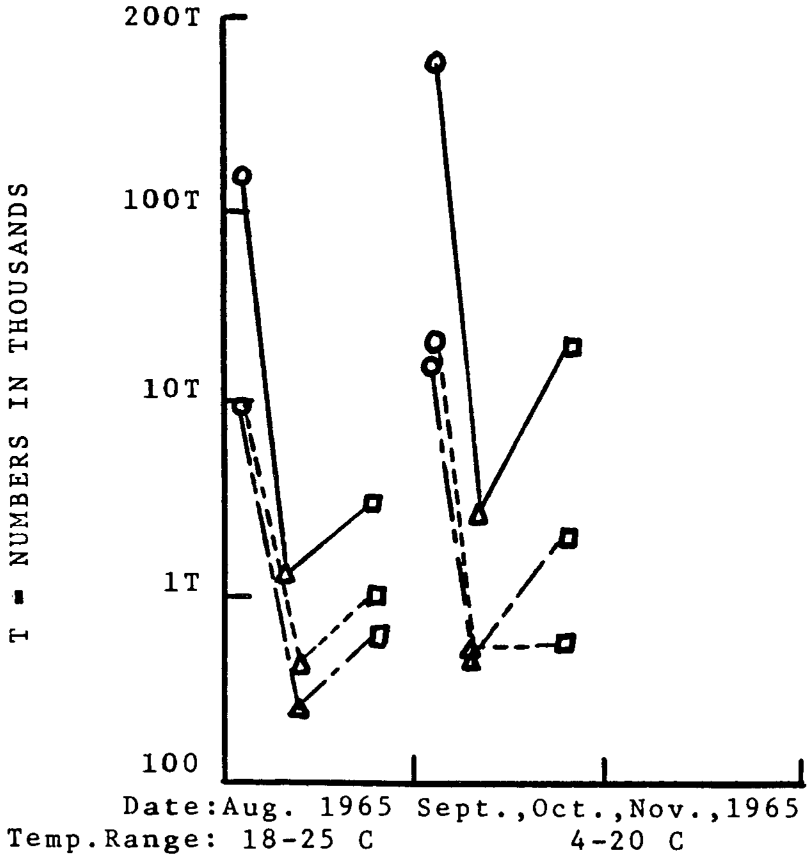

FIGURE 2.--Numbers of indicator bacteria per ml in sewage and pond effluents based on geometric mean counts of weekly samples during test periods. Community B - Two ponds in series.———, coliforms; —·—·—, fecal coliforms; -------, fecal streptococci; O, sewage; Δ, Pond 1; □, Pond 2 effluents.

Table 2

Survival of Indicator Bacteria, Salmonellae, and Enteric Viruses In Two Oxidation Ponds Operated in Series - Community B

Date	Temperature Range	Coliforms	Fecal Coliforms	Fecal Streptococci	Isolation of: Salmonellae	Virus
			Pond 1			
8/65	18-25 C	1.3*	4.6*	7.0*	-t	-t
9/65-11/65	20-4 C	2.6	3.8	3.5	1/10**	4/4**
			Pond 2			
8/65	18-25 C	2.7	8.3	9.0	-	-
9/65-11/65	20-4 C	11.4	12.9	3.4	4/10	4/4

* Per cent survival of these bacteria in pond effluents based on numbers originally present in the raw sewage. Percentages represent geometric mean of membrane filter counts for the total number of weekly samples collected during the test periods.

t No test.

** Number of times isolated from samples tested.

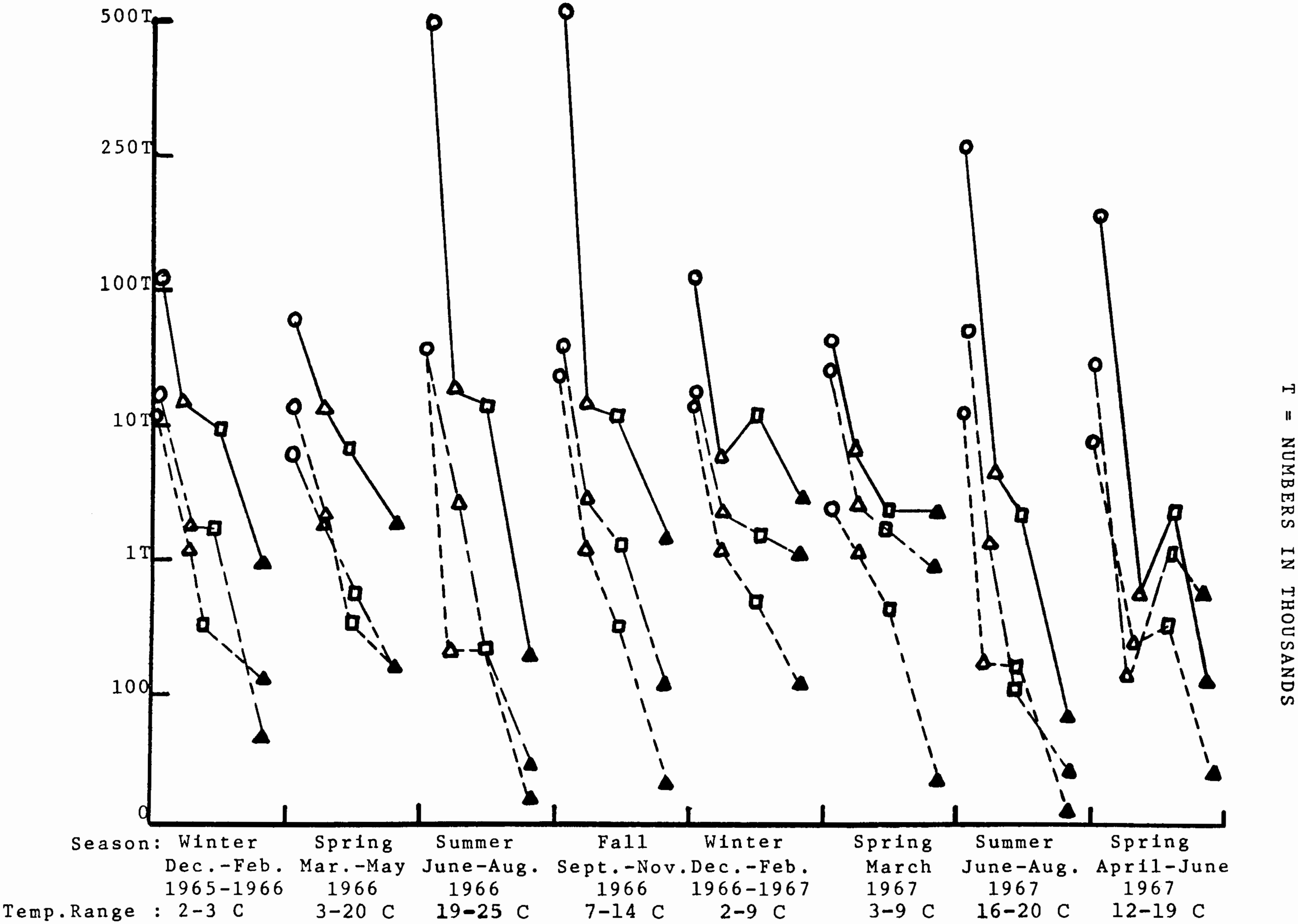

FIGURE 3.--Numbers of indicator bacteria per ml in sewage and pond effluents based on geometric mean counts of weekly samples during test periods. Community B - Three ponds in series. ———, coliforms; — - —, fecal coliforms; -----, fecal streptococci; O, sewage; △, Pond 1; □, Pond 2; ▲, Pond 3 effluents.

Table 3

Survival of Indicator Bacteria, Salmonellae, and Enteric Viruses In Three Oxidation Ponds Operated in Series - Community B

Date	Temperature Range	Coliforms	Fecal Coliforms	Fecal Streptococci	Isolation of: Salmonella	Virus
			Pond 1			
12/65-2/66	2-3 C	9.9*	11.2*	10.5*	2/10**	8/8**
3/66-5/66	3-20 C	15.2	41.2	32.0	6/14	14/14
6/66-8/66	19-25 C	6.4	8.6	0.8	8/11	5/5
9/66-11/66	7-14 C	3.4	2.1	2.1	2/3	1/2
12/66-2/67	2-9 C	5.6	18.4	9.9	3/4	0/1
6/67-8/67	16-26 C	2.7	2.3	3.6	5/6	-t
			Pond 2			
12/65-2/66	2-3 C	5.9	10.7	6.1	2/10	10/10
3/66-5/66	3-20 C	12.1	9.8	4.6	4/14	12/12
6/66-8/66	19-25 C	5.3	8.7	0.2	8/11	5/5
9/66-11/66	7-14 C	2.2	1.2	1.2	2/3	1/1
12/66-2/67	2-9 C	8.3	7.8	4.2	2/4	1/2
6/67-8/67	16-26 C	1.1	0.2	2.5	5/6	-
			Pond 3			
12/65-2/66	2-3 C	0.6	0.3	1.6	0/10	-
3/66-5/66	3-20 C	0.5	0.1	2.6	10/14	12/12
6/66-8/66	19-25 C	0.1	0.1	0.1	4/11	-
9/66-11/66	7-14 C	0.3	0.3	0.3	1/3	-
12/66-2/67	2-9 C	3.2	4.0	1.1	1/4	0/1
6/67-8/67	16-26 C	0.03	0.06	0.09	2/4	-

* Per cent survival of these bacteria in pond effluents based on numbers originally present in the raw sewage. Percentages represent geometric mean of membrane filter counts for the total number of weekly samples collected during the test periods.

** Number of times isolated from samples tested.

t No test.

used in series. As the data presented in Figures 2 and Table 2 indicate, the die-off rate of the indicator bacteria in Pond 1 was similar to that reported for the Community A one pond system. However, the numbers of indicator bacteria in effluents from Pond 2 were somewhat greater than the numbers present in the Pond 1 effluents. During the period of this study, there was only a small discharge of effluent from Pond 2 due to seepage of the liquid waste through the gravel bottom of this pond providing a stagnant condition which apparently was suitable for some growth of the indicator bacteria. Salmonellae were isolated from 1 out of 10 samples from Pond 1, and 4 out of 10 samples from Pond 2 respectively. Enteric viruses were isolated from all of the four samples tested from each of the ponds. The results of tests for indicator bacteria and enteric pathogens when the oxidation ponds for Community B were operated in a three-pond series are indicated in Figure 3 and Table 3 over an 18-month period. It is apparent from this data that there was some decrease in the numbers of the indicator bacteria in effluents from Pond 2 as compared to Pond 1, and that a considerable further die-off occurred in Pond 3. It can also be noted that the greatest die-off of these organisms occurred during the summer months. For example, for Pond 3 effluents the percentage survival of coliforms, fecal coliforms, and fecal streptococci was 3.2, 4.0, and 1.1 for the test period December 1, 1966 to February 28, 1967 when the temperatures of the pond ranged from 2-9 C, and 0.03, 0.06 and 0.09 respectively for the period June

1, 1969 to August 31, 1969, when the temperatures ranged from 16 to 26 C. Salmonellae and enteric virus isolations were still detected in effluent samples from all three ponds.

Studies were initiated on effluents from the oxidation ponds provided for the disposal of the domestic waste from Community C shortly after they were put into operation. Figure 4 and Table 4 give the numbers of indicator bacteria and the enteric pathogen isolations for sewage and effluent samples when the system was operated on the basis of two ponds in series over a 5-month period. The results were similar to those reported for the Community B two-pond system. In general, there was no major increased die-off of these organisms in Pond 2 over that occurring in Pond 1. During the month of July there was an increase in the number of coliforms in the Pond 2 effluent compared to the Pond 1 effluent. Isolations of salmonellae and enteric viruses were similar to both pond effluents.

The results obtained when this oxidation pond system was operated using three ponds in series are tabulated in Figure 5 and Table 5 based on tests made over a 9-month period. The percentage survival of the indicator bacteria in effluents from Ponds 1 and 2 are similar to those reported previously although there was an appreciable additional die-off in Pond 2 during the period July 1, to November 30, 1967. Again there was greater survival of these organisms during December to March when the temperatures were 2-12 C. The almost total die-off of indicator bacteria and salmonellae was quite dramatic in Pond 3 effluents during the period July 1, to October 30, 1967. For example,

Table 4

Survival of Indicator Bacteria, Salmonellae and Enteric Viruses In Two Oxidation Ponds Operated in Series - Community C

Date	Temperature Range	Coliforms	Fecal Coliforms	Fecal Streptococci	Isolation of : Salmonella	Virus
			Pond 1			
6/66	17-25 C	15.0*	13.7*	2.5*	3/3**	1/1**
7/66	18-25 C	5.5	5.0	1.4	4/4	1/2
8/66	19-24 C	0.8	1.3	0.5	2/5	1/2
9/66	19-24 C	0.5	3.8	0.3	2/2	2/2
10/66	13-19 C	8.3	10.0	3.0	4/6	3/3
			Pond 2			
6/66	17-25 C	4.8	6.4	1.2	2/3	1/1
7/66	18-25 C	14.4	27.6	1.0	2/3	1/2
8/66	19-24 C	1.6	1.1	0.5	3/5	2/2
9/66	19-24 C	0.02	0.4	0.04	2/2	1/1
10/66	13-19 C	2.0	2.7	2.1	8/8	3/3

* Per cent survival of these bacteria in pond effluents based on numbers originally present in the raw sewage. Percentages represent geometric mean of membrane filter counts for the total number of weekly samples collected during the test periods.

** Number of times isolated from samples tested.

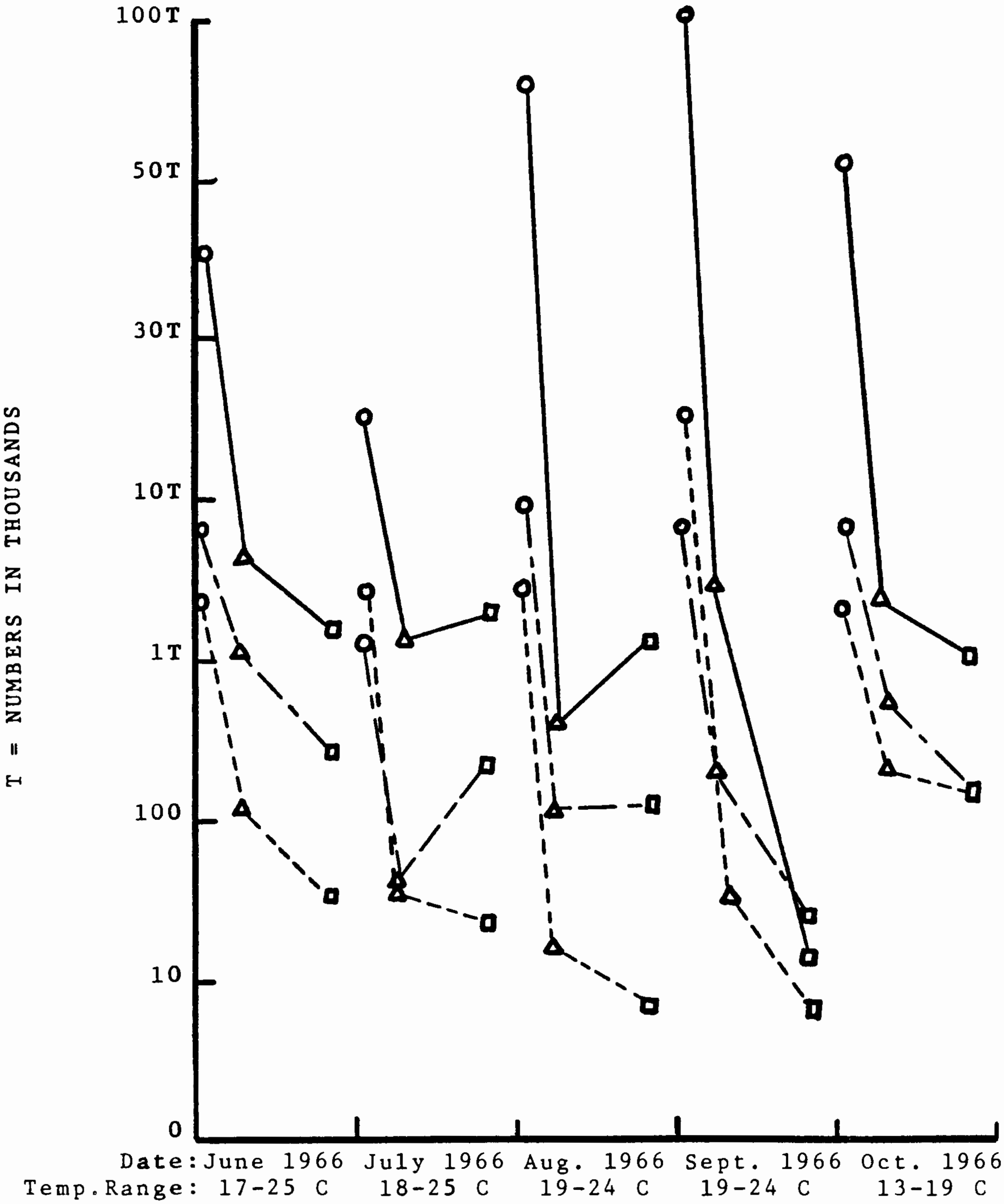

FIGURE 4.--Numbers of indicator bacteria per ml in sewage and pond effluents based on geometric mean counts of weekly samples during test periods. Community C - Two ponds in series. ————, coliforms; — — — —, fecal coliforms; -------, fecal streptococci; O, raw sewage; Δ, Pond 1; □, Pond 2 effluents.

Table 5

Survival of Indicator Bacteria, Salmonellae, and Enteric Viruses In Three Oxidation Ponds Operated in Series - Community C

Date	Temperature Range	Coliforms	Fecal Coliforms	Fecal Streptococci	Isolation of: Salmonellae	Virus
			Pond 1			
7/67	19-26 C	1.7*	4.0*	0.75*	-t	-t
8/67	19-25 C	10.1	12.2	2.5	-	-
9/67	17-21 C	3.9	8.5	9.5	-	-
10/67	9-16 C	2.2	1.8	9.0	-	-
11/67	4-14 C	4.9	6.3	9.2	-	-
12/67	2-12 C	7.9	14.5	31.2	-	-
1/68	3-10 C	17.0	85.0	50.5	-	-
2/68	1-10 C	24.5	7.4	13.5	-	-
3/68	2-10 C	34.0	10.0	6.4	-	-
			Pond 2			
7/67	19-26 C	0.03	0.02	0.05	-	-
8/67	19-25 C	0.24	0.1	1.0	-	-
9/67	17-21 C	0.06	0.3	0.7	-	-
10/67	9-16 C	0.17	0.2	1.3	-	-
11/67	4-14 C	0.8	0.5	1.4	-	-
12/67	2-12 C	12.0	16.0	7.8	-	-
1/68	3-10 C	4.3	12.0	38.3	-	-
2/68	1-10 C	8.9	9.0	6.9	-	-
3/68	2-10 C	25.3	22.8	4.2	-	-
			Pond 3			
7/67	19-26 C	0.02	0.02	0.04	1/4**	-
8/67	19-25 C	0.02	0.003	0.003	0/4	-
9/67	17-21 C	0.002	0.001	0.001	0/2	-
10/67	9-16 C	0.005	0.004	0.08	0/5	3/3**
11/67	4-14 C	0.05	0.05	1.1	1/4	2/2
12/67	2-12 C	2.0	1.6	2.8	1/3	-
1/68	3-10 C	1.6	6.0	17.1	1/4	1/1
2/68	1-10 C	10.0	10.0	5.2	0/4	4/4
3/68	2-10 C	3.7	5.1	3.9	0/4	-

* Per cent survival of these bacteria in pond effluents based on numbers originally present in the raw sewage. Percentages represent geometric mean of MPN counts for coliforms and plate counts for fecal streptococci of weekly samples collected during the test periods.

t No test.

** Number of times isolated from samples tested.

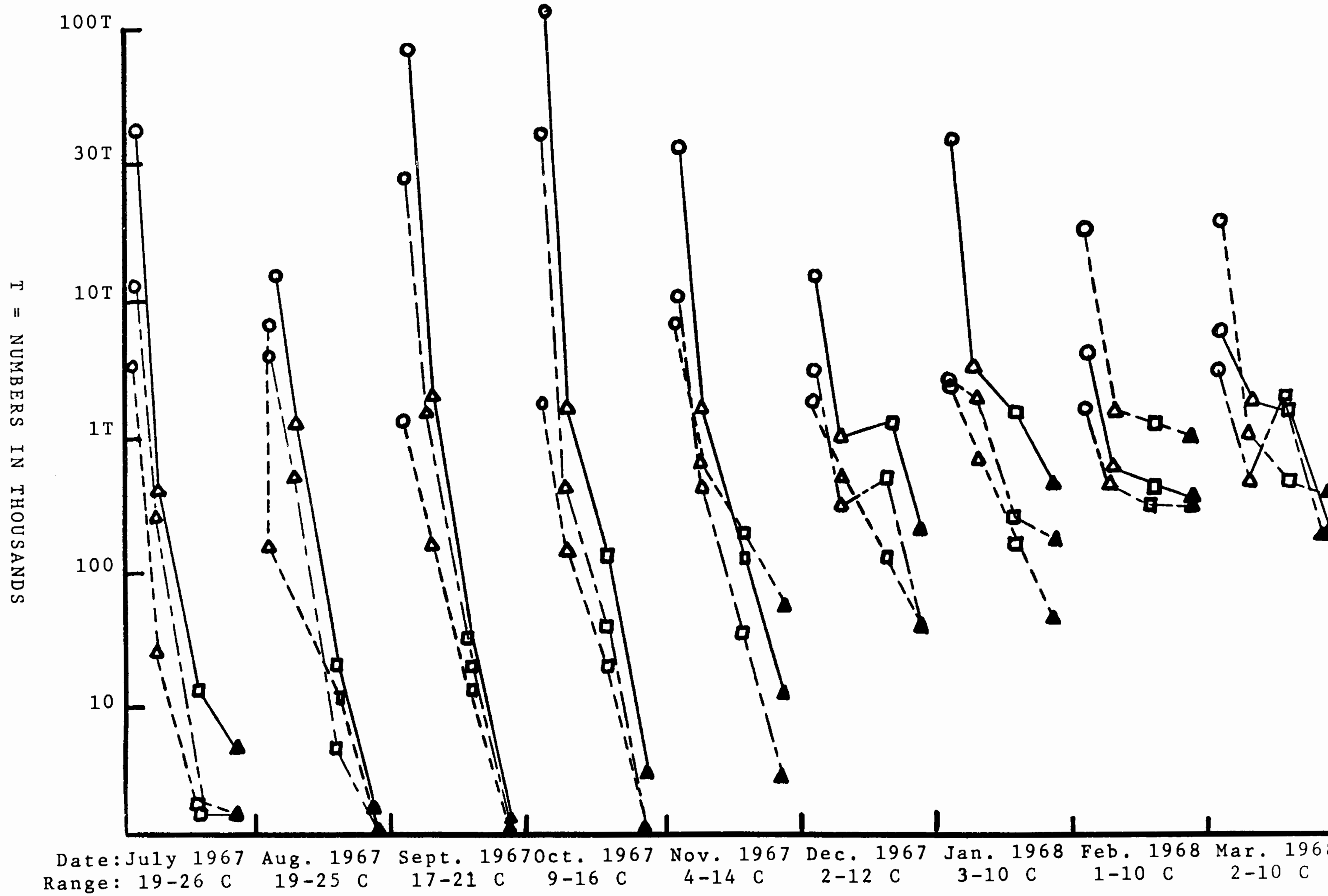

FIGURE 5.--Number of indicator bacteria per ml in sewage and pond effluents based on geometric mean counts of weekly samples during test periods. Community C - Three ponds in series. ________, coliforms; ___ _ ___ _, fecal coliforms; ------, fecal streptococci; O, sewage; △, Pond 1 effluents; □, Pond 2 effluents; ▲, Pond 3 effluents.

based on weekly tests made during the month of September when the temperature ranged from 19-25 C, the per cent survival of coliforms, fecal coliforms, and fecal streptococci was 0.002, 0.001, and 0.001 respectively. No salmonellae were isolated from 2 samples tested during this period. It is also of interest to note that for samples tested during August, when the per cent survival of coliforms, fecal coliforms, and fecal streptococci was 0.005, 0.004, and 0.08 respectively, no salmonellae were isolated from 5 weekly samples tested but enteric viruses were isolated from all 3 effluents examined. It might be noted here that Poliomyelitis Type I, II, and III viruses were isolated most frequently from the various pond effluents tested. Isolation of Coxsackievirus B4 and ECHO virus Type 6 were also accomplished. It should also be pointed out that procedures used for the detection of both salmonellae and enteric viruses involved the concentration of these organisms by filtration of the samples for salmonellae and suspension of gauze pads at the point of sewage or effluent discharge for the viruses. Thus, no information was provided as to the numbers of these organisms in the raw sewage or the pond effluents.

Finally, the results of studies on the survival of indicator bacteria and salmonellae are presented in Figure 6 and Table 6 when these ponds were operated in a four-pond series for an 8-month period. These data were based on tests of effluents from Ponds 2 and 4 only. The Pond 4 effluent was discharged directly into an estuary without further treatment.

Table 6

Survival of Indicator Bacteria and Salmonellae
In Four Oxidation Ponds Operated in Series - Community C
(Data for Ponds 2 and 4 only)

Date	Temperature Range	Coliforms	Fecal Coliforms	Fecal Streptococci	Isolation of : Salmonellae
			Pond 2		
11/66	5-15 C	1.8*	2.1*	1.4*	2/4**
12/66	2-13 C	1.9	1.6	3.2	2/4
1/67	2-11 C	6.0	6.7	3.4	1/4
3/67	3-9 C	7.7	7.3	1.2	2/3
4/67	6-11 C	5.6	8.1	5.4	0/1
5/67	10-15 C	-t	-t	-t	-t
6/67	17-24 C	3.0	0.81	0.69	1/4
			Pond 4		
11/66	5-15 C	0.15	0.12	0.05	0/4
12/66	2-13 C	0.12	0.12	0.05	0/4
1/67	2-11 C	0.5	1.1	2.2	0/4
3/67	3-9 C	5.3	1.0	4.6	2/3
4/67	6-11 C	0.05	0.08	0.4	1/3
5/67	10-15 C	0.01	0.0	0.04	0/5
6/67	17-24 C	0.02	0.05	0.1	0/4

* Per cent survival of these bacteria in pond effluents based on numbers originally present in the raw sewage. Percentages represent geometric mean of membrane filter counts for the total number of weekly samples collected during the test periods.

** Number of times isolated from samples tested.

t No test.

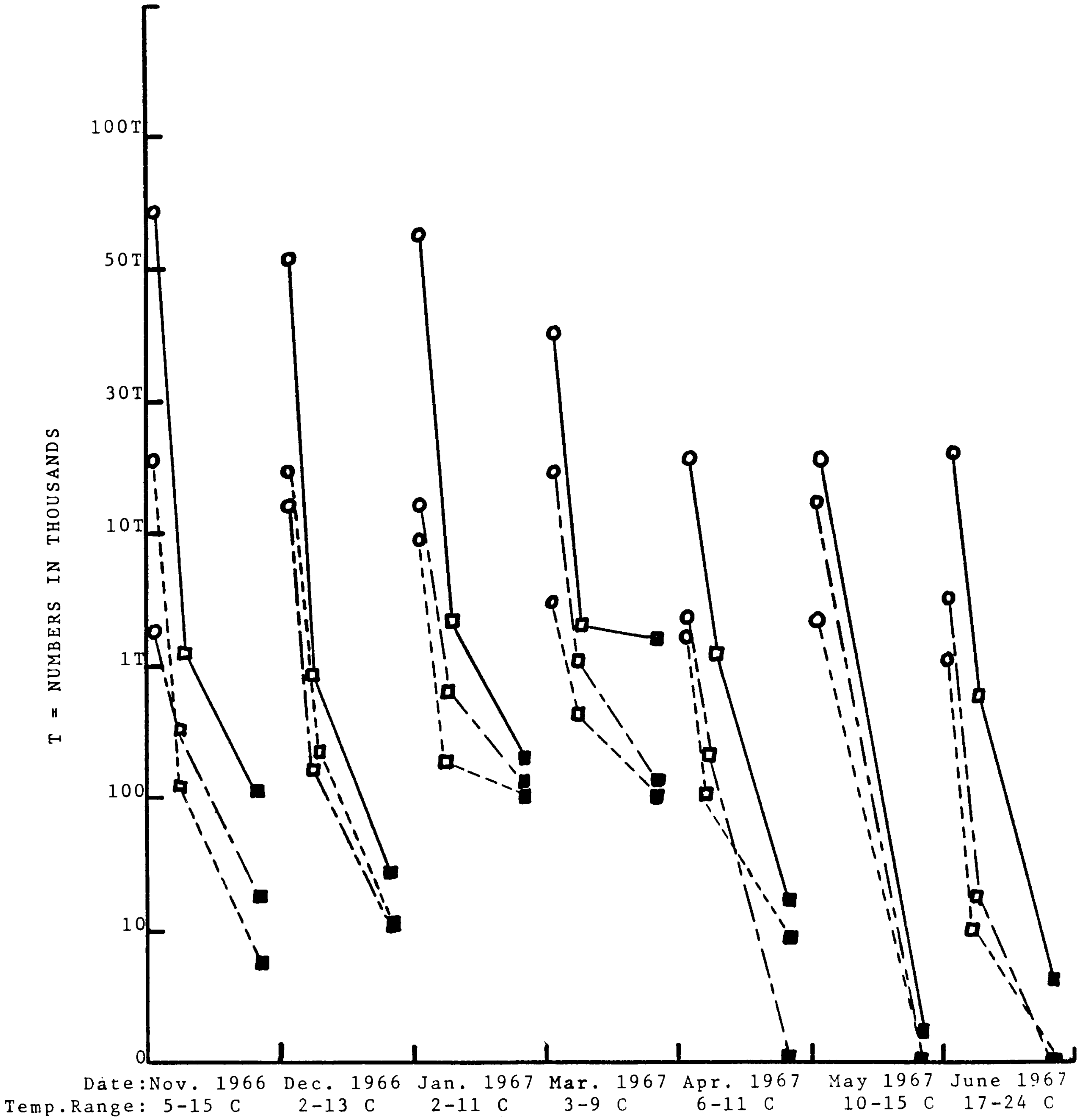

FIGURE 6.--Numbers of indicator bacteria per ml in sewage and pond effluents based on geometric mean counts of weekly samples during test periods. Community C - Four ponds in series. ———, coliforms; — — — —, fecal coliforms; -------, fecal streptococci; O, sewage; □, Pond 2; ■, Pond 4 effluents.

It is evident from this data that only very small numbers of indicator bacteria were present in the Pond 4 effluents. The greatest die-off in the ponds can again be noted during the warmer season when, for example, in Pond 4 effluents, 8 coliforms, no fecal coliforms, and an average of less than 1 fecal streptococcus per ml were detected in the samples tested during the month of June. However, it is also interesting to note that even during the winter period of December and January only small numbers of indicator bacteria had survived in Pond 4 as compared to Pond 3 effluents as reflected in the data presented in Figure 5 and Table 5. Salmonellae were not detected in effluents from Pond 4 tested during the months of November, December, January, May, and June. It should be noted that the ponds under study froze over during late December, January, and February, thus altering their oxidation status.

During these studies, a limited attempt was made to establish antibiotic factors that might relate to the die-off of the indicator bacteria in the pond systems. Tests for the presence and concentration of coliform and fecal streptococci bacteriophages in the effluents indicated that their numbers decreased during the warm months and increased during the winter season. *Bdellovibrio* organisms could only be isolated from surface mud surrounding the ponds and not from the pond effluents. The major non-enteric bacterial flora that developed in the Community C oxidation ponds consisted of the genera Flavobacter, Achromobacter, and Pseudomonas. When *E. coli* and *Streptococcus faecalis* were inoculated into broth filtrates of several hundred isolates represented in these genera, no toxic effects could be demonstrated. In many of the filtrates, these indicator bacteria actually increased in numbers. Thus, bacteriophages, *Bdellovibrio* and the bacteria chiefly involved in the digestion of organic matter in the ponds did not appear to have a major toxic action on the indicator bacteria. A number of membrane filter filtrates of the pond effluents did show a toxic effect for coliforms and fecal streptococci but this was not consistent with high or low die-off rates in the ponds.

Summary

The die-off of coliforms, fecal coliforms, and fecal streptococci in domestic wastes discharged into the 5 to 10 acre oxidation ponds studied ranged from 95 to 99 per cent when these ponds were operated as one or as two ponds in series at temperatures of 17 to 26 C. The percentage survival of these organisms was much greater during the winter season in such ponds when the temperature ranged from 1 to 10 C. Salmonellae and enteric viruses were isolated from a majority of the effluent samples tested from these ponds during all seasons of the year.

When the ponds were operated in a series of three or four ponds, very few viable indicator bacteria remained in the effluents from the last pond in the series when the temperature of the ponds ranged from 10 to 26 C. Under such conditions, counts as low as 2 per ml or less were obtained for coliforms, fecal coliforms, and fecal streptococci. Salmonellae could be detected in only 1 out of 24 effluent samples tested from the third or fourth pond in the series during the summer periods. However, enteric viruses were isolated from a number of these effluent samples. The numbers of indicator bacteria were appreciably higher and the enteric pathogen isolations more frequent in effluents from the ponds during the winter seasons. Thus, it must be recognized that effluents from oxidation ponds may not be free from bacterial or viral pathogens and such effluents may create a health hazard if discharged without further treatment into waters used for bathing or shellfish growing areas.

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