VIRUS SURVIVAL IN GROUNDWATER

by

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INTRODUCTION

Virus survival in the subterranean environment is believed to be influenced by three interacting factors: the nature of the virus, the nature of the soil and the climate. Different types and strains of virus exhibit varying abilities to survive, based on differences in their genetic makeup. The effect of soil type on virus survival has been extensively studied using various soils (Hurst et al, 1980). The results of that study are presented in Table 1.

TABLE 1. Factors which affect virus survival in soil

1.	As viral adsorption to soil increases, virus survival is prolonged.
2.	Virus survival increases with increasing levels of exchangeable aluminum.
3.	Virus survival decreases with increasing pH and resin extractable phosphorus.
4.	As temperature increases, virus survival decreases.
5.	Aerobic soil microorganisms adversely affect virus survival, while anaerobic microorganisms have no effect.

Climatic conditions also play a role in virus survival. Rainfall is important in that it can cause desorption of virus from soil particles, thus allowing migration to the groundwater (Gerba and Lance, 1978; Wellings et al, 1975). Temperature has also been shown to influence virus survival in the marine environment. In surface waters, sunlight is thought to be an important factor in virus inactivation.

Virus survival in marine waters has been extensively studied, due to the large number of outbreaks of viral illness associated with consumption of contaminated shellfish. Little information is available concerning the persistence of viruses in groundwater. Keswick et al (1982) found that human enteric viruses are capable of surviving longer than 24 days in groundwater. Their experiments, however, were carried out in McFeter's survival chambers, which may have excluded contact with components of the groundwater that could influence virus survival, such as antagonistic bacteria.

The purpose of this investigation is to study the survival of viruses in groundwater, with the ultimate goal of developing a model to predict safe distances between drinking water wells and septic tanks.

MATERIALS AND METHODS

Groundwater samples were collected from seven different sites (Table 2). The samples were collected aseptically in sterile polypropylene containers, and packed on ice for shipment to the laboratory.

TABLE 2. Sample collection sites

Site Description	Groundwater Temperature	Depth
New York 1 - monitoring well northeast of landfill	12°C	< 100 ft
New York 2 - monitoring well north of landfill	12°C	< 100 ft
North Carolina 1 - well near septic system	14°C	deep water table
North Carolina 2 - well near septic system	14°C	shallow water table
Tucson - drinking water well	23°C	> 200 ft
Arizona - monitoring well	23°C	> 200 ft
Wisconsin - drinking water well	12°C	50 ft

Fifty-ml aliquots of water were placed in sterile, polypropylene tubes and seeded with one of the following viruses: MS-2 (a bacteriophage) and one of two animal viruses, Poliovirus-1 or Echovirus-1. To determine the influence of microorganisms on virus survival, duplicate tubes containing water passed through 0.45 μm and 0.2 μm filters were used.

The tubes were incubated at 4°C, 12°C or 23°C. One-ml aliquots were withdrawn on days 1, 2, 3, 5, 7, 10, 15, 20, 25, 30, 40, 50 and 60. Samples containing animal viruses were frozen for future assay.

Determination of the number of MS-2 phage in samples was performed using the agar overlay plaque technique (Adams, 1959). The bottom layer consisted of trypticase soy agar. The overlay contained trypticase soy broth and 1% agar. One ml E. coli ATCC 15597 and a 1-ml sample were added to the overlay which was poured onto the bottom layer. Plaques were counted after 24 h of incubation at 37°C.

RESULTS AND DISCUSSION

Initial experiments were performed to determine the reproducibility of results. MS-2 phage were seeded into 9 tubes containing 50 ml of groundwater and 3 tubes were incubated at each temperature. As no major differences among the replications were noted (Table 3), subsequent experiments were done using only one tube per set of experimental conditions.

TABLE 3. Reproducibility of virus survival determination

Incubation Temperature	Replication	Survival -Log N/N_o* After 10 Days
4°C	a	0.43
	b	0.31
	c	0.37
12°C	a	1.1
	b	1.15
	c	0.95
23°C	a	1.85
	b	1.84
	c	1.89

*N=number of plaque forming units (pfu) per ml after specified length of time; N_o=initial number of plaque forming units per ml.

To determine whether the age of the water sample had any effect on results, water was inoculated with MS-2 one day after collection and after it had been stored for 30 days at 4°C. No large differences in survival of MS-2 were seen (Table 4).

TABLE 4. Effect of age of water on survival of MS-2

	Survival - Log N/N_0	
Day	1 Day Old	30 Days Old
0		
1	0.041	0.079
2	0.56	0.47
3	0.74	0.69
5	1.6	1.2
7	2.2	2.2
10	2.2	2.5
15	2.6	2.7
20	3.2	2.9
25	4.0	4.1

The influence of microorganisms on virus survival is shown in Table 5. The results varied greatly from sample to sample. More investigation needs to be done to more clearly define the role of microorganisms in virus survival.

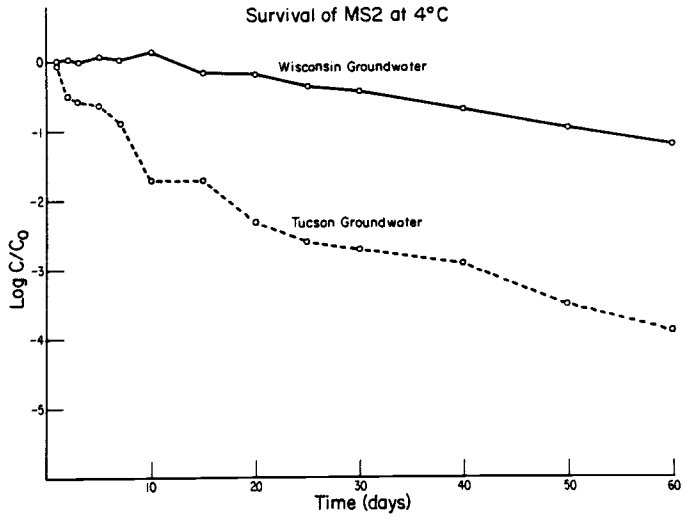
TABLE 5. Effect of microorganisms on the survival of MS-2

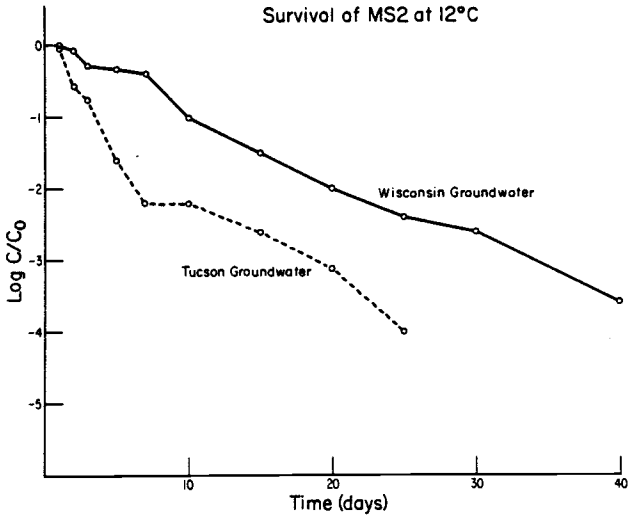
	Survival - Log N/N_0*	
Sample	Filtered	Non-filtered
North Carolina 1	1.8	0.84
North Carolina 2	0.86	3.5
Arizona	3.2	1.6
New York 1	0.95	1.0
New York 2	1.5	0.97

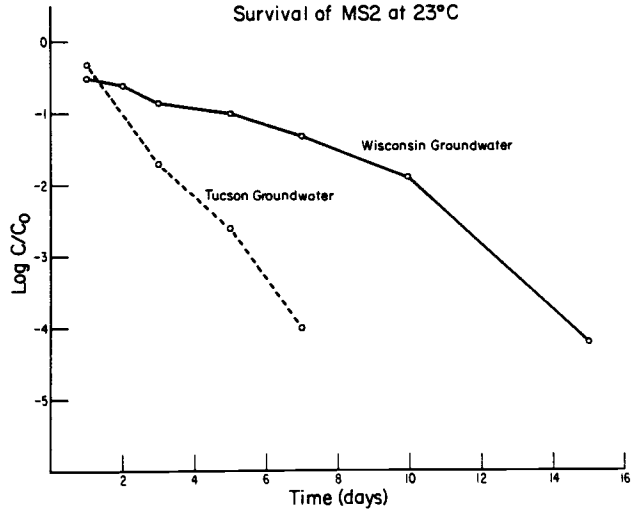
*After 30 days, incubation temperature 12°C.

The temperature of incubation played a major role in the survival of the virus. Figures 1, 2 and 3 show the inactivation rate of MS-2 in the Tucson and Wisconsin groundwater samples. It was found that as incubation temperature increased, the survival rate decreased. However, the rate of the decrease was strikingly different for the two groundwater samples at all three temperatures.

FIGURES 1, 2 and 3. Survival of MS-2 in Tucson and Wisconsin groundwaters at 4°C, 12°C and 23°C

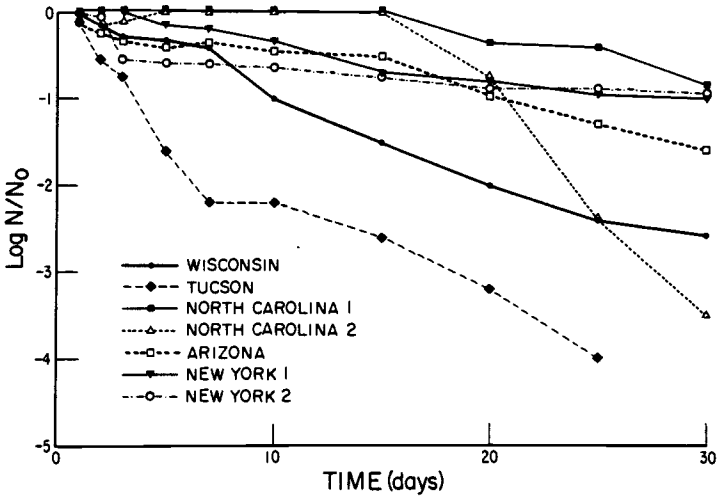






The survival rate of MS-2 at 12°C in all seven groundwater samples is shown in Figure 4. A wide variation in survival rates occurred among the different waters.

FIGURE 4. Survival of MS-2 at 12°C



CONCLUSIONS

From these results it was concluded that: (1) persistence of viruses in groundwater varies with location of sample collection, (2) viruses survive longer in groundwater samples incubated at lower temperatures [4°C] than at 12°C or 23°C, (3) the influence of microorganisms on virus survival varies in different groundwater samples, and (4) virus survival in groundwater is longer than in surface water.

However, because of the wide variation in survival rates from site to site under the same experimental conditions (temperature and the presence or absence of microorganisms), other factors which influence survival of viruses must be present in the water. These factors need to be studied to explain this variation.

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REFERENCES CITED

Adams, M. H. 1959. Bacteriophages. New York: Interscience Publishers.

Gerba, C. P. and J. C. Lance. 1978. Poliovirus removal from primary and secondary sewage effluent by soil filtration. Appl. Environ. Microbiol., 36:247-251.

Hurst, C. J., C. P. Gerba and I. Cech. 1980. Effects of environmental variables and soil characteristics on virus survival in soil. Appl. Environ. Microbiol., 40:1067-1079.

Keswick, B. H., C. P. Gerba, S. L. Secor and I. Cech. 1982. Survival of enteric viruses and indicator bacteria in groundwater. J. Environ. Sci. Health, A17:903-912.

Wellings, F. M., A. L. Lewis, C. W. Mountain and L. V. Pierce. 1975. Demonstration of virus in groundwater after effluent discharge onto soil. Appl. Microbiol., 29:751-757.