LOSS OF INFECTIVITY OF POLIOVIRUS 1 IN RIVER WATER UNDER SIMULATED FIELD CONDITIONS

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Abstract—The effects of light, virus concentration, and turbidity on the rate of loss of infectivity (LOI) of poliovirus 1 were investigated in two test systems, which utilized flowing river water Two levels of each variable were used in a 2^3 confounded factorial design The seeded systems were sampled at regular intervals to establish LOI rates Virus infectivity was measured by plaque assay Loss of infectivity followed a two-component curve, an initial, rapid phase followed by a second, slower component The slopes of the two components were examined by the analysis of variance to determine the potential influence of each variable Both light and turbidity exerted a significant influence on the LOI rate in the second component of the LOI curve and also in the transition period between the two components, however, during the initial rapid phase none of the variables influenced the LOI rate (at the 0 05 significance level) This research demonstrates the significance of light as a virucidal component in the aquatic environment

INTRODUCTION

Initial work on the waterborne transmission of enteric viruses has been directed at the detection and enumeration of viral contaminants in sewage effluents and receiving waters (Akin *et al*, 1971) More recently, attention has been directed toward the behavior of enteric viruses in the aquatic environment (Berg, 1976, Berg, 1977, Carlson *et al*, 1968, Floyd & Sharp, 1977, Gerba & Schaiberger, 1975a, Schaiberger, 1975b)

Current wastewater treatment techniques do not completely remove viral hazards from the effluents of treatment facilities (Berg, 1976, Berg, 1977) The continuing association of enteric viruses with a number of human diseases points up the lack of epidemiological evidence to establish the extent of the viral hazard in our potable water supplies (DeMichele, 1974) Increasing reliance on recycled water will accelerate the need to understand the factors which influence the survival of these viruses in water

The purpose of this study is to evaluate the influence of two variables known to affect virus behavior in the aquatic environment, turbidity (Bitton, 1975) and virus concentration (Akin *et al* 1971), and a third variable, light (ambient solar radiation), which has not been previously examined for its effect on viruses in the aquatic environment

MATERIALS AND METHODS

Experimental facilities

Two recirculating flow systems were used (Figs 1 and 2) The tray system consisted of four plastic trays (46 × 27 × 4 5 cm) fitted with pumps which recirculated the 5 5 l capacity approximately 11 times in 10 min The trays were placed in a water bath for temperature control

The experimental channel system was a recirculating artificial stream constructed from four-foot aluminium sections lined with 15 mil vinyl to form two parallel channels 214 6 m long and 15 cm deep Each channel was approximately 18 cm wide Including the recirculating hardware the system capacity was 3000 l A more complete description of the channel facility has been published (Gannon *et al*, 1966)

Light

The tray experiments were conducted in June 1975 Solar intensity was recorded on a pyrheliometer using an arbitrary scale to provide relative values for comparing experimental blocks of tray data Except for a 5 h period of heavy rain, the daylight hours were typified by full sun and occasional scattered clouds. The temperature of the water in both dark and ambient trays ranged from 16 to 23 5°C with a mean of 18 7°C There did not appear to be a significant difference between the light (ambient) and dark tray temperatures

Turbidity

Turbidity was measured as nephelometric turbidity units (NTU) Natural turbidity was less than 2 5 NTU and was used as the low level High level turbidity was provided by raising the turbidity to 36 NTU with a stock solution of organically loaded bentonite The bentonite stock solution was prepared in 3% beef broth, then centrifuged and resuspended three times in autoclaved river water to remove excess dissolved beef broth Preliminary work comparing virus recovery from river water, with river water plus bentonite showed a slight recovery reduction from the river water plus bentonite
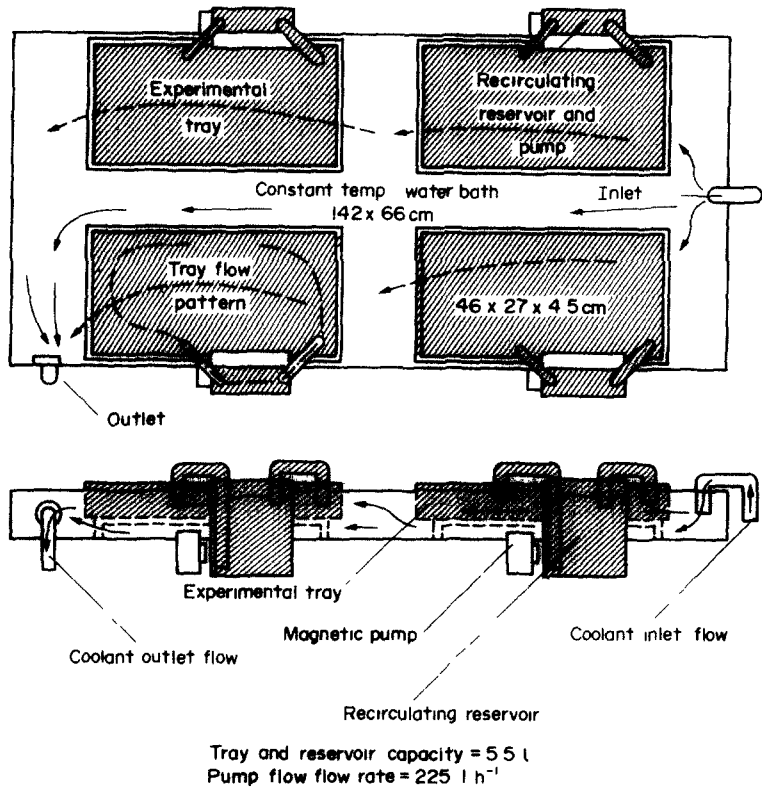
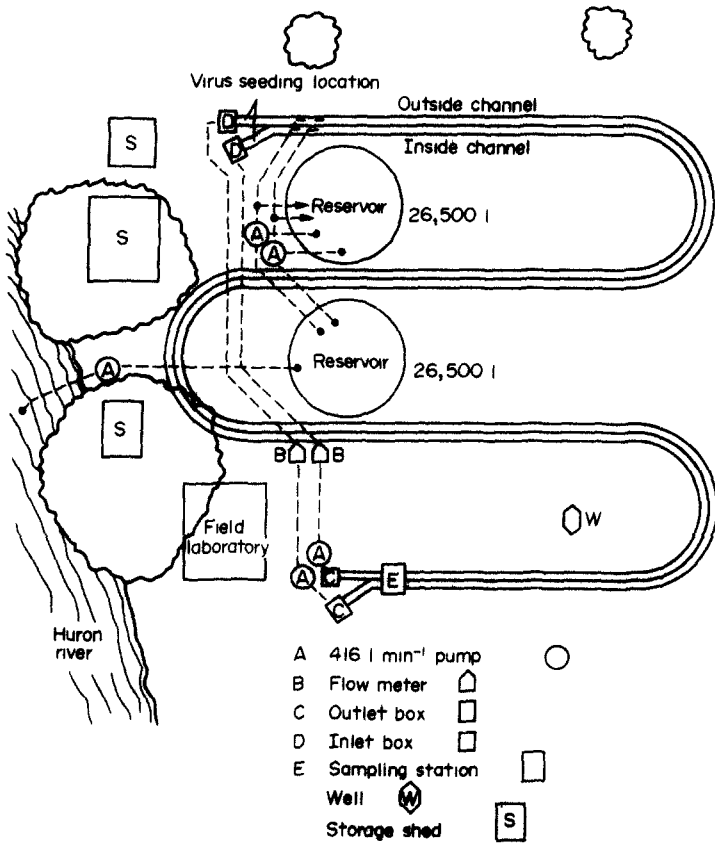Fig 1 Experimental tray system recirculating flow pattern

Fig 2 Experimental channel system

Virus concentration levels

The high and low starting virus levels in the tray study were approximately 10^5 and 10^4 PFU ml^{-1}, respectively In the channel experiments, the starting levels ranged from 700 to 4200 PFU ml^{-1}

Experimental design and analysis

The basic statistic used to evaluate the influence of each variable and potential variable interactions was the loss of infectivity (LOI) rate The virus concentration of each sample was plotted against time and the LOI curves were determined by employing the least squares method of curve fitting The LOI curve was assumed to be of the form

$$y = a e^{bx}$$

This equation may be linearized into

$$\ln y = \ln a + bx$$

where

y = the number of PFU ml^{-1} of inoculated river water at time = x h
b = the rate of LOI, and
x = the time elapsed in hours

The experimental tray design utilized each variable at two levels All possible combinations of three variables at two levels were used twice during the 16 experimental units The units were organized into four blocks which were conducted on alternate weeks to allow prompt assay of the virus-seeded river water The specific block design (of high and low variable levels) presented in Table 1 strengthened the analysis

of the potential effects of each variable and two-variable interactions at the expense of information on three-factor interaction, the latter being confounded with the block effects

Three channel experiments were conducted with similar variable levels to examine the potential of the facility for LOI studies

Sampling intervals

The LOI rates were calculated from virus assays at zero, 0 25, 1, 6, 12 h and each 12 h interval thereafter All values were based on the sample assay of the seeded water except the zero hour data which were calculated from the viral assay of the stock virus inoculum and the dilution values of the tray volumes The first tray samples were taken after the mixing period (15 min)

Water source

For the channel experiments, water was pumped directly from the adjacent Huron River into 26,500 l reservoirs for mixing to provide a homogeneous supply For the tray experiments, river water was collected and stored in a 750 l carboy for a common source Conductivity, pH and nutrient data were obtained prior to each set of experiments to establish the uniformity between experiments

Virus preparation

Culture of cells and virus Poliovirus 1 (vaccine strain LSc2ab) was propagated in Vero cells (ATCC CCL81) in 0 5 gallon roller bottles Vero cells were also used to assay virus in the channel study, as de-

Table 1 LOI slope statistics for the first and second components at selected intervals

Experimental tray*	High level‡ variables	Interval			
		0–1 h	0–6 h	1–72 h	6–72 h
BItI†	L	−1 49	−0 73	−0 17	−0 12
BIt2	V	−1 37	−0 76	−0 10	−0 08
BIt3	TLV	−1 45	−0 73	−0 18	−0 15
BIt4	T	−1 54	−0 72	−0 09	−0 06
BIIt1	No high levels	−1 60	−0 82	−0 11	−0 07
BIIt2	TV	−1 51	−0 82	−0 09	−0 07
BIIt3	TL	−1 56	−0 82	−0 24	−0 15
BIIt4	LV	−1 61	−0 82	−0 32	−0 25
BIIIt1	T	−1 59	−0 79	−0 09	−0 06
BIIIt2	V	−1 53	−0 77	−0 10	−0 08
BIIIt3	TLV	−1 53	−0 79	−0 19	−0 14
BIIIt4	L	−1 48	−0 83	−0 31	−0 23
BIVt1	No high levels	−1 31	−0 73	−0 14	−0 10
BIVt2	TV	−1 26	−0 72	−0 09	−0 07
BIVt3	TL	−1 23	−0 68	−0 23	−0 14
BIVt4	LV	−1 24	−0 76	−0 32	−0 25

† B—block, t—tray
‡ T = turbidity, L = light, V = virus
High turbidity > 30 NTU
Low turbidity < 2 5 NTU
High light—ambient solar radiation
Low light—total exclusion
High virus—110,000–130,000 PFU ml^{-1} starting at time 0
Low virus—11,000–13,000 PFU ml^{-1} starting at time 0

scribed below The Vero cells were grown MEM in Hanks' balanced salt solution (HBSS) with 10% fetal calf serum and an antibiotic supplement consisting of 100 units penicillin G $100\,\mu g$ streptomycin $100\,\mu g$ kanamycin and $4\,\mu g$ amphotericin B per ml For virus preparation roller bottle monolayers were rinsed three times with 10 ml of HBSS and each bottle then inoculated with 5 ml of undiluted dye-free stock virus and 25 ml of serum-free, dye-free MEM When cultures showed advanced cytopathology, bottles were frozen and thawed three times Cellular debris was removed by centrifugation at $300\,g$ for 20 min Pooled supernatant fluid was stored at $-20^{\circ}C$ until used

Virus assay

BGM cells were used to assay virus in the tray study BGM cells were grown in MEM with 10% fetal calf serum, and $5\,\mu g\,ml^{-1}$ of gentamicin and amphotericin B, subcultured as described by Dahling et al (1974) For maintenance, serum concentration was reduced to 5% and penicillin-streptomycin (100 units and $100\,\mu g\,ml^{-1}$) substituted for gentamicin Levels of infectious virus in water were determined by plaque assay (Melnick & Wenner, 1969) using two-ounce prescription bottles The overlay medium consisted of $1\,5\%$ Bacto-Agar (Difco, Inc) in MEM 2% heat inactivated fetal calf serum, five-fold concentrate of the antibiotic supplement described above $0\,005\%$ neutral red and $25\,mM\,MgCl_2$ Cultures were examined daily after 48 h (for a total of 7 days) and all plaques marked and recorded Data were recorded as plaque forming units (PFU) per ml

Virus concentrating technique

For the channel experiments the membrane filter process described by Wallis et al, (1972) was modified to allow rapid handling of the more turbid samples The ease of manipulation, minimal time requirements and sterile concentrate provided by the technique were the basic factors considered in selecting the process To the 250 ml sample, 2 ml of $0\,1\%$ methyl orange was added as a pH indicator The sample was then acidified to approximately pH 4 with 1N HCl Following acidification 2 ml of $0\,05\,M$ $AlCl_3$ and 10 ml of $1\,25\%$ celite (Hill et al, 1974b) were added to the sample and thoroughly mixed The sample was then vacuum filtered through a combination of two 47 mm diameter filters as a single unit, an AP25 fiberglass prefilter followed by an $0\,45\,\mu m$ membrane filter The fiberglass prefilters were pretreated with a 1% Tween 80 solution followed by a thorough rinse with distilled water to minimize virus adsorption to the fiberglass The river water was vacuum filtered to adsorb the virus content of the sample onto the membrane filter A sterile test tube was aseptically placed beneath the filter base and the sample was eluted through the filters with 4 5 ml of $0\,05\,M$ glycine-NaOH buffer at pH 11 5 An additional 4 5 ml of $0\,05\,M$ glycine-HCl buffer at pH 2 0 was passed through the filter to neutralize the concentrated

sample The 9 ml of eluate was transferred into a sterile screw-capped test tube containing 1 ml of a 10-fold concentrate of HBSS and 50-fold concentrate of standard antibiotics The sample volume had thus been reduced from 250 ml to 10 ml and was rendered isotonic for tissue culture application Any bacterial contamination which may have occurred at the field station was suppressed by the inclusion of antibiotics in the concentrated sample Preliminary tests for recovery efficiency utilized 250 ml of river water seeded with 2066 PFUs per 1 0 ml and resulted in a $42\,6\%$ recovery rate No concentration or clarification techniques were necessary at the virus levels used for the tray study Samples were assayed immediately or stored at $4^{\circ}C$ for no longer than 12 h

RESULTS

Tray experiments

The plotted data points appeared to fit a two stage LOI curve pattern which has been previously observed (Akin et al, 1971, Hiatt, 1964, Malina et al, 1975) Figures 3-10 contain the curves of replicated experimental units, averaged for more convenient presentation The curves illustrate the virus inocula levels used, duration of LOI, and the pattern of two component slopes Several time intervals were examined to determine the most representative two component curves Table 1 presents the slopes of the various LOI component intervals The transition or breakpoint between the more rapid, early LOI slope and the slower final LOI slope occurred during the
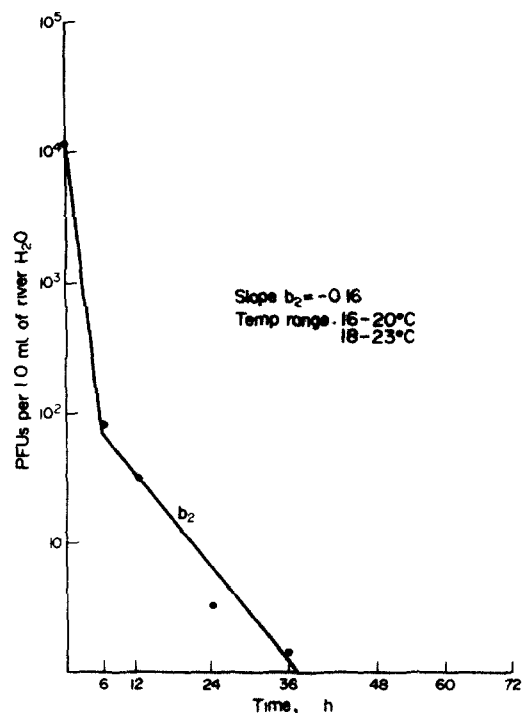

Fig. 3 Average tray loss of infectivity BItI and BIIIt4 (low turbidity, high light, low virus)

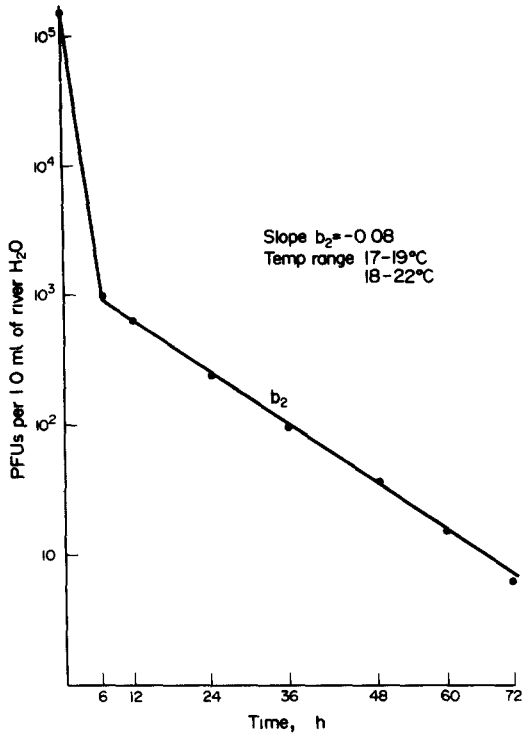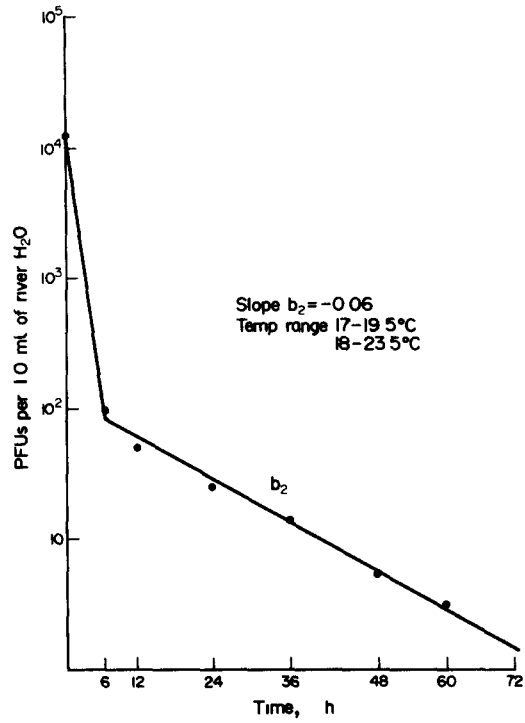Fig 4 Average tray loss of infectivity BIt2 and BIIIt2 (low turbidity, low light, high virus)

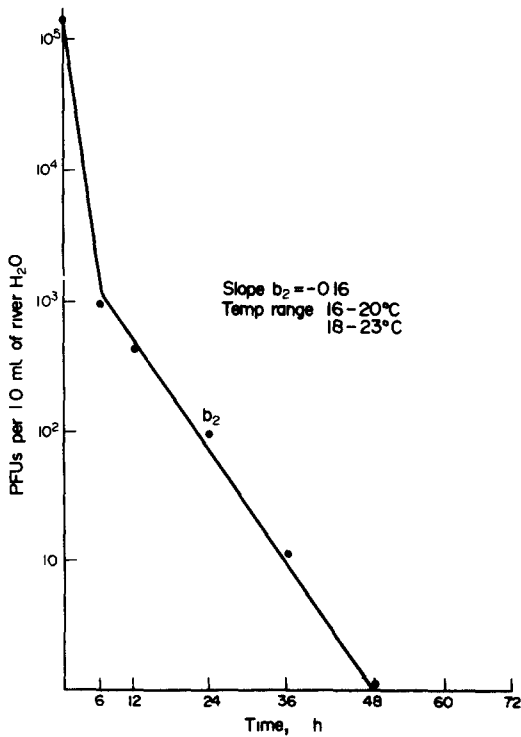Fig 6 Average tray loss of infectivity BIt4 and BIIIt1 (high turbidity, low light, low virus)

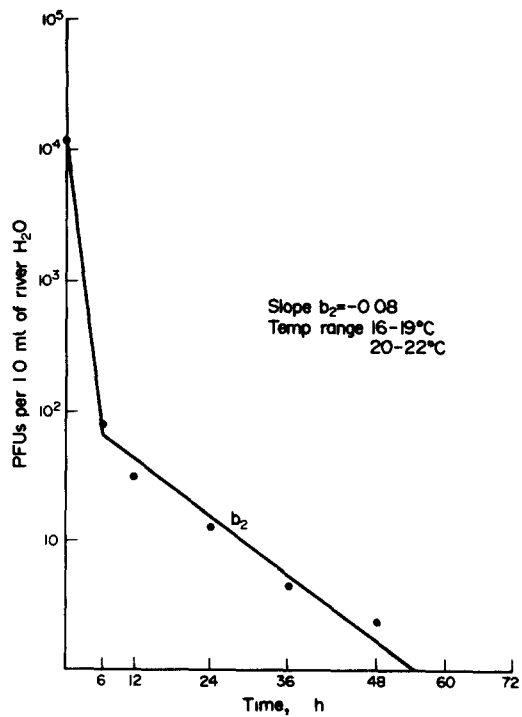Fig 5 Average tray loss of infectivity BIt3 and BIIIt3 (high turbidity, high light, high virus)

Fig. 7 Average tray loss of infectivity BIIt1 and BIVt1 (low turbidity, low light, low virus).
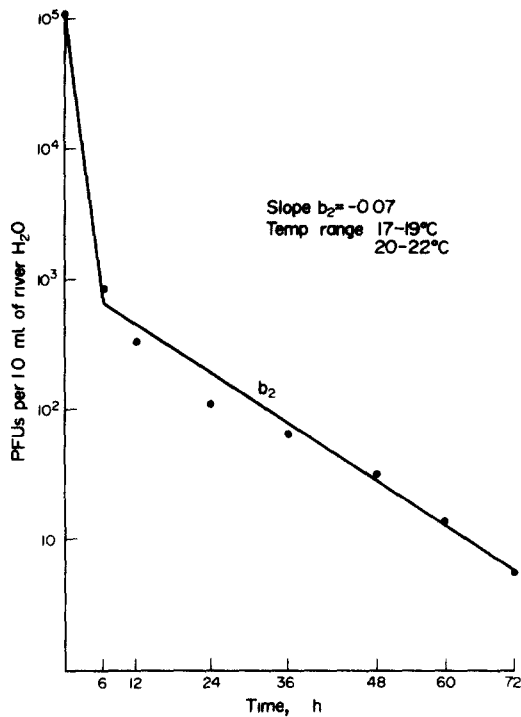
Fig 8 Average tray loss of infectivity BIIt2 and BIVt2
(high turbidity, low light, high virus)

1-6 h interval No data were available from within this interval so slope statistics were developed using both the 1 h point and the 6 h point as the break-point The amount of variability in the slope statistics was less for the second component when the break-point was arbitrarily placed at the 6 h point so it was used throughout as a matter of convenience

Analysis of variance

Both light and turbidity were indicated as sources of variation which affected the LOI rate during the second component (6-72 h) and the interval expanded to include the transition period (1-72 h) The F values for turbidity derived from the analysis of variance, exceeded the critical F value at the 0 05 significance level for both of the noted intervals The F values for light were even more significant during the same intervals and exceeded the critical F values at the 0 01 level Table 2 contains the calculated F values None of the variables nor variable interactions were implicated as statistically significant sources of LOI rate variation during either of the intervals used as potential early LOI components (0-1 h and 0-6 h)

Channel experiments

The LOI curves developed for the channel experiments also exhibited the two component curve pattern observed in the tray study Table 3 presents the slope statistics, including run 3 which utilized both channel and tray facilities conducted with parallel variable levels
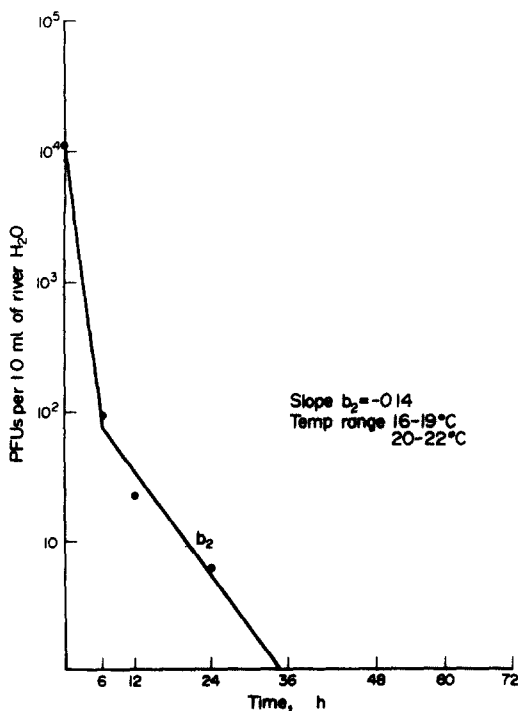

Fig 9 Average tray loss of infectivity BIIt3 and BIVt3
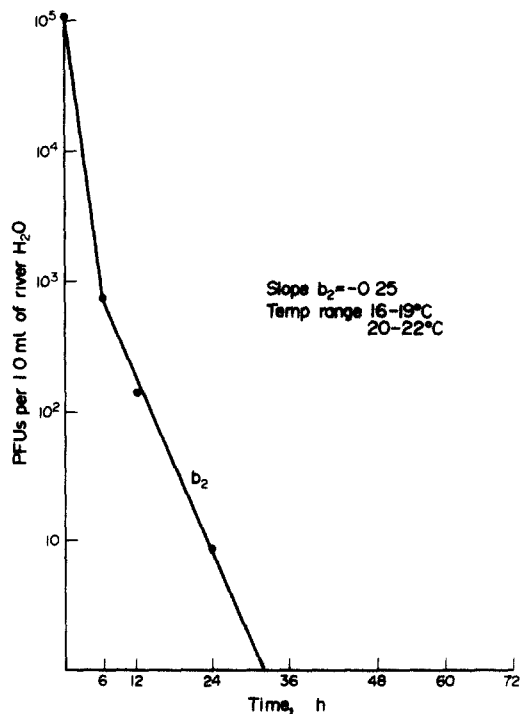(high turbidity, high light, low virus)

Fig 10 Average tray loss of infectivity BIIt4 and BIVt4
(low turbidity, high light, high virus)

Table 2 *F* values for variables and variable interactions for tray experiments

Source of Variation	F Value (6–72 h)	F Value (1–72 h)
Turbidity	8 18*	6 86*
Light	49 92†	66 23†
Turbidity and light	2 83	1 81
Virus	1 81	0 01
Turbidity and virus	1 02	2 21
Light and virus	1 39	0 61
Replications‡	1 02	1 45
Blocks within replications	1 37	2 83

* Exceeds the critical *F* value at the 0 05 significance level

† Exceeds the critical *F* value at the 0 01 significance level

‡ The *F* value for "replications" indicates the extent of variation between experimental units which duplicate each other (BI and BIII, BII and BIV) See variable levels, Table 1 (Remington & Shork, 1970)

Figure 11 demonstrates the poor curve fit for the channel experiments when the data for the zero to six hour interval are used for the initial curve component.

DISCUSSION

The statistical analysis of the tray data did not identify the starting virus concentration as a significant factor in the determination of the rate of loss of infectivity (LOI) However, both turbidity and light were implicated as significant sources of variability

While the initial virus concentration was not significant (at the 0 05 level) for the rate of LOI, an interesting difference in the duration of the early LOI components was noted between the channel tray curves Differences between the two facilities rule out a definitive statement, but the lower starting virus levels used in the channel experiments (700–4200 PFU ml^{-1} of river water) may have played a role in the reduction of duration of the early LOI curve component (Akin *et al*, 1971) Any such inference from the current study must be regarded as speculative, however, the low virus levels used in the tray study and the levels of the channel inocula were of the same order of magnitude as those reported by others when a similar reduction in the duration of the early LOI component was noted, and suggested that the virus level affected the duration of the initial phase (Akin *et al*, 1971)

It has been pointed out that two phenomena account for the reduction in the levels of infectivity observed in enteric viruses in the aquatic environment, actual loss of infectivity brought about by impairment of the infective capability of the virus particle, and/or the apparent loss of infectivity which results from conditions that cause infectious particles to join together into clumps (which act as a single infectious focus) (Akin *et al*, 1971, Floyd *et al*, 1977, Gerba & Schaiberger, 1975a, Schaiberger, 1975b, Hill *et al*, 1974a, Moore *et al*, 1975, Schaub *et al*, 1974, Young & Sharp, 1977)

The apparent LOI has been divided into situations that involve (1) the adsorption of virus particles onto other particulate matter in intimate contact with the water, and (2) the aggregation of virus particles into virus clumps (Akin *et al*, 1971, Bitton, 1975, Carlson *et al*, 1968, Fowlks, 1959, Floyd & Sharp, 1977, Gerba & Shaiberger, 1975a, Hill *et al*, 1974b) Ionic

Table 3 Channel experiments LOI slope statistics

Experimental unit	Experimental conditions	(0–1 h) Slope	Interval (1–last h) Slope	r^2*
Run 1				
channel I	Low turbidity, low light and low virus	−1 53	−0 18	0 96
channel 0	High turbidity, high light and low virus	−1 57	−0 19	0 98
Run 2				
channel I	High turbidity, low light and low virus	−2 43	−0 09	1 00
channel 0	Low turbidity, high light and low virus	−2 42	−0 10	0 99
Run 3				
channel I	Low turbidity, high light and high virus	−0 72	−0 09	0 97
tray I	Low turbidity, high light and high virus	−0 74	−0 09	1 00
channel 0	High turbidity, low light and high virus	−0 94	−0 07	−0 99
tray 0	High turbidity, low light and high virus	−0 92	−0 08	1 00

* r^2 is a measure of how well the data points fit the calculated slope line The 0–1 h slopes are all 1 00 since only two data points were available for calculating the slope
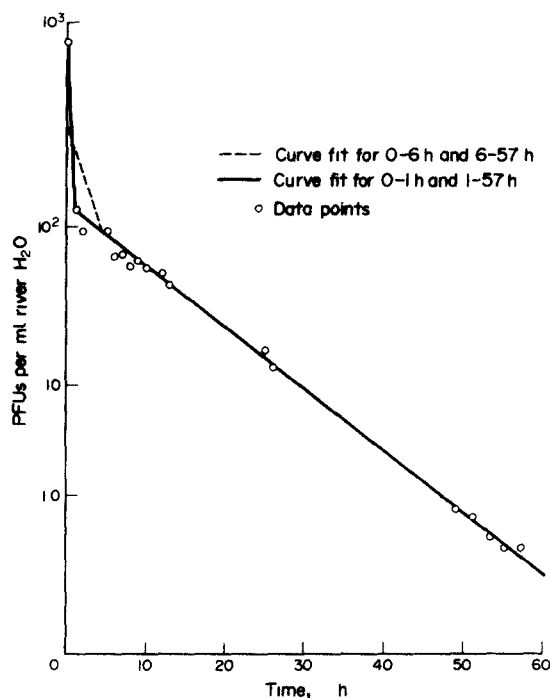
Fig 11 Channel study comparison of two LOI curve intervals (high turbidity, low light and low virus level)

strength and substances which compete for the available bindings sites influence both adsorption and aggregation (Akin et al, 1971)

The appearance of the early component of the LOI curves suggests that the aquatic environment provided by the river water was sufficiently different from the stock virus preparation to mask the potential influence of the variables on the rate of LOI during the early portion of the experiments Thus, the influence of turbidity and light on the LOI rate became apparent only after the factor(s) responsible for the early LOI component subsided

Turbidity was implicated at the 0 05 significance level as a source of LOI rate variation during the second curve component and during the interval which included the second component and the transition period, but not during the early component The increased LOI rate associated with the higher level of turbidity suggests the possibility of solids associated infectivity and reiterates the necessity for using caution in assuming that the potential infectivity level is no higher than the assayed value (Wallis & Melnick, 1967) The number of reported recoveries over 100%, no doubt result from solids separation in the sampling process and illustrate the point and reveal the incomplete state of knowledge of the phenomena governing the behavior of enteric viruses in the aquatic environment (Akin et al, 1971)

The effect of solar radiation has not been previously examined with regard to natural waters and enteric viruses U V light has been investigated as a

virucidal treatment for contaminated waters in the shellfish industry (Hill et al 1969) and the mechanisms responsible for the virucidal effects have been examined (Kalter & Millstein 1974, Schaub et al 1974 Wellings et al 1974) In addition the extent and penetration of solar radiation into the aquatic environment has been investigated (Ruttner 1973) and suggests the potential for ambient solar radiation to exert a virucidal effect

As was the case with turbidity the rapid LOI rates associated with the early component masked the detection of any potential influence of light during that interval Data from the trays which received ambient solar radiation was also examined for evidence of a diurnal LOI pattern, with a diminished night-time LOI rate Unfortunately the rates were so rapid that only three of the eight ambient light trays had more than one daylight slope interval before the infectivity dropped below the detection level The diurnal slope analysis was thus inconclusive However the statistical evaluation of slope data produced highly significant variation associated with the effects of solar radiation following the early LOI component Additional research is needed to determine if the effect is direct or as has been suggested is indirect such as enhanced biological activity

A similar acceleration in the loss of infectivity has been reported for viruses seeded onto vegetation via spray irrigation (Konowalchuk & Spiers, 1971) The results of the current research indicate that solar radiation may be added to the list of variables which determine the infectivity of poliovirus in the aquatic environment

The mechanisms by which turbidity affects the rate of LOI needs to be further defined In natural waters, where turbidity levels may be higher and depths greater than those used in the current research, the penetration of light would be diminished and hence turbidity would shade and protect virus particles from the virucidal effects of light Further research is needed to determine the impact of light in natural settings such as impoundments with relatively clean water

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