COLIFORMS, FECAL COLIFORMS, AND FECAL STREPTOCOCCI AS INDICATORS OF WATER POLLUTION

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Abstract. The presence and survival of coliforms, fecal coliforms, and fecal streptococci were studied in sewage treatment plants, heavily polluted rivers, a lake, and other drinking water sources. In all cases the fecal streptococci were generally more resistant to the natural water environment and to purification processes than the other indicator organisms and, at points distant from the original source of pollution were often the only indicators of the fecal nature of the pollution. In two of the systems studied the survival of the fecal streptococci paralleled the survival of enteric viruses better than the coliforms. The fecal streptococci may thus in certain cases provide a better estimate of the probable virus content in lightly contaminated water than the other two indicators.

1. Introduction

The most widely accepted bacterial indicators of fecal pollution in water have been the coliform group of bacteria (WHO International Drinking Water Standards, 1963; Standard Methods, 1965). However, although all coliforms *may* be of fecal origin, they are also to be found in other sources, such as plants, soil, etc. Recently, some attention has been paid to the fecal coliform group as pollution indicators (Geldreich, 1967; Deaner and Kerri, 1969). These coliforms are capable of growth at 44.5° *C* and although they are often all assumed to be *Escherichia coli* this is probably an oversimplification since other strains are often present among the colonies that grow at this temperature (Silberstein *et al.*, 1951; Mishra *et al.*, 1968; Buras and Kott, 1969). Many international and national standards now incorporate both these indicators and tolerate a higher number of coliforms than previously permitted, provided that the fecal coliforms are strictly limited in any given class of water (WHO International Drinking Water Standards, 1963; Standard Methods, 1965; Water Quality Criteria, 1968).

A third group of bacteria, the fecal streptococci, has also been advocated as an indicator of fecal pollution (Slanetz and Bartley, 1964). However, many workers find them to be of limited value as sole indicator but useful in conjunction with either the coliforms or fecal coliforms in establishing the source of pollution (Barbaro *et al.*, 1969; Geldreich and Kenner, 1969).

During a period of $2\frac{1}{2}$ yr the survival of these three indicators was studied under various ecological conditions in Israel and their relative importance as pollution indicators and in particular as predictors of viral pollution assessed for each type of water system examined.

A major section of these studies was carried out within the framework of an overall

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study of the pollution of the Jordan River – Lake Tiberias watershed (Shuval *et al.*, 1971) while another section formed part of a study on the hydrobiology of the Nahal Soreq stream (Dor and Shecter, 1972).

2. Materials and Methods

The following systems were analyzed:

(a) Biological filtration sewage treatment plants,
(b) Self-purifying open sewage stream in dry river bed,
(c) Polluted river, and
(d) Sources of drinking water.

All samples were taken by the grab method either with sterile plastic jerrycans (for large samples) or with sterile stoppered glass bottles (samples up to 200 ml). 0.1 ml 10% sodium thiosulphate per 100 ml sample was added to all containers used for sampling chlorinated supplies. Samples were placed in iced containers and transported as rapidly as possible to the laboratory in Jerusalem. The samples were tested on the day of sampling whenever this was feasible but those which had to travel some distance were held overnight at 4C and tested the next day. Experiments showed that none of the bacterial counts altered as a result of this overnight storage, provided that heavily polluted samples (e.g. untreated sewage effluent) were diluted prior to storage. If left undiluted the coliform and fecal coliform counts in these samples tended to increase by one order of magnitude.

Bacterial Tests

Pilot experiments showed that the membrane filtration (MF) method and the multiple tube most probably number (MPN) method gave comparable results. Thus for ease of handling large numbers of samples, only the MF method was used except in special cases where it proved to be unsuitable (due to interference by other micro-organisms, colloidal matter, etc.) when the MPN method was employed.

Gelman Metricel 0.45 μm filters were used. Coliform bacteria were cultured on *m*-Endo media (Difco) and counted after 24 hr incubation at 35C. Fecal coliforms were incubated on M–FC broth (Difco) for 24 hr in a water bath at 44.5C.

In initial experiments fecal streptococci were incubated for 48 hr at 35C on m-Enterococcus Agar. In later experiments these plates were incubated for 4 hr at 35C followed by 44 hr at 44.5C to eliminate the growth of atypical microcolonies (Mead, 1966; Burman, 1967).

3. Results

1. *Sewage Treatment Plants*

Two plants were investigated: The Tiberias Municipal Plant and the Hadassah Medical Center Plant in Jerusalem. Both plants have primary settling tanks and high rate biological filtration units. The Hadassah plant also has facilities for chlorinating the effluent. Samples for bacteriological assay were taken from several points in the system:

(a) Raw sewage.
(b) After primary settling (Tiberias only).
(c) After biological filtration.
(d) After chlorination (Hadassah plant only).

In the Tiberias plant a parallel study on the same samples was carried out by the Virology Unit of the Environmental Health Laboratory, to follow the effect of the sewage treatment on the virus content of the sewage.

TABLE I

Removal of bacterial indicators and viruses by the Tiberias Sewage Treatment Plant, December 1968–June 1969

Indicator	Raw Sewage	After primary settling		After filtration	
	Bacteria/100 ml	Bacteria/100 ml	% Removal	Bacteria/100 ml	% Removal
Coliforms	3.8×10^9 [a]	6.6×10^8	83	8.4×10^6	92
Fecal coliforms	1.6×10^8	6.1×10^7	62	5.9×10^6	74
Fecal streptococci	8.4×10^6	5.9×10^6	30	2.7×10^6	66
Viruses [b]					
pfu/100 ml	95	–	–	30	31

[a] All bacterial numbers are the log. average of 12 samples.
[b] Virus results from a parallel study carried out by the Virology Unit of the Environmental Health Laboratory Dept. Med. Ecol., Hebrew University Medical School.

The results in Table I show that the efficiency of the Tiberias plant is rather low. The average overall removal of coliforms was 92%, of which 83% was removed during the primary settling stage. With fecal coli the overall efficiency fell to 74% and for the fecal streptococci the average overall efficiency fell to 66% with most of the removal occurring during the biological filtration. (The efficiency of virus removal was even lower with an overall average removal of only 31% – see discussion below.) The Hadassah plant (Table II) was more efficient and even before chlorination 99% of the coliform, 98.6% of the fecal coliform and 96% of the fecal streptococci had been removed. Again the streptococci were more resistant to treatment and even after chlorination this trend persisted.

TABLE II

Removal of bacterial indicators by the Hadassah Sewage Treatment Plant December 1968-March 1971

	Raw Sewage	Filtered effluent		Chlorinated effluent
	Bacteria/100 ml	Bacteria/100 ml	% Removal	Bacteria/100ml
Coliforms	9.5×10^8 [a]	9.5×10^6	99	14
Fecal coliforms	1.3×10^8	1.9×10^6	98.6	6
Fecal streptococci	4.8×10^5	2.0×10^4	96	2.8

[a] All bacterial counts are the log. average of 116 samples.

Similar trends were recorded in pilot experiments following the fate of the 3 indicator organisms in 2 oxidation ponds. Over a 13 day period the coliform and fecal coliform were reduced by 4 orders of magnitude and the fecal streptococci by only 3.

2. *Open Sewage Channel*

Untreated sewage effluent from western Jerusalem flows directly into a seasonal river bed – the Nahal Soreq Wadi, creating a man-made permanently flowing river based solely on sewage effluent, except in the winter months when the flow is augmented by storm run off. Between the sampling stations 1 and 6 the stream flows 30 km.

Most of the sampling was carried out during the winter months when the average water temperature was below 20C; however, due to the geographical situation of the stream there was a 4C rise between stations 1 and 6. All three indictors show significant decreases in number (Figure 1). Again the fecal streptococci had the highest

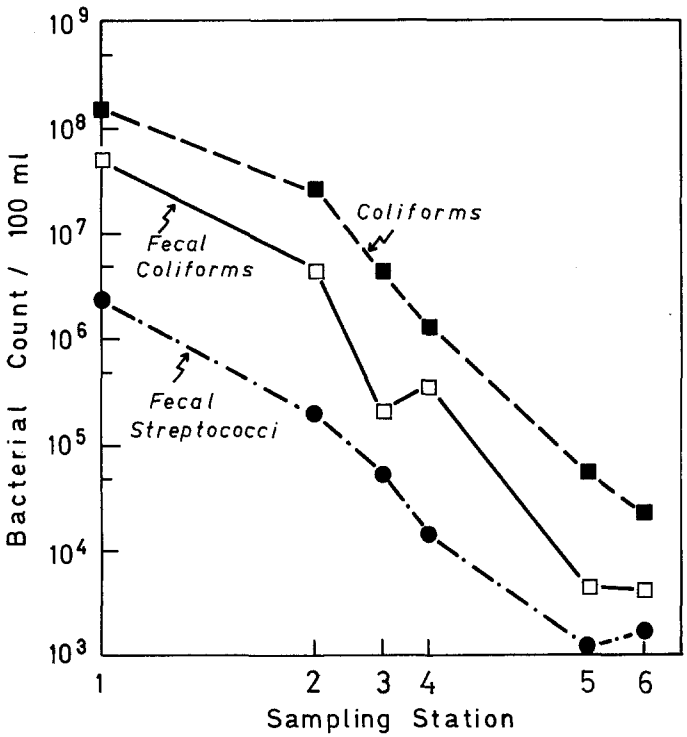

Fig. 1. Average concentration of bacterial pollution indicators at various points in Nahal Soreq (Oct. 1970–May 1971).

survival rate (Figure 2). An inverse relationship was found between the water temperature and the bacterial survival rate which is increased by one order of magnitude during the winter months (Figure 2).

Since the higher temperatures are usually associated with other conditions, such as

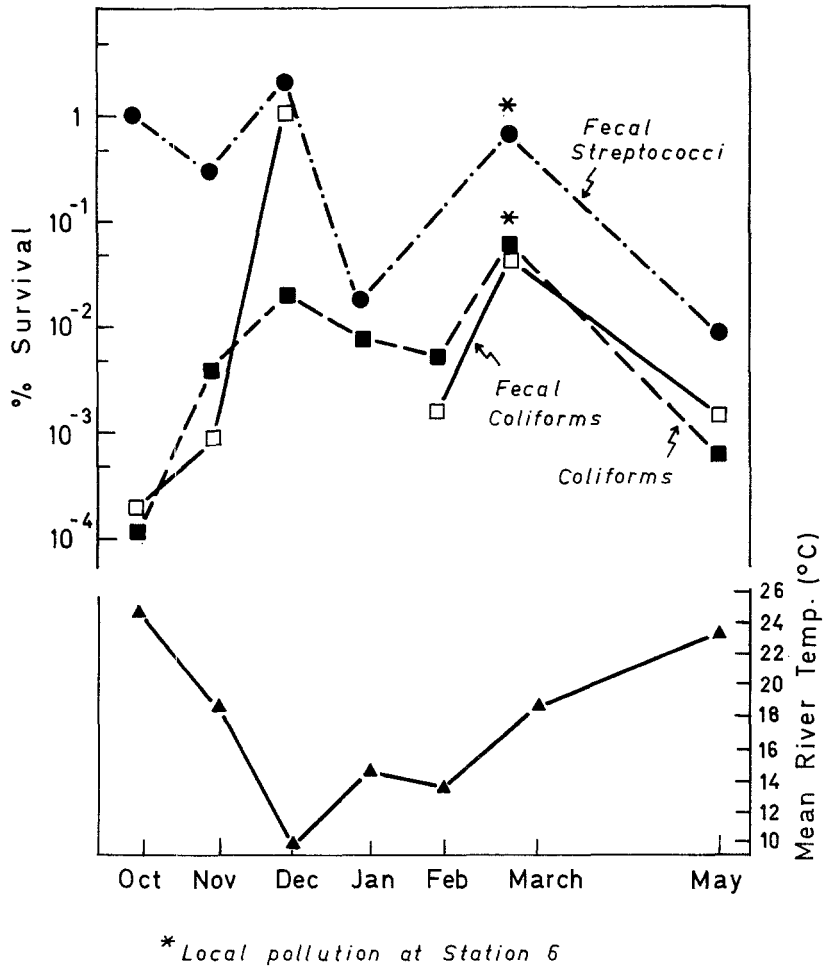

Fig. 2. Relationship between the percentage survival of indicator organisms between stations 1 and 6 and the mean water temperature in Nahal Soreq (Oct. 1970–May 1971).

longer and more intense light radiation, possible increase in antibacterial agents due to increased microbiological activity and lower flow rates, it is not possible at this stage to say which of these changed conditions has the most marked effect on bacterial survival. The relationship between viruses and the bacterial indicators is shown in Figure 3. The disappearance of viruses closely parallels that of the fecal streptococci while the coliforms and fecal coliforms disappear more rapidly. Occasional increases in the number of micro-organisms at points below station 1 were due to intermittent local sources of pollution.

3. *Polluted River*

The upper Jordan river receives the untreated effluent from the town of Qiryat Shemona and from several settlements along its course before it flows into Lake

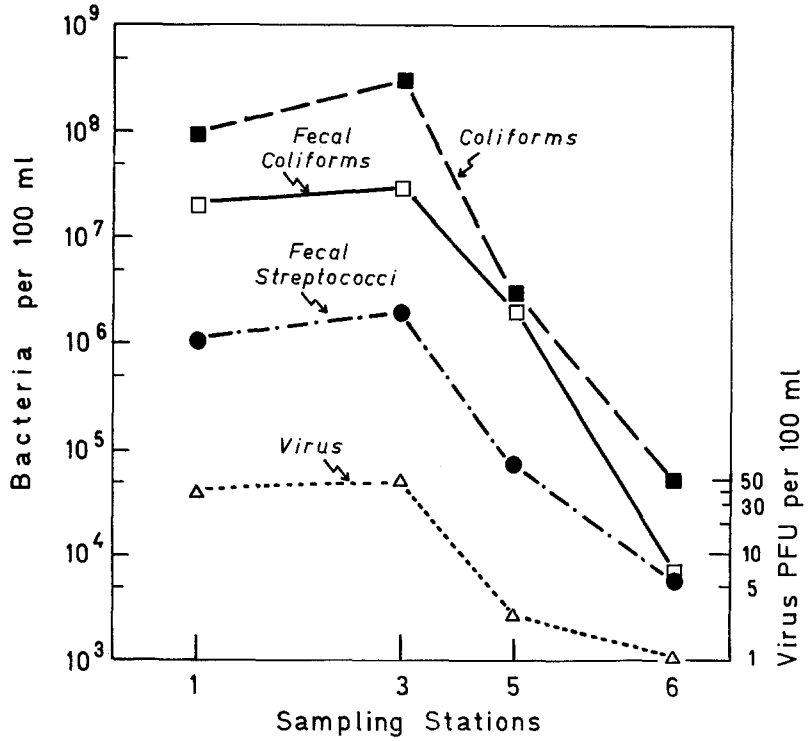

Fig. 3. Concentration of bacterial pollution indicators and virus units in Nahal Soreq (23rd March 1971).

Kinneret at a point 34 km from Qiryat Shemona. Figure 4 shows the fate of the three indicator organisms as the river flows southward. The drop in number between sampling stations 1 and 2 is due to dilution after which there is a period of self purification (stations 2 to 5) before the river enters the lake and bacteria are again greatly reduced in number by dilution. Some seasonal variation was observed in the degree of self purification, which appeared to be more effective in the summer months (Figure 5). Again, this may be due to a combination of the higher temperature, greater light radiation and/or the slower flow (10 to 20 m^3 in the summer, 40 to 100 $m^3\ s^{-1}$ in the winter) which would allow a greater degree of sedimentation. (Similar relationships between temperature and purification were observed in oxidation ponds by Post (1969).) In the case of the Jordan river, another reason for the higher bacterial indicator count in the winter may be due to the effluent from the oxidation ponds of settlements in its catchment area. In summer this effluent is used for irrigation. In addition, the winter rains may cause more polluted storm run-off to reach the Jordan river.

4. *Sources of Drinking Water*

a. *Lake Kinneret.* This lake serves as the source for the Israel National Water Carrier and for the town of Tiberias. Table III shows the bacterial counts obtained from samples taken over a period of $1\frac{1}{2}$ yr. It can be seen that the fecal streptococci

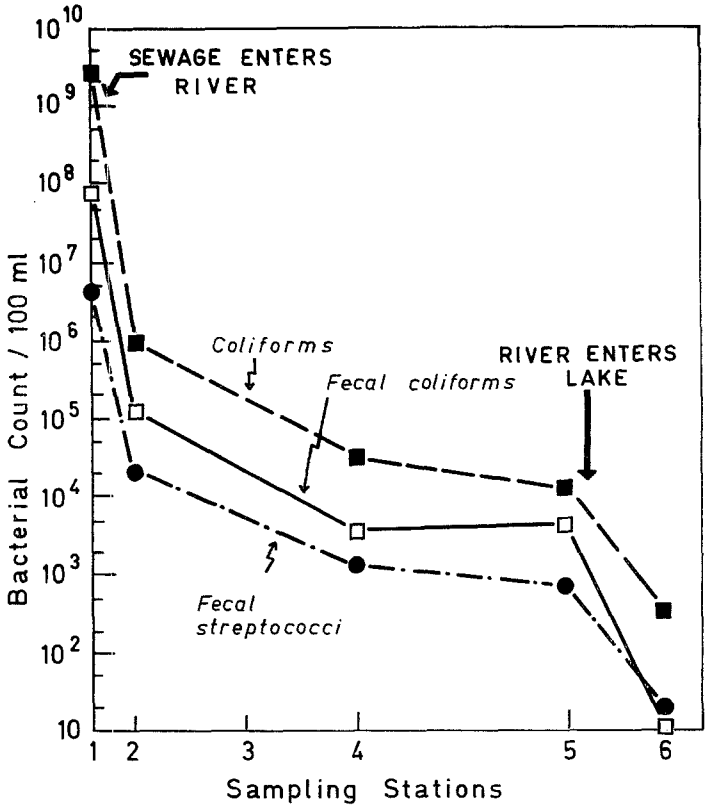

Fig. 4. Average concentration of bacterial pollution indicators in the Jordan River. (Dec. 1968–Nov. 1969).

occurred in greater numbers than the fecal coliforms in 75% of the samples, exceeded the coliforms in 30%, and were present in the absence of fecal coliforms in 40%.

b. *Polluted Springs and Wells.* Two storage wells in the main western Jerusalem water supply pipeline (at points prior to final chlorination), and 2 natural springs were investigated. The results are shown in Table IV. The natural springs were heavily polluted. Spring I had high concentrations of all three indicators at all times with the fecal streptococci having a higher count than the fecal coliforms in 14% of the cases. Spring II was less heavily contaminated and although fecal coli were sometimes absent, fecal streptococci were always detected and were higher than the fecal coliforms in 66% of the samples and even outnumbered the coliforms in 33%. Varying degrees of pollution were detected in the storage wells with the coliforms varying from 1000 to 0 coli per 100 ml. Fecal coliforms were detected only in one sample but fecal streptococci occurred in 3. Overall, the fecal streptococci outnumbered the fecal coliforms in 42% of the samples and fecal streptococci were present in the absence of fecal coliforms in 15.3%. They were then the sole indicators of the fecal nature of the pollution.

TABLE III

Bacteria indicators in Lake Kinneret

Station	Coliforms	Bacteria/100 ml Fecal coliforms	Fecal streptococci
	HNCB[a]	HNCB	6
	0	HNCB	8
	40	10	240
	20	10	40
At the entrance	110	0	2
to Kinnarot	1000	0	10
pumping station	1800	450	340
	40	–	1
	20	10	20
	80	24	0
	500	0	6
	30	0	7
	230	0	8
	6100	260	80
	70	10	6
Tiberias	70	0	86
drinking water	40	6	122
intake	390	0	10
	30	0	30
	60	20	4

[a] HNCB high 'non-coliform' background of atypical colonies made count impossible.

TABLE IV

Bacterial pollution indicators in wells and springs

Source	Coliforms	Bacteria/100 ml Fecal coliforms	Fecal streptococci
Spring I	1×10^6	3×10^2	7.5×10^3
	3.1×10^3	6.8×10^2	6.5×10^2
	1.6×10^4	1.3×10^4	3.4×10^2
	9×10^3	1.5×10^3	4.0×10^2
	9×10^4	2.7×10^4	1×10^2
	6.8×10^3	6×10^3	1.2×10^2
	4.1×10^4	1.9×10^4	2.5×10^2
Spring II	2×10^2	20	2×10^2
	4×10^2	0	1.3×10^2
	2.4×10^3	6.8×10^2	1.2×10^2
	3×10^4	2×10^3	1×10^3
	1×10^3	0	5.9×10^3
	0	0	90
Wells I and II	20	0	2
	100	0	13
	0	0	0
	0	0	0
	2×10^3	0	0
	1.5×10^2	1	1.3×10^2

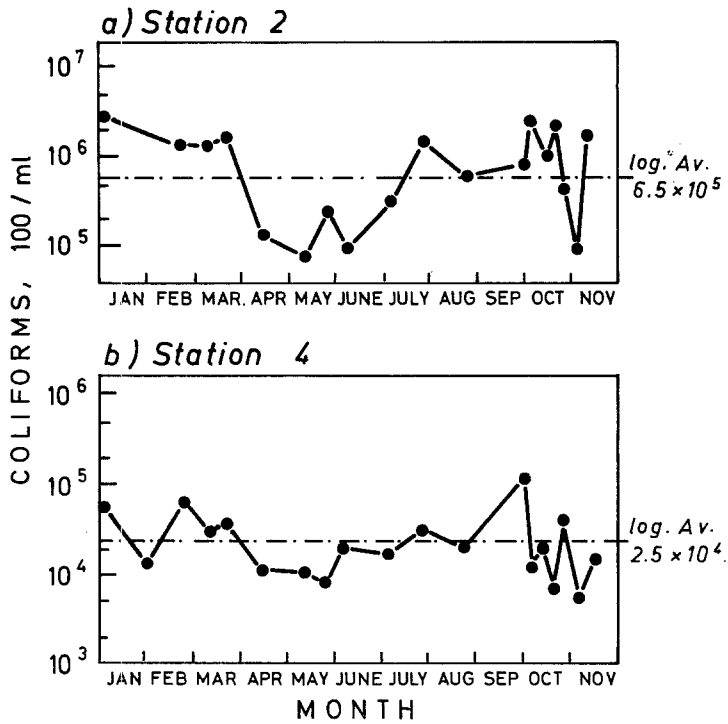

Fig. 5. Variation in coliform counts at points along the upper Jordan River (1969).

4. Discussion

All three indicators tested were satisfactory for assessing the degree of pollution in heavily contaminated water. However, the three indicators differ in their resistance to natural and man-made purification processes. All the systems studied indicate that under local conditions the fecal streptococci are more resistant and therefore more persistent than either the coliforms of the fecal coliforms. This is in agreement with the findings of other workers (Geldreich and Kenner, 1969; Lee *et al.*, 1970). Similar observations have been made in polluted sections of Israeli coastal waters, where the coliform streptococci ratio was 70:1 at points immediately above the sewage outfall and 19:1 in lightly contaminated water 750 m from the outfall. The fecal coliforms and fecal streptococci ratios for the same points were 22:1 and 3:1 (Yoshpe-Purer and Shuval, 1971).

The use of fecal streptococci as pollution indicators has been criticized on account of their lower initial concentration, and the variety of the methods proposed for their isolation, and the uncertainty as to the selectivity of some of these methods (Water Quality Criteria, 1968; Burman, 1967). However, improved membrane filter techniques (The Bacteriological Examination of Water, 1969; Burman, 1967; Mead,

1963), now make it possible to determine whether the streptococci are of human or animal origin or arise from some other source (Burman, 1967).

In raw sewage and freshly polluted water the streptococci are present as chains of cells, so that the unit measured by the MF method is not a single cell but a chain. As the bacteria travel further from the point of pollution all except one cell within the chain may die, or the chain may fragment. Thus in lightly polluted water the number of streptococci estimated may be based on single cells or fragments of streptococcal chains and may thus underestimate the die-away of the single streptococcal cells. Coliforms in raw sewage may be present as clumps of cells and the same considerations could thus apply to them. Nevertheless, in the experiments described above fecal streptococci were often the only indication of the fecal origin of the pollution at points distant from the source. For example, in 40% of the Lake Kinneret samples and 15% of the samples taken from other drinking water sources, the fecal streptococci were the only indicators of the fecal origin of the contamination.

Although the maintenance of strict bacterial standards in drinking water has in the past protected the human population from outbreaks of water-borne diseases, one or two recent outbreaks of virus infections have been traced to water thought to be safe by the coliform standards (Dennis, 1959).

The detection of viruses is, at present, a rather lengthy and expensive procedure, especially when large volumes of water must be tested (Shuval and Katzenelson, 1972). By using the relationship between the number of viruses and indicator bacteria in polluted water it may be possible to estimate the probable volume of relatively unpolluted water likely to contain one virus unit. In the samples of polluted water studied above, all three organisms show a low virus to bacteria ratio. For coliforms this is approximately $1:10^6$, for fecal coliforms $5:10^5$ and for fecal streptococci $2.5:10^4$. In addition viruses have been shown to be relatively more stable than coliforms both in the natural water environment and in various treatment processes (Shuval, 1970). In heavily polluted water it makes very little differences which of the three bacterial indicators is used for estimating the virus content of the water. The choice of indicator organism may be important however in lightly polluted samples where the number of fecal streptococci present in any given sample approaches the number of coliforms. If the calculation is based on the presence of 10 coliforms 100 ml, the estimated volume of water containing one virus unit will be 10^5 l; but if the presence of 10 fecal streptococci is taken as the basis for the calculation there will be 1 virus in about 5×10^2 l. By using the latter estimate we may obtain a safer estimate for the viral content of the water. These calculations assume that the virus: bacteria ratio remains constant under all conditions. Further work is necessary to justify this assumption, but it is interesting to note that in the Tiberias sewage plant and the Nahal Soreq river, the disappearance rate of fecal streptococci most closely paralleled that of viruses. Until such time that virological assay of large volumes of water becomes practical on a routine basis it may prove worthwhile to fully utilize the information obtained from these relatively simple bacteriological tests to estimate, albeit approximately, the virus content of lightly polluted water.

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