Critical Review

Review of Factors Affecting Microbial Survival in Groundwater

DAVID E. JOHN*,† AND JOAN B. ROSE*,‡

College of Marine Science, University of South Florida, 140 Seventh Ave South, St. Petersburg, Florida 33701, and Department of Fisheries and Wildlife, 13 Natural Resources Building, Michigan State University, East Lansing, Michigan 48824

This review quantitatively examines a number of published studies that evaluated survival and inactivation of public-health-related microorganisms in groundwater. Information from reviewed literature is used to express microbial inactivation in terms of $\log_{10}$ decline per day for comparison to other studies and organisms. The geometric mean value for inactivation rates for coliphage, poliovirus, echovirus, coliform bacteria, enterococci, and *Salmonella* spp. were similar at approximately 0.07−0.1 $\log_{10}$ day^{-1}, while geometric mean inactivation rates for hepatitis A virus, coxsackievirus, and phage PRD-1 were somewhat less at 0.02−0.04 $\log_{10}$ day^{-1}. Viruses show a temperature dependency with greater inactivation at greater temperatures; however this occurs largely at temperatures greater than 20 °C. Coliform bacteria dieoff in groundwater does not show the temperature dependency that viruses show, likely indicating a complex interplay of inactivation and reproduction subject to influences from native groundwater organisms, temperature, and water chemistry. The presence of native microorganisms seems to negatively impact *E. coli* survival more so than viruses, but in most cases, nonsterile conditions led to a greater inactivation for viruses also. The effect of attachment to solid surfaces appears to be virus-type-dependent, with PRD-1 more rapidly inactivated as a result of attachment and hepatitis A and poliovirus survival prolonged when attached.

Introduction

Groundwater resources are heavily used for domestic drinking water supplies in the United States and most of the world. Nationally, 40% of the U.S. domestic water supply originates from groundwater. Furthermore, over 40 million people use groundwater to supply their drinking water via individual wells (*1*). Worldwide, groundwater represents a significant majority of the drinking water supply in many nations, including Denmark, Portugal, Italy, Switzerland, Belgium, and The Netherlands, all of which derive more than two-thirds of their drinking water from groundwater (*2*).

Aquifers have until the last several decades been generally considered protected from potential sources of microbial or chemical contamination typically found in surface waters. Increasing population densities, development, and industrialization, leading to increased withdrawals from aquifers, however, have focused a greater attention on the quality of groundwater as a growing concern. Microbial contamination of groundwater is responsible for numerous disease outbreaks; in the U.S., at least 46 outbreaks of disease caused by contaminated groundwater have been documented between 1999 and 2002, representing 81% of all drinking water microbial illness outbreaks (*3*, *4*). These figures include reported microbial disease outbreaks and those cases involving acute gastrointestinal illness of undetermined etiology. These outbreaks represent a total of 2739 cases of illness and several deaths. This is likely an underestimation of the total incidence of groundwater-caused illness in the United States, since many self-resolving and isolated cases go unreported to health officials.

The main pathogenic microorganisms of concern may be grouped into enteric viruses, bacteria, and protozoa. Waterborne viruses include enteroviruses, coxsackievirus, echovirus, rotavirus, norovirus, and hepatitis A and E. Bacteria of concern are chiefly enteropathogenic *E. coli*, *Salmonella* and *Shigella* spp., *Campylobacter jejuni*, and *Aeromonas hydrophila*, among others. The main protozoa that have been transmitted by groundwater are *Cryptosporidium parvum* and *Giardia lamblia* (*5*).

Along with a heightened state of awareness about potential groundwater contamination has come interest within the regulatory, public health, and research communities for more information about the sources, transport, and fate of waterborne microorganisms in relation to aquifers and groundwater. In particular, a large body of research has examined the transport of viruses through the vadose zone and within aquifers. Numerous factors have been identified that impact the transport of bacteria and viruses in groundwater. Among them are the size and isoelectric point of virus particles (*6*), organic content of the groundwater (*7*), saturated versus unsaturated groundwater flow (*8*, *9*), ionic strength and pH of the groundwater (*10*), aquifer substrate grain size (*10*, *11*), and other size-dependent exclusion factors such as filtration and cell size (*11*, *12*). Other hydrological factors are also important such as flow velocity and the heterogeneity of the aquifer substrate (*12*). Transport studies are often used to define modeling equation components, which can then be useful for predicting transport rates and distances through a particular aquifer based on the values of various parameters (*13*, *14*). Percolation through the vadose zone has been shown to be a significant factor in removing possible contaminants before ground water reaches the saturated zone of an aquifer (*11*). However, information on survival of microbes once in

* Address correspondence to either author. Phone: (727)553-1647 (D.E.J.); (517)432-4412 (J.B.R.). Fax: (727)553-1189 (D.E.J.); (517)-432-1699 (J.B.R.). E-mail: djohn@marine.usf.edu (D.E.J.); rosejo@msu.edu (J.B.R.).

† University of South Florida.

‡ Michigan State University.

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the groundwater zone is particularly important in areas with shallow aquifers or in situations where possibly contaminated surface water may come in direct contact with the aquifer. Thus, also of concern is the survival of all groups of microorganisms once in aquifers.

A review by Hurst (*15*) compiled data from published reports on factors that influenced survival or inactivation rates of enteroviruses and rotaviruses in surface freshwaters. Quantitative data such as rates were not presented, although a figure of temperature effects was given. The temperature effect for data summarized by Hurst varies, and no average was given. The author believes that for these studies there are likely other factors beyond those analyzed accounting for differences in temperature effects. Factors that were determined to have a statistically significant effect on waterborne virus survival include:

- chloride concentration over the range from <0.5 to 16.3 mg/L
- pH over the range from 6.0 to 7.8
- total organic carbon from <1 to 17 mg/L
- hardness from 29 to 339 mg $CaCO_3$
- temperature from 4 to 37 °C
- turbidity from <2.5 to 36 NTU.

Waterborne microorganisms of public-health concern can enter aquifers via several sources and mechanisms, including percolation from surface water, sinkholes, septic systems, leaky sewer lines, or direct injection of wastewater effluent or surface water through wells. The prevalence of such sources also varies from nation to nation depending on regulations and infrastructure. One technology of rising importance that involves injection of surface water to aquifers for storage and later recovery for use is termed aquifer storage recovery (ASR). At least 53 ASR systems were operational in the United States as of January, 2002, with about 100 more in development or planning stages (*16*). ASR technology is also an integral part of the Comprehensive Everglades Restoration Plan. Currently, the U.S. EPA and local agencies require that water injected via ASR wells meets all primary and secondary drinking water standards (*17*). However, interest among proponents of ASR technology in the U.S. and elsewhere exists in determining the feasibility of relaxing pretreatment requirements for stored water, assuming that the water will be treated as any surface water after withdrawal. In addition, natural attenuation of potentially harmful microorganisms, present in source waters, occurring due to biological, physical, and geochemical factors present in the subsurface environment should be evaluated to further an understanding of the potential improvement of stored water quality.

This review seeks to summarize the current state of knowledge on inactivation of many organisms of concern in groundwater from a quantitative perspective. Since no standard exists for reporting results of studies on microbial inactivation, data have been reported by various authors in many different ways. The purpose of this review was to facilitate some level of comparison among numerous disparate studies to evaluate any consistencies and trends within the body of published research on the topic. The information presented herein summarizes methods and findings of studies on microbial survival in groundwater and collates these findings into expressions of inactivation rates in terms of $\log_{10}$ decline in the viable or culturable organisms per day. This common statistic was chosen since most professionals in the engineering and regulatory field commonly gauge microbial removal in terms of $\log_{10}$ concentration ratio changes. In many cases, authors have reported inactivation data in these terms; in other cases. the data as presented were converted to $\log_{10}$ day^{-1} declines based on times to achieve a given level of reduction or approximated from graphical data. Observations on kinetics of inactivation from various studies have been noted when possible, and in general, rates converted from graphical data express an average rate resulting from the total decline in viable counts observed over the study period. Individual survival studies are grouped here based on the organisms that were evaluated. Last, we present analyses of data extracted from reviewed research that combine inactivation rates for the organisms studied to express ranges and other summary statistics and summarize information from reviewed studies on potential factors affecting microbial inactivation in groundwater.

Viruses

Studies published by Yates and others in 1985 and 1990 have reported the effects of numerous parameters on virus survival in groundwater (*18*−*20*). Virus inactivation in groundwater samples was compared for determining possible effects of differing chemical and physical parameters as well as the presence or absence of indigenous bacteria (analyzed by filter-sterilizing subsamples of test water with 0.2-μm filters). Parameters and their effects on poliovirus 1, MS-2, and echovirus 1 survival included temperature, total dissolved solids (TDS), hardness, turbidity, pH, and nitrate concentrations. In Yates et al. (*20*), all of the above factors plus heterotrophic bacteria counts were evaluated for effects on MS-2 and poliovirus 1 inactivation. Temperature was the only factor significantly correlated to the inactivation rates of all viruses, while greater calcium hardness was also correlated to more rapid decay of MS-2. Experiments to evaluate inactivation along with changes in indigenous bacterial population densities over the experimental time frame did reveal that MS-2 decline was significantly correlated with an increase in indigenous bacterial numbers. This may be considered an indirect function of time as the presence or absence of bacteria (raw vs filtered) was not found to significantly affect decay rates of either MS-2 or poliovirus. Large variations in decay rates did exist between samples incubated at the same temperatures. For some samples, inactivation was more rapid in unfiltered water while for others it was more rapid in filtered waters. In others still, no significant difference existed between filtered and unfiltered waters.

Survival of hepatitis A virus (HAV), poliovirus, and echovirus in groundwater was evaluated with respect to the effect of temperature, soil, and presence of autochthonous microorganisms by Sobsey et al. (*21*). Survival studies were performed on viruses in soil-free systems and suspensions of two types of clay, a clay loam, sand, a loamy sand, and organic muck, with suspending water consisting of groundwater, primary sewage effluent, and secondary sewage effluent. Testing was performed at 5 and 25 °C. Autoclaved subsets of these conditions were also contrasted. Since these solid media−water conditions were established as suspensions that ranged from 3% to 75% w/v with the water, conditions were not modeling those of saturated, consolidated aquifer substrate per se, but the study can give insight on the relative survival of liquid-phase viruses to those in a mixture of solid media. In the context of this review, we have focused only on the experiments involving groundwater from this study, which were largely confined to just HAV. Poliovirus and echovirus were tested in groundwater only with the clay loam and organic muck and not in groundwater alone. It was not clear from the paper exactly which conditions were tested at 5 °C since no inactivation results were actually presented except to say that HAV did not decline over 8 weeks in groundwater or soil suspensions and none of the three viruses declined in the effluent samples at 5 °C. Results of experiments using the two soil suspensions in which all three viruses were tested revealed that HAV survived longer than echovirus and poliovirus at 25 °C in both soils, suggesting poliovirus and echovirus are not effective indicators for predicting the survival of hepatitis A virus. It is also worth

noting that among the sterile/nonsterile pair comparisons the nonsterile replicate resulted in more rapid inactivation for four out of six HAV experiments, one out of two poliovirus experiments, and two out of two echovirus experiments. Comparison of HAV survival in water-only experiments and soil suspensions at 25 °C indicated that survival was generally enhanced in soil suspensions except in the loamy sand in which survival was equivalent to soil-free conditions. The differences between survival in soil suspensions and groundwater without soil were not related to the degree of adsorption to the various soils or sterile versus nonsterile conditions in any apparent way.

A brief study by Yahya et al. (*22*) evaluated inactivation of the bacteriophages MS-2 and PRD-1 in four different groundwater samples, incubated at the ambient temperature of the aquifer for each sample, which was either 7 or 23 °C. Conclusions derived from this study are that little difference in inactivation rates between MS-2 and PRD-1 was observed at 7 °C, while there was a more pronounced increase in the decay rate of MS-2 at 23 °C than was observed for PRD-1. Still, PRD-1 showed faster rates of decline at 23 °C than at 7 °C. Alvarez et al. (*23*) evaluated inactivation of MS-2 and poliovirus in groundwater samples that were either filtered through a 0.2-μm pore filter or used raw. For each virus, inactivation in the filtered groundwater subsample was only slightly faster.

Survival of bacteriophage and enteric viruses in groundwater and saturated soil microcosms was evaluated by Blanc and Nasser in the context of a larger effort to gauge temperature and wastewater effluent effects on survival and adsorption of viruses (*24*). This study on inactivation of MS-2, PRD-1, poliovirus 1, and hepatitis A virus is useful for contrasting survival in water-only microcosms against solid substrate saturated with groundwater. Two temperatures, 10 and 23 °C, were compared, and soils used were a "loamy soil" and a "sandy soil." Although sampling methods were not described, the implication from described results was that survival in the saturated soil experiments included both sorbed and free viruses rather than viruses from only the pore water. In comparisons of the same virus and conditions at the two temperatures, inactivation was more rapid at 23 °C for all cases but 2 out of 12 (HAV in groundwater without soil and HAV in sandy soil). MS-2 was most affected by the change of temperature. The comparison of saturated soil to water column survival indicated that inactivation of poliovirus and hepatitis was slower in both saturated soils than in water alone at 10 °C. At 23 °C, inactivation of poliovirus was the same in the loamy and slower in the sandy soil, while hepatitis inactivation was still slower in both saturated soils. In contrast, inactivation of PRD-1 was more rapid in microcosms with soil. Inactivation of MS-2 was the same in saturated soil except for the sandy soil at 10 °C in which inactivation was more rapid than in the water column. These relative differences were not directly related to the differing degrees of adsorption and appear to be more virus-type-dependent. In comparisons of the relative survival of the various viruses, MS-2 was not an appropriate indicator for the survival of poliovirus and hepatitis in saturated soil, due to its more rapid inactivation. Inactivation of the pathogenic viruses in groundwater with or without soil was less than 0.1 $\log_{10}$ day^{-1} on average in all cases, while that of MS-2 was at least that rapid in a number of cases, including all microcosms at 23 °C. Inactivation of PRD-1 was more similar to that of the two pathogenic viruses but was still more rapid than HAV and polio in saturated soil. Inactivation of polio was less rapid than HAV at 10 °C in groundwater without soil and more rapid in groundwater with soil (both types). Inactivation of the two was similar at 23 °C in the groundwater control and sandy soil, while polio inactivation was more rapid in the loamy soil. Unfortunately, HAV adsorption to the soils was not reported, so insight on the effect of adsorption differences between these two viruses was not possible.

Jansons et al. evaluated several types of viruses for survival in dialysis tube devices while suspended in several bore holes containing groundwater (*25*). Viruses evaluated were coxsackievirus B5, echovirus 6, 11, and 24, and poliovirus 1. Survival of viruses in seven bore holes was evaluated, using a single virus type in each bore hole. Poliovirus 1 was used in three bore holes, and the rest of the viruses in one each. A plume of groundwater recharge created a gradient of dissolved oxygen (DO) and temperature, such that bore holes more influenced by the reclaimed water had a higher DO concentration and lower temperature than those of the native aquifer water. This gradient allowed a comparison of poliovirus survival in response to the three different DO concentrations. It was found that inactivation was greater in the bore hole with higher mean DO concentrations, such that inactivation rates were 0.09 $\log_{10}$ day^{-1} in a mean of 5.4 mg/L DO and 0.03 $\log_{10}$ day^{-1} in water with a mean DO of 0.2 mg/L. The temperature was on average the same in these holes at 15.7 and 15.9 °C. No other direct comparisons of the same organisms could be made in the groundwater. The authors also speculated that microbial activity in the poliovirus 1 dialysis tube at higher DO concentrations could have led to more rapid inactivation, due to the detection of high numbers of *Pseudomonas maltophila* in samples from this microcosm that were not detected in the other bore holes.

The effect of hydrostatic pressure on poliovirus 1 survival was evaluated by Bitton et al. (*26*) using groundwater and seawater. Groundwater samples spiked with virus were stored for 24 h at 24 °C at initial pressures of atmospheric pressure (control), 500, 1000, 2000, 3000, and 4000 psi (range of 34−272 atm). Little effect was observed in the groundwater samples as a result of pressure. Survival of poliovirus 1 ranged from 82.5% of the control at 3000 psi to 100% of the control at 4000 psi. These data were not included in quantitative summary analyses in this review.

Bacteria

McFeters et al. evaluated survival of several types of pathogenic and indicator bacteria in diffusion chambers suspended in flowing well water (*27*). Bacteria evaluated included groups of coliform bacteria and enterococci from both isolates and mixed populations derived from sewage and fecal samples, along with several isolates of *Shigella* and *Salmonella* species and *Vibrio cholerae*. The use of diffusion chambers excluded native groundwater organisms, so essentially this study evaluated survival in filtered groundwater. The in situ groundwater temperature averaged approximately 10 °C. In this study, the earliest on the survival of microorganisms in groundwater, inactivation rates were reported as half-times, or the time for a 50% reduction of viable organisms in hours, while more modern inactivation studies generally employ decay rates with units of day^{-1} (either as $\log_{10}$ or ln changes in the ratio of surviving organisms). Also, survival experiments reported were of a short duration from 2 to 4 days. Most likely because of this scale difference, the inactivation half-times reported in this study if converted to the day time frame were relatively rapid, equating to rates of 0.3 $\log_{10}$ day^{-1} or faster. For quantitative comparisons of converted rates to others for this review, only two reported values were used that were derived from experiments on mixed natural populations of fecal coliform and fecal streptococci (enterococci) that lasted 4 days and were approximated from graphical data depicting about 1.5−2 log decline rather than 50%. Qualitative observations based on the author's conclusions were that indicator bacteria (coliform and enterococci) showed the same or slightly slower inactivation compared to the pathogens and enterococci

showed slightly slower inactivation than fecal coliform; thus enterococci was reported to be a superior indicator of pathogen survival.

Survival of several pathogenic and indicator/facultative pathogenic bacteria in a single groundwater source was evaluated by Filip et al. (*28*). Experiments were performed with microcosms using filtered water samples (0.45 μm) held at 10 °C, the natural temperature of the originating aquifer. Organisms analyzed for their survival in this water were *Escherichia coli*, *Salmonella typhimurium*, *Pseudomonas aeruginosa*, *Yersinia enterocolitica*, *Staphylococcus aureus*, *Streptococcus faecalis*, *Bacillus cereus*, *Bacillus megaterium*, and *Clostridium perfringens*. Survival of *E. coli*, *S. typhimurium*, and *P. aeruginosa* was also evaluated in suspensions (50% w/v) of two size-fractionated sands and a mixed-grain-size sand in groundwater. The sands were not sterilized prior to suspension, and more descriptive details of the sand were not given. Sampling of these suspensions included both attached and free cells. Adsorption to the sand was not measured. Inactivation rates ($\log_{10}$ day^{-1}) in groundwater without sand were approximately 0.03 for *E. coli*, 0.01 for *S. faecalis*, 0.04 for *S. typhimurium*, 0.2 for *S. aureus*, 0.008 for *Y. enterocolitica*, and 0.6 for *B. megaterium*. From the authors' original graphs, *B. cereus* declined about 3.6 $\log_{10}$ in 10 days but showed no further decline to 100 days. *C. perfringens* showed little to no decline over 100 days, and *P. aeruginosa* increased in concentration to 11 days and thereafter declined by approximately 1 $\log_{10}$ over 100 days. Since these declines were not even approximately linear, they were not included in summary inactivation rate data for this review. In the sand suspensions, *E. coli* inactivation was very slightly more rapid than in groundwater without sand (0.04−0.05 $\log_{10}$ day^{-1} in sand suspensions vs 0.03 $\log_{10}$ day^{-1} in water), while *S. typhimurium* inactivation was slightly slower in the sand suspensions (0.03−0.04 $\log_{10}$ day^{-1} in sand suspensions vs 0.05 $\log_{10}$ day^{-1} in water). *P. aeruginosa* inactivation in the sand suspensions was nonlinear, so rates were not estimated for this review, but the overall decline was less than that in the water-only experiments for the smaller grain sand and was greater than that in the water in the larger grain and mixed sand suspensions. Differences were not large however.

The effect of eliminating native microorganisms from groundwater (by autoclaving) on survival of seeded *E. coli* in anaerobic groundwater was determined in a study by Banning et al. (*29*). At 28 °C, inactivation of *E. coli* was much less rapid in the presterilized groundwater than in the raw sample, with inactivation rates of 0.5 $\log_{10}$ day^{-1} in raw and 0.01 $\log_{10}$ day^{-1} in sterilized water.

Personne et al. (*30*) evaluated oxygen effects on survival of fecal coliform and enterococci bacteria at a single temperature (15 °C) using nontreated groundwater and subsamples that were sealed to prevent gas exchange. It should be noted that initial microbial concentrations in this study were quite low at about 1×10^3 cfu/100 mL, whereas other studies typically employ initial concentrations several orders of magnitude greater to enable measurement of a greater degree of inactivation. Due to this difference and since survival results were presented only graphically and were not even approximately first order, inactivation rates were not estimated for comparison in this review. Qualitatively, their data indicated no differences between the aerobic and "anaerobic" conditions, and fecal coliform inactivation was much more rapid than that of enterococci.

The effect of solid media on *E. faecalis* inactivation was evaluated by Pavelic et al. using diffusion chambers suspended in situ in a groundwater well (*31*). The well employed in this study contained low-concentration dissolved oxygen water (0.11 mg/L) at 20 °C. Some diffusion chambers were packed with porous media from the aquifer while others contained only the bacteria to determine any effect on inactivation. The water initially used to suspend *E. faecalis* cells for loading into test chambers and the porous media were presterilized, so no effects due to native microbial activity were present. Sampling of the chambers with aquifer substrate extracted only pore water, so no attached bacteria were sampled. No differences in inactivation due to the solid media were detected, with inactivation rates being approximately 0.7 $\log_{10}$ day^{-1} in either case.

Temperature effects on survival of *E. coli* in saturated soils were reported by Sjogren (*32*). Although this study employed distilled water as a water source rather than groundwater, its data were incorporated in this review due to the good temperature comparison for *E. coli* and importance of data on survival in saturated porous media for groundwater considerations. Two sandy loam soils were evaluated, one slightly alkaline in suspension (pH 6.8−8.3) and the other slightly acidic (pH 5.5−7.2). Adsorption analyses were not performed, and only the saturated soil experiments were considered in this review. Results from this work indicated that little temperature effect was present for *E. coli* inactivation up to 20 °C. Inactivation rates for temperatures from 5 to 20 °C were all from 0.03 to 0.06 $\log_{10}$ day^{-1} in the two soil types evaluated, while at 37 °C inactivation was more rapid at about 0.2 $\log_{10}$ day^{-1} in both saturated soils. The *E. coli* died off more rapidly in the more alkaline soil at 10 and 20 °C (1.5−2 times faster than acidic soil), but there was little difference at 5 and 37 °C.

Comparisons of Viruses and Bacteria

Keswick et al. (*33*) evaluated survival of several indicator organisms and animal viruses in situ in well water using polycarbonate membrane survival chambers that allowed exchange of water and dissolved compounds while retaining test organisms within the chamber and excluding autochthonous microbes. Organisms evaluated were coxsackievirus B3, poliovirus 1, echovirus 7, rotavirus SA-11, and bacteriophage f2 along with the bacteria *E. coli*, *S. typhimurium*, and fecal streptococci (enterococci). Seeded chambers were placed in a covered container receiving a continuous flow of groundwater. The water temperature over the 24-day duration of the experiment was reported to vary from 3 to 15 °C in the article, but given the relatively short duration of the experiment and the well depth of 275 ft, this seems unlikely and may be a misprint with actual temperature ranging from 13 to 15 °C or even 3−5 °C. Unfortunately, no further details were described that would reveal if the reported temperature was correct. The bacteriophage f2 and *E. coli* both declined at a faster rate than two of the animal viruses (coxsackievirus B3 and poliovirus 1), while the sewage-isolate fecal streptococci exhibited a similar inactivation rate to poliovirus 1 and coxsackievirus B3.

Bitton et al. (*34*) evaluated survival of a number of indicator organisms and pathogens in a single Florida groundwater source. Inactivation of *E. coli*, *Streptococcus (Enterococcus) faecalis*, *Salmonella typhimurium*, bacteriophage f2, and poliovirus 1 was determined in seeded groundwater flasks, incubated at 22 °C. *E. coli* and *S. typhimurium* were much more rapidly inactivated in this groundwater than poliovirus, but inactivation of *S. faecalis* was approximately similar to poliovirus. In addition, a field study was performed in which samples were taken from six shallow monitoring wells tapping groundwater that received primary septic tank effluent. Septic discharge was halted due to excessively dry conditions, and sampling of the shallow wells was conducted with the cessation of discharge to evaluate the survival of indicator bacteria present in the groundwater. Inactivation of these fecal and total coliform in the field study closely paralleled that of poliovirus 1 in the laboratory.

Nasser and Oman (*35*) examined temperature effects on several organisms in groundwater. Organisms evaluated were

TABLE 1. Summary Statistics on Viral Inactivation Rates[a]

organism	poliovirus	echovirus	hepatitis A[b]	coxsackievirus	coliphage	PRD-1
n (values)	56	18	22	8	77	13
temp. range (°C)	3−30	12−25	4−30	3−28	4−30	7−23
geometric mean	0.07	0.1	0.02	0.02	0.1[c]	0.04[d]
mean	0.2	0.1	0.03	0.09	0.3	0.09
std dev	0.3	0.1	0.04	0.1	0.5	0.2
median	0.07	0.09	0.02	0.03	0.08	0.03
Q_1	0.04	0.06	0.01		0.03	0.01
Q_3	0.1	0.2	0.04		0.3	0.05
IQR	0.06	0.14	0.03		0.27	0.04
range	0.005−1.7	0.05−0.6	0−0.1	0.002−0.3	0−2.5	0−0.8
outer fence F_1	<0	<0	<0		<0	<0
outer fence F_3	0.4	0.4	0.13		0.9	0.2
no. strong outliers	5	1	0		5	1
number studies	10	3	3	3	13	4
references	*19−21, 23−25, 33−35, 37*	*19, 21, 25*	*21, 24, 35*	*25, 33, 37*	*18−20, 22−24, 33−38, 41*	*22, 24, 36, 41*

[a] All values are in log decline per day. Rates were either taken directly from reported data, converted from data reported in other formats, or estimated from graphical information. [b] Does not include results from Sobsey et al. at 5 °C, which were not shown due to no decline. [c] One value of "0" excluded from geometric mean calculation. [d] Two values of "0" excluded from geometric mean calculation.

hepatitis A virus, male-specific bacteriophage (F+ phage), *E. coli*, and poliovirus 1. Surprisingly, inactivation of *E. coli* was most rapid at 4 °C. Otherwise, survival was negatively impacted by temperature based on comparative observation of figures in this study. Graphically depicted inactivation rates of the four organisms were generally in the range from 0.01 to 0.05 $\log_{10}$ day^{-1}, although low poliovirus and hepatitis A virus inactivation rates of about 0.005 and 0.001 $\log_{10}$ day^{-1}, respectively, were reported at 4−10 °C.

Dowd and Pillai (*36*) evaluated survival of two bacteria and two bacteriophage in groundwater microcosms. Experiments were run with *Salmonella typhimurium* and a *Klebsiella* species and MS-2 and PRD-1 bacteriophage. Survival microcosms were incubated at 21 °C. Inactivation results were reported graphically for bacterial organisms, while the bacteriophage inactivation was stated as approximately 0.8 $\log_{10}$ day^{-1} for both phage strains. Bacterial inactivation rates were about 0.1 $\log_{10}$ day^{-1} for *Klebsiella* and 0.6 $\log_{10}$ day^{-1} for *S. typhimurium*.

The effect of several parameters that may influence inactivation of microbes in groundwater was evaluated by Gordon and Toze (*37*). Comparative evaluations of inactivation effects from temperature (15 vs 28 °C), native microorganisms (filter-sterilized vs raw water), and aerobic versus anaerobic conditions were made for *E. coli*, MS-2, poliovirus 1, and coxsackievirus 1 in groundwater microcosms. This study thus represents a valuable comparative look at several factors within the context of a single study with consistent experimental techniques. The most influential factor affecting the survival of all tested organisms was the presence or absence of native groundwater microorganisms. Differences due to temperature were present but secondary to the effect of filter-sterilizing the water. Oxygen effects were evaluated at a single temperature (28 °C), and these comparisons revealed that the most rapid inactivation for all organisms was in raw, aerobic water. For the enteric viruses (poliovirus and coxsackievirus), inactivation in filtered aerobic and anaerobic and raw anaerobic water was all similarly slow. However, for *E. coli* and MS-2, inactivation in raw anaerobic water was not as rapid as in aerobic water but was still quite rapid, while in filtered aerobic and anaerobic water inactivation was similarly slow. The indication from these data is that the native microbial population significantly influences survival of contaminants.

Pang et al. (*38*) evaluated inactivation of free F+ RNA coliphage, fecal coliform, and *E. coli* in groundwater batch studies and used modeling equations and results from column and batch adsorption and removal studies to estimate inactivation of sorbed microbes for comparisons. The experimental and mathematical procedures for making evaluations of this type are described in detail in this study and in a review by Schijven and Hassanizadeh (*39*). Inactivation rates of these organisms at 20 °C indicated that in the liquid phase (in water column) F+ RNA coliphage were more rapidly inactivated than the bacteria by a factor of about 3 with $\log_{10}$ day^{-1} rates of about 0.08 for bacteria and 0.27 for the phage. Estimates of sorbed inactivation rates revealed much more rapid inactivation with rates >1 $\log_{10}$ day^{-1} for all organisms. The difference between free and estimated attached inactivation rates were by a factor of 5 for the coliphage and 15−20 for the fecal coliform and *E. coli*.

Compilation of Inactivation Rate Data from Reviewed Studies

To summarize inactivation data, most rates determined from reviewed studies described above were compiled for each type of organism. Values where no inactivation was measurable were not included in some analyses. From these compilations, some summary statistics were determined to gauge the ranges and central tendencies of the data. To facilitate data analyses, organisms were grouped into several categories: coliphage (all types), poliovirus, echovirus, hepatitis A virus, PRD-1 bacteriophage, coliform bacteria (including fecal, total, and *E. coli*), enterococci/streptococci, and *Salmonella* spp. All of the rates compared were converted to $\log_{10}$ N/N_0 inactivation values.

Tables 1 and 2 contain summary statistics for virus and bacteria inactivation data, respectively. In each table, statistics calculated include the number of observations, means, medians, and standard deviations. Also, the values corresponding to Q_1 and Q_3 (lower and upper bounds of the middle 50% of observed values, respectively), interquartile ranges (IQRs, range of the middle 50% of values), and minimum and maximum inactivation rate values from the reviewed studies are listed. The locations of the outer fences F_1 and F_3 were determined and given, which were based on the value of Q_1 or $Q_3 \pm (3 \times \text{IQR})$, respectively. The location of the outer fences identifies any rate values that are strong outliers compared to the rest of the reviewed inactivation rates for that organism group, and the number of strong outliers is given for each group. Also, temperature ranges of the studies were included, and the number of studies evaluated for each organism group are shown. Statistics for the quartiles, fences, and outliers were only performed on organism groups for which there were at least 10 values.

TABLE 2. Summary Statistics on Inactivation Rates for Bacteria

organism	coliform bacteria	enterococci/ fecal streptococci	*Salmonella* spp
n (values)	35	7	6
temp. range (°C)	3−37[a]	3−22*	10−22
geometrtic mean	0.08	0.1	0.07
mean	0.3	0.3	0.1
std dev	0.4	0.3	0.2
median	0.05	0.2	0.05
Q_1	0.03		
Q_3	0.2		
IQR	0.17		
range	0.01−1.5	0.01−0.8	0.03−0.6
outer fence F_1	< 0		
outer fence F_3	0.9		
no. strong outliers	4		
number studies	10	5	3
references	*27−29, 32−38*	*27, 28, 31, 33, 34*	*28, 34, 36*

[a] Temperature includes range of 3−15 °C for diffusion chamber study by Keswick et al. (*33*).

Given the uncertainties and complexities inherent in studies on the survival of millions or billions of biological organisms under a variety of different environmental conditions and the fact that some rate values were approximated from graphical presentations of results, statistics in these and other tables summarizing rates in this review are rounded to one significant figure. This also helps to emphasize the interpretation of these results in terms of large-scale trends, relationships, and periods needed for inactivation, the resolution for which should not realistically be narrowed below several days to weeks when regarding estimates of microbial survival in the relatively stable environment of aquifers.

For virus survival studies, coliphage were evaluated in 13 studies reviewed here, for a total of 77 observations. Polioviruses were included in 10 studies for 56 observations. The temperature ranges covered by data for both viruses was from 3 or 4 to 30 °C. Geometric and arithmetic means were 0.07 and 0.2 $\log_{10}$ day^{-1} for poliovirus, respectively, and 0.1 and 0.3 $\log_{10}$ day^{-1} for coliphage, respectively. The value of the lower bound to the middle 50% of reviewed inactivation rates (Q_1) is nearly equal for these two virus types. Mean values for echovirus inactivation rates were also similar to poliovirus. However, hepatitis A virus inactivation rates are somewhat less than these other viruses. Longer survival of HAV has also been reported in individual studies comparing hepatitis inactivation to that of other virus types (*21, 24*). From a structural standpoint, HAV and poliovirus are related, as both are picornaviruses with naked icosahedral capsids and single stranded RNA genomes. Potential effects of more specific structural differences that would influence survival capacity have not been identified.

For coliform bacteria, including *E. coli* and nonspecific total and fecal coliform results, inactivation rates from 10 studies were compared, in which the temperature ranged from 3 to 37 °C, for a total of 35 rate values. Data values ranged from about 0.01 to 1.5 $\log_{10}$ day^{-1}. If only the middle 50% of values are considered, then inactivation rates for coliform ranged from 0.03 to 0.2 $\log_{10}$ day^{-1}, which relate to 90% inactivation times of 5 to about 30 days. Four values were identified as strong outliers from the other data, which were two from Gordon and Toze survival experiments on *E. coli* in unfiltered groundwater at 28 °C and two from Pang et al., one *E. coli* and one mixed fecal coliform, which were estimated inactivation rates of bacteria sorbed to pumice in groundwater at 20 °C. The data for coliform bacteria were widely distributed in general, with the standard deviation in excess of both the arithmetic and geometric means. With a range of values over 2 orders of magnitude, the geometric mean is a better representation of the central tendency of these data. The geometric mean for coliform inactivation rates converts into a time for 90% reduction on the order of 10−14 days.

Inactivation rates for enterococci and/or fecal streptococci from five reviewed studies were compiled into summary statistics for a total of seven observations (Table 2). As discussed above, most enterococci data from the McFeters study (*27*) were not compiled with these since those data were reported in "half-times" and the actual decline expressed is of a different magnitude. Temperatures in these studies ranged from 3 to 22 °C. Although fewer rates were evaluated, on average enterococci inactivation is similar to that of coliform bacteria with a slightly greater geometric mean but a smaller range. However, several studies that compared the two types of bacteria found that enterococci inactivation proceeded more slowly than *E. coli* or fecal coliform (*27*, *33*, *34*). *Salmonella* bacteria inactivation rates were compiled from three studies reviewed here, for six total observations. Temperatures for *Salmonella* experiments ranged from 10 to 22 °C. On the basis of these *Salmonella* rates, 90% inactivation times would range from 2 to about 30 days. On average, *Salmonella* inactivation appears slower than the other two indicator bacteria groups.

Comparisons of inactivation rates reveal some interesting points. For one, the geometric mean value for inactivation rates for coliphage, poliovirus, echovirus, coliform bacteria, enterococci, and *Salmonella* spp. are very similar at approximately 0.07−0.1 $\log_{10}$ day^{-1} (Tables 1 and 2). In addition, the Q_1 values (lower boundary of the middle 50% of data points) of the data sets for coliform bacteria, coliphage, poliovirus, and echovirus are also similar, ranging from 0.03 to 0.06 $\log_{10}$ day^{-1} inactivation. T_{90} times corresponding to this range are about 2.5 weeks to 1 month. Also, the median and geometric mean inactivation rate values for hepatitis A, coxsackievirus, and phage PRD-1 are all similar from about 0.02 to 0.04 $\log_{10}$ day^{-1}. (The geometric mean for PRD-1 would be lower if the two observations of no decline were included also.) These would equate to longer periods for 90% decline of 4−7 weeks. And the Q_1 values of hepatitis A and PRD-1 are the same (0.01 $\log_{10}$ day^{-1}), giving a predicted period for 90% decline of 100 days.

Naturally, these inactivation rates were derived in many different ways from numerous studies at different temperatures and other conditions, and 25% of the calculated or approximated rates for each organism are below the Q_1 values. Their similarity does seem quite striking considering these observations represent 236 inactivation rate values. However, it should also be noted that all groups of organisms for which rates were compared show ranges of values representing over an order of magnitude. So although from these ranges of estimated and calculated inactivation rates there are similarities in central tendencies between these enteric organisms, survival in the groundwater environment is clearly subject to various factors that affect the relative dieoff of each type of organism.

Analysis of Factors Affecting Inactivation in Groundwater

Temperature. Several works that have been reviewed here report significant enhancement of inactivation due to higher temperature, and a review of virus behavior in groundwater by Schijven and Hassanizadeh (*39*) also describes temperature-based inactivation. For our review, rates were grouped for each organism into temperature ranges for comparison of rate values derived from various temperature conditions. Table 3 contains temperature-grouped inactivation rates for the viruses and coliform bacteria. For coliphage, poliovirus,

TABLE 3. Inactivation Rates of Viruses and Coliform Bacteria, Grouped into Temperature Ranges

organism	temperature group (°C)	mean rate (log day $^{-1}$)	geometric mean rate (log day^{-1})	median rate (log day^{-1})	std. dev (log day^{-1})	range (log day^{-1})	*n* (values)	reference
poliovirus	0−10	0.02	0.01	0.01	0.02	0.005−0.05	5	*24, 35*
	11−15	0.09	0.08	0.07	0.05	0.03−0.2	20	*19, 20, 37*
	16−20	0.1	0.09	0.08	0.05	0.03−0.2	9	*19, 20, 25, 35*
	21−25	0.2	0.1	0.07	0.3	0.02−0.7	13	*19−21, 24, 25, 34*
	26−30	0.4	0.08	0.09	0.6	0.006−1.4	8	*23, 35, 37*
hepatitis A	0−10	0.02	0.01[a]	0.001	0.04	0−0.08	5	*24, 35*
	20−30	0.04	0.03	0.03	0.04	0.009−0.1	17	*21, 24, 35*
echovirus	11−15	0.1	0.09	0.08	0.06	0.05−0.2	7	*19*
	16−20	0.1	0.09	0.10	0.04	0.05−0.2	4	*19, 25*
	21−25	0.2	0.1	0.07	0.2	0.06−0.6	7	*19, 21, 25*
coxsackievirus	0−20	0.06	0.02	0.03	0.09	0.002−0.2	4	*25, 33, 37*
	25−30	0.1	0.04	0.05	0.2	0.007−0.3	4	*37*
rotavirus	3−15	0.4					1	*33*
coliphage	0−10	0.03	0.03[a]	0.02	0.03	0−0.1	14	*19, 22, 24, 35, 41*
	11−15	0.1	0.07	0.07	0.2	0.03−1.0	30	*18−20, 37*
	16−20[b]	0.2	0.1	0.08	0.4	0.02−1.3	9	*19, 20, 35, 38*
	21−25	0.4	0.3	0.3	0.3	0.16−1.4	14	*19, 20, 22, 24, 34, 36*
	26−30	0.8	0.2	0.4	1.0	0.006−2.5	9	*23, 35, 37*
PRD-1	0−10	0.02	0.02[a]	0.02	0.01	0−0.04	7	*22, 24, 41*
	21−25	0.2	0.1[a]	0.05	0.3	0−0.8	6	*22, 24, 36*
coliform bacteria	0−10	0.07	0.05	0.04	0.1	0.03−0.4	10	*27, 28, 32, 35*
	15−20	0.4	0.1	0.08	0.6	0.02−1.5	9	*32, 35, 37, 38*
	21−37	0.3	0.1	0.2	0.4	0.007−1.4	13	*29, 32, 34−37*

[a] Excludes value of "0". [b] Includes sorbed inactivation rate (maximum value).

and coliform bacteria, inactivation rate values were plotted against experimental incubation temperature (or in situ temperature as appropriate) in Figures 1A−C. These diagrams provide a good visual summary of temperature effects on inactivation rates for these organisms. It seems that for coliform bacteria little correlation between temperature and inactivation rate is apparent; however this does not consider other aspects of the microcosm (native bacteria, solid media). Greater inactivation rate values (above 0.5 $\log_{10}$ day^{-1}) are generally found above 20 °C. A general temperature effect is somewhat more apparent for coliphage inactivation rates in Figure 1B but not for poliovirus in Figure 1A. Also, when looking at the temperature groupings for the virus in Table 3, mean inactivation rates were consistently more rapid above 20 °C than those below this temperature. Such a trend was not apparent for coliform bacteria, but averages were more rapid above 10 °C than those below this temperature.

A column graph of mean inactivation rates for each temperature group (Figure 2) depicts the trend in viral inactivation rates with respect to temperature. Figure 2 also shows the greater inactivation rates for echovirus and hepatitis A at temperatures above 10 °C. However, the increase is not as dramatic as for poliovirus and coliphage. One point, although, is that the total number of rate values represented by the means for echovirus and hepatitis A are much fewer than those for poliovirus 1 and the coliphage.

Several investigators observed that virus inactivation increases with increasing temperature within their respective studies, as shown in Table 4, which summarizes descriptions of relative temperature effects for each organism. Possible mechanisms whereby viruses are inactivated at higher temperatures are more rapid denaturation of viral capsid proteins with increased temperature or potential degradation by extracellular enzymes that could be more active at higher temperature. Potential indirect effects from indigenous groundwater bacterial activity are discussed in more detail below. Unfortunately, only two studies reviewed compared inactivation at more than one temperature for coliform-type bacteria (*32*, *37*). From these two studies, inactivation of *E. coli* was seen to increase above 20 °C in deionized (DI)-water-saturated soils, but no significant difference was observed for *E. coli* inactivation in filtered or raw groundwater between 15 and 28 °C in the study by Gordon and Toze (*37*). Temperature-based inactivation at elevated temperatures may be a vague concept for bacteria, because coliform bacteria have been shown to potentially replicate in the aquatic environment at higher temperatures given adequate nutrient levels (*40*). Temperature likely interacts with other parameters of the environment in a more complicated manner for bacteria. For instance, the effects of nutrient availability (bioavailable carbon), competing or predatory/parasitic organisms (other bacteria, protozoa, and phage), and perhaps deleterious chemical compounds all may be temperature-dependent. In the case of fecal coliform bacteria, inactivation of the population may actually be a combination of death and reproduction, thus subject to a complex interplay of growth, predation by larger organisms, competition for nutrients, bacterial antagonism, enzymatic or chemical inactivation, and phage lysis. While evidence exists showing the ability of fecal coliform to replicate in the environment, there is a lack of such evidence for the other main indicator bacterial group, enterococci, or for pathogenic bacteria of enteric origin such as *Salmonella* or *Shigella*.

Although studies that compare different temperatures for virus inactivation show consistently greater inactivation rates at higher temperatures, the diagrams in Figures 1A and 1B also demonstrate considerable variability at different temperature levels, as is also shown by the large standard deviations about the means for each temperature range in Table 3. It seems evident that although temperature is clearly a major controlling factor for virus inactivation in groundwater it is not the only factor. Thus, it is important to recognize that when considering virus inactivation in groundwater it may be inaccurate, given the current state of knowledge, to predict rates of decline based on temperature and virus type alone (*39*, *41*). Ultimately, additional factors should be further described to allow better predictions when incorporating other groundwater conditions.

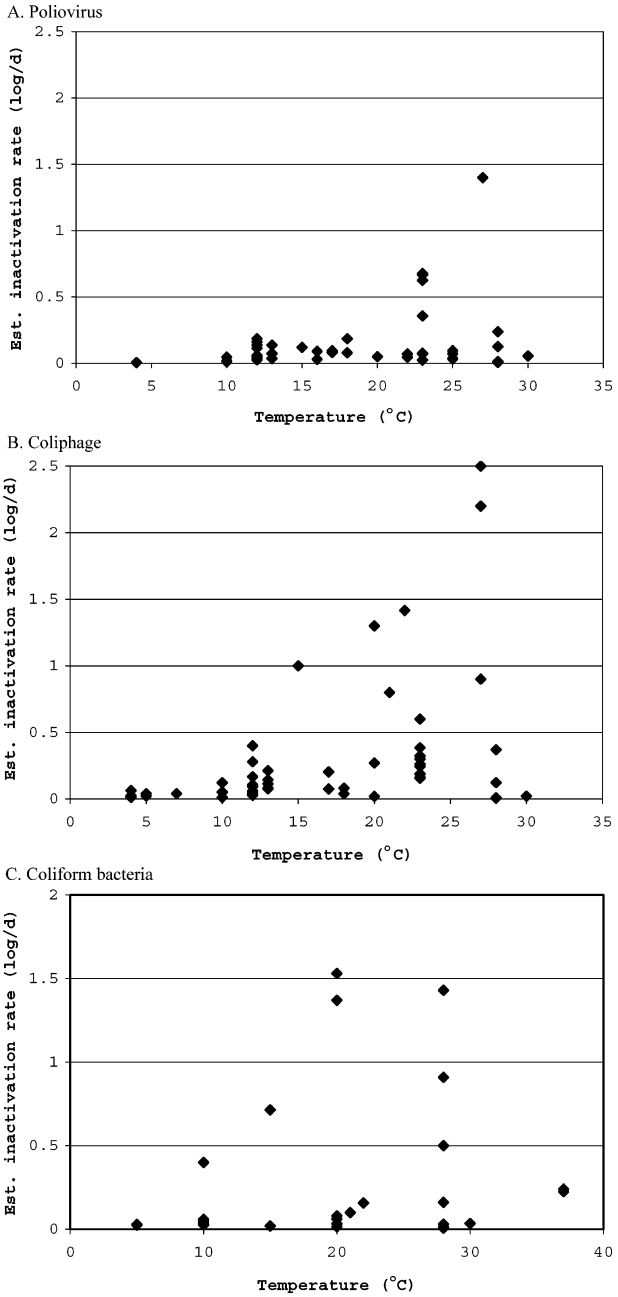

FIGURE 1. Plots of reported or approximate inactivation rates vs temperature for (A) poliovirus, (B) coliphage, and (C) coliform bacteria.

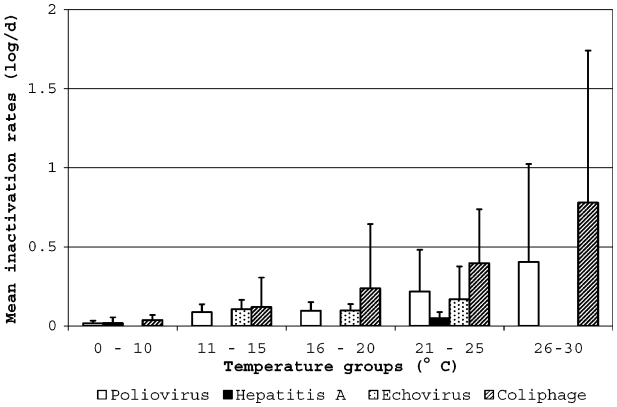

FIGURE 2. Virus inactivation rates from reviewed studies, averaged by temperature group. Error bars show standard deviation about the means for each temperature range. Hepatitis A values include one value at a temperature of 30 °C.

Native Groundwater Organisms. In the case of contaminant microorganisms input to the groundwater environment, the enteric microorganisms are not going into a sterile microcosm but an environment already inhabited by native groundwater microorganisms. The presence and activity of such organisms may play an important role in controlling the survival of non-native contaminant microbes. Of the studies reviewed here, several evaluated survival of test bacteria and viruses in either filter-sterilized water microcosms (which generally have removed bacteria and protozoa but not viruses) or in membrane diffusion chambers (which exclude native bacterial and generally viral organisms). A few also evaluated survival in autoclaved water samples (*21*, *29*). All of the studies that filter-sterilized water employed 0.2-μm pore filters except Filip et al. (*28*) who used a 0.45-μm filter. As a note on this point, since groundwater bacteria may be oligotrophic and generally smaller, they have been noted to pass through 0.45-μm filters as seen by Lillis and Bissonnette (*42*), who observed heterotrophic plate count bacteria capable of passing through 0.45-μm filters and captured on 0.2-μm filters in all of several tested groundwater samples, but never more than 10% of the total cultivable counts found on the larger pore membranes. No reports of viable groundwater bacteria able to pass through 0.2-μm filters have been found, and 0.2-μm filtration is thought to effectively remove bacteria from suspension.

As a comparison of inactivation rates in light of the presence of native groundwater bacteria, Table 5 groups inactivation rates from poliovirus, coliphage, and coliform bacteria into averages from raw conditions and bacteria/protozoa-free conditions. These are pooled from the various studies reviewed here. Since temperature appears to be a determining factor in viral inactivation rates, these are also subdivided based on broad temperature ranges. From Table 5, it appears that virus inactivation rates in light of raw versus sterile conditions do not show a clear trend. At lower temperatures, both arithmetic and geometric means of inactivation rates are greater in the sterile conditions for poliovirus and coliphage. At higher temperatures, variability is greater for pooled inactivation rates, and arithmetic means for both virus categories are greater in sterile conditions while geometric means are lower in sterile conditions. Due to the broad range of inactivation rates, geometric means may be more representative, but still a consistent trend is not apparent. Inactivation rates averaged by raw or treated water for coliform bacteria indicate that both arithmetic and geometric mean inactivation rates are more rapid by a factor of over 3 in raw water.

Perhaps a better gauge of effects from native groundwater organisms may be obtained by studies that specifically considered this as a potential factor. Such studies are summarized in Table 6. For viruses, studies by Gordon and Toze (*37*) and Sobsey et al. (*21*) reported inactivation rates to be lower in the absence of groundwater bacteria, while that of Alvarez et al. (*23*) reported slightly higher inactivation rates in filtered groundwater. However, in the study by Gordon and Toze, the impact was only observed for poliovirus and coxsackievirus in aerobic water conditions, not in anaerobic. Both reviewed studies that contrasted raw versus treated water for *E. coli* survival (one 0.2-μm filter-sterilized, one autoclaved) found that inactivation was reduced in the absence of native groundwater microorganisms. Although not listed in Table 6, Yates et al. (*20*) found that a more-rapid decline of MS-2 was significantly correlated to increasing heterotrophic bacteria counts over time, even though the presence or absence of bacteria was not significantly correlated to MS-2 inactivation rates. Jansons et al. also observed survival variability that could be related to groundwater

TABLE 4. Relative Effects of Experimental Temperature on Inactivation Rates As Described in Individual Studies

organism	effect on inactivation rate	conditions contrasted	reference
MS-2 (coliphage)	increased	w/increasing temp.	Yates et al. (*19*)
MS-2 (coliphage)	increased	w/increasing temp.	Yates and Gerba (*18*)
MS-2 (coliphage)	increased	w/increasing temp.	Yates et al. (*20*)
MS-2 (coliphage)	increased	at 20 vs 10 °C	Blanc and Nasser (*24*)
poliovirus 1	increased	w/increasing temp.	Yates and Gerba (*18*)
poliovirus 1	increased	w/increasing temp.	Yates and Gerba (*19*)
poliovirus 1	increased	w/increasing temp.	Yates et al. (*20*)
poliovirus 1	increased	at 25 vs 5 °C	Sobsey et al. (*21*)
poliovirus 1	increased	w/increasing temp.	Nasser and Oman (*35*)
poliovirus 1	increased	at 20 vs 10 °C	Blanc and Nasser (*24*)
echovirus	increased	at 25 vs 5 °C	Sobsey et al. (*21*)
hepatitis A	increased	at 20 vs 10 °C	Blanc and Nasser (*24*)
hepatitis A	increased	at 25 vs 5 °C	Sobsey et al. (*21*)
hepatitis A	increased	w/increasing temp.	Nasser and Oman (*35*)
coxsackievirus B1	increased	at 28 vs 15 °C	Gordon and Toze (*37*)
E. coli in DI-water-saturated soils	increased	at 37 vs ≤20 °C	Sjogren (*32*)

TABLE 5. Comparison of Estimated Inactivation Rates Pooled from Poliovirus, Coliphage, and Coliform Bacteria into Raw and Treated Conditions[a]

organism and conditions		mean rate (log day^{-1})	std. dev (log day^{-1})	geometric mean (log day^{-1})	*n* (values)	reference
poliovirus	raw; 0−15 °C	0.07	0.05	0.05	19	*19, 20, 24, 35, 37*
	treated; 0−15 °C	0.11	0.07	0.09	7	*20, 33*
	raw; 16−30 °C	0.2	0.3	0.1	24	*19−21, 23−25, 34, 35, 37*
	treated; 16−30 °C	0.4	0.6	0.08	6	*20, 23, 37*
coliphage	raw; 0−15 °C	0.08	0.2	0.05	33	*18−20, 22, 24, 35, 37, 41*
	treated; 0−15 °C	0.2	0.1	0.1	11	*18, 20, 33*
	raw; 16−30 °C	0.4	0.5	0.2	25	*19, 20, 22−24, 34−38*
	treated; 16−30 °C	0.6	0.9	0.1	7	*20, 23, 37*
coliform bacteria	raw, all temperatures	0.3	0.5	0.1	27	*28, 29, 32, 34−38*
	treated, all temperatures	0.1	0.2	0.03	8	*27−29, 33, 37*

[a] Bacteria and larger organisms were generally removed by filtration or seclusion of test organisms in diffusion chambers or dialysis tubes and in some cases by autoclaving (which would also inactivate viruses). Thus, "treated" does not apply to the possible presence of bacteriophage in general.

TABLE 6. Summary of Trends Observed in Comparison of Microbial Inactivation from Raw to Bacteria-Free Groundwater

organism	relative inactivation rate in raw vs treated	reference
MS-2 (coliphage)	decreased	Alvarez et al. (*23*)
MS-2 in aerobic and anaerobic	increased	Gordon and Toze (*37*)
poliovirus 1	decreased	Alvarez et al. (*23*)
poliovirus 1 in aerobic only	increased	Gordon and Toze (*37*)
poliovirus 1	increased	Sobsey et al. (*21*)
echovirus	increased	Sobsey et al. (*21*)
coxsackievirus B1 in aerobic	increased	Gordon and Toze (*37*)
hepatitis A	increased	Sobsey et al. (*21*)
E. coli in aerobic and anaerobic	increased	Gordon and Toze (*37*)
E. coli in anaerobic	increased	Banning et al. (*29*)

bacterial activity (*25*). They speculated that more rapid poliovirus inactivation in the higher dissolved oxygen bore was possibly related to high counts of the bacteria *Pseudomonas maltophila*. In support of the potential role of pseudomonad bacteria in affecting viral survival, a study by Nasser et al. (ref *43*, not reviewed for quantitative inactivation rates here) found increased inactivation of coxsackievirus and hepatitis A in soil saturated with suspensions of extracellular exudates from *Pseudomonas aeruginosa* cultures compared to buffered saline controls, suggesting inactivation as a result of possible enzymatic attack.

The most comprehensive insight into possible mechanisms for inactivation was provided by Gordon and Toze, who also compared the effect of peptone and glucose amendment on virus and *E. coli* survival, in both prefiltered and raw water. (Nutrient-amendment results were not included in quantitative comparisons for this review.) Nutrient addition decreased inactivation of *E. coli*, poliovirus, and coxsackievirus over nonamended trials, and decay was only observed at all in amended trials when groundwater bacteria were present. Those authors speculated that the most important factor for survival of contaminant viruses and bacteria was the activity of native groundwater microbes, and temperature and redox conditions indirectly affected this activity such that higher temperature and aerobic conditions allowed for increased activity of native organisms, leading to a faster decay of test organisms. The explanation offered for the reduced decay in nutrient-amended trials

when native organisms were present was that the peptone and glucose offered protection from inactivation by enzymatic attack or acted as alternate nutrient sources for the native bacteria, instead of attacking the seeded viruses or bacteria themselves for food.

Thus, while there is some indication from the literature that virus survival in the subsurface may be affected negatively by the activity of other microorganisms in some situations, this phenomenon appears to be more consistent for bacterial survival. Bacteria would intuitively be subject to a wider range of threats from other organisms: They may be attacked by either protozoa or phage, along with poorly understood interactions with other bacteria. In support of predation by eukaryotic organisms, Davies et al. demonstrated that chemical inhibition of protozoan predators greatly enhanced *E. coli* survival in stream sediments and actually allowed for a net increase in culturable counts (*44*). Also, since coliform bacteria such as *E. coli* are thought to replicate in the environment under the proper conditions, observed inactivation is probably a combination of dieoff and low-level reproduction, which could be enhanced without competition for nutrients from other bacteria. Native heterotrophs would likely be more adept at utilizing environmental concentrations of nutrients, and this would also explain why Gordon and Toze found reduced decay of *E. coli* when nutrients were added. Autoclaving water may have the additional effect of increasing the bioavailability of organic compounds via thermal degradation, possibly leading to enhanced reproduction of bacteria like *E. coli*. Future experimentation evaluating the complex interactions of native groundwater organisms with introduced enteric microbes and the environmental factors that influence these interactions will continue to be highly valuable and is critical to any mechanistic studies of microorganism survival in the subsurface.

Dissolved Oxygen Level. Due to lack of atmospheric contact, reducing conditions are often encountered in groundwater aquifers. However, the majority of studies reviewed here did not consider the potential impact of dissolved oxygen concentrations on survival of contaminant microorganisms in groundwater. Naturally, modeling anaerobic conditions in a time-series survival experiment is considerably more difficult. However, while not enough inactivation studies in anaerobic groundwater conditions were found to make pooled inactivation rate comparisons, DO considerations were addressed in several reviewed studies. The study by Gordon and Toze (*37*) did directly compare aerobic to anaerobic conditions, finding that poliovirus and coxsackievirus inactivation in anaerobic groundwater was much slower than that in aerobic groundwater, while MS-2 and *E. coli* inactivation was slightly reduced in anaerobic water compared to that in aerobic water. Jansons et al. (*25*) reported more rapid inactivation of poliovirus in the bore hole with higher DO concentrations, but the difference was not deemed statistically significant. Although not a study performed in groundwater, a report by Roslev et al. (*45*) found that in drinking water aerobic incubation significantly increased inactivation of coliform bacteria and somatic coliphage but not fecal enterococci. Personne et al. determined that there was no difference between aerobic and anaerobic incubations on inactivation of fecal coliform and enterococci (*30*). The effect of dissolved oxygen is potentially an interactive one with the impact of native microorganisms. If low DO levels impede predatory eukaryotes or antagonistic bacteria, survival of fecal organisms will be enhanced. Enteric bacteria are naturally microaerophilic due to conditions in the gut, so intuitively lower DO levels would not be directly detrimental.

Solid Media and Attachment. Another important consideration regarding the groundwater environment is the presence of a solid matrix composed of the aquifer mineral material. However, many studies on microbial survival in groundwater have not employed saturated solid material and have measured inactivation only in the water column. This may be partly due the added difficulty in sampling and in differentiating inactivation from sorption to mineral surfaces. However, the presence of aquifer substrate presents a different environment to bacteria and viruses that may impact observations on their survival in two important ways.

First, inactivation kinetics of sorbed microbes may be different to those free in the pore water of saturated media, which is important for considerations of reversible attachment and release kinetics used in contaminant transport models. Microbes that are attached to mineral surfaces as a result of irreversible attachment are not necessarily relevant to considerations of microbial survival in aquifers, because these have been effectively taken out of the system of concern. A review on viruses by Schijven and Hassanizadeh (*39*) presents a detailed explanation of modeling considerations for evaluating inactivation with respect to sorbed and free viruses. From studies discussed in that review, many of which involved water types other than groundwater such as sewage effluents and marine/estuarine water, the authors surmise that generally inactivation of attached virus particles is reduced over inactivation of free particles in the liquid phase, but there are exceptions reported. Two more recent studies made comparisons between measured inactivation of free organisms in the groundwater solution phase and modeled estimates of inactivation rates for attached organisms. One of these (Pang et al., ref *38*) determined that for F+ RNA coliphage, a fecal coliform population, and an *E. coli* isolate inactivation of sorbed organisms was considerably more rapid than that for free ones. Likewise, Ryan et al. (*41*) interpreted modeling results from a combination of field and laboratory experiments involving iron-oxide-coated sand to indicate that attachment of radiolabeled PRD-1 and MS-2 bacteriophage resulted in surface inactivation at a rate much faster than that in solution at their aquifer temperature of 15 °C.

In general, apparently conflicting observations likely indicate that besides experimental differences and interpretations of data obtained via indirect measurements, modeling, and assumptions, differences among organisms, mineral media, and water conditions play a role in the degree and impact of attachment. Mechanistically speaking, Ryan et al. hypothesize that strong electrostatic attractions between virus particles and charged surfaces on minerals result in surface-based inactivation via denaturation of viral proteins. This would be largely dependent on the water pH, viral characteristics (isoelectric point), dissolved solids and organic content of the water, and the nature of the minerals in the aquifer substrate, making impacts very site-specific. If attachment to mineral media has a major impact on enteric microbial survival, including enteric bacteriophages, then studies that compared inactivation in the presence of soil or aquifer substrate should reveal more rapid inactivation in suspensions with solid media than in water alone if sampling of suspensions captured both sorbed and free microbes. Furthermore, this impact should also be correlated to the degree of adsorption to solid media. Several studies that evaluated such conditions were reviewed here. Among these, results from Filip et al. (*28*) revealed little difference between inactivation in sand suspensions versus water without sand for *E. coli*, *S. typhimurium*, and *P. aeruginosa*. Adsorption was not measured. In studies of hepatitis A virus, by Blanc and Nasser (*24*) and Sobsey et al. (*21*), HAV inactivation was found to be less rapid in soil suspensions than in water without soil, and adsorption was substantial (up to 99%) in Sobsey et al. (not reported in Blanc and Nasser). This also was the case for poliovirus. In concert with the findings of Ryan et al., however, PRD-1 inactivation was found to be

more rapid in soil suspensions than water without soil by Blanc and Nasser. These observations lend credence to speculation that aspects of viral structure may be a deciding factor in the impact of attachment/adsorption to inactivation, with PRD-1 among those detrimentally affected by attachment. This concept needs further detailed study, with the objective of specifically determining the impact of structural components on surface- or attachment-mediated inactivation or lack thereof. Why the results of Pang et al. indicated extremely rapid inactivation of coliform-type bacteria when adsorbed is not clear. Generally, fecal coliform, which include *E. coli*, are thought to be harbored in surface water sediments in much greater numbers (*46*−*48*), which would be counterintuitive if dramatically faster inactivation of attached fecal coliform were a universal phenomenon.

The second general consideration for the impact of aquifer substrate on enteric microbial survival is the indirect effects presented by this environment, such as the potential impact on inactivation by predatory/competing organisms or redox conditions on survival of organisms traveling through the pore water of solid media. Only one study found (*31*) directly evaluated the presence or absence of aquifer substrate on survival of bacteria (enterococci) in their diffusion chambers, finding no difference in survival of bacteria from the extracted pore water. However, these chambers employed prefiltered water and sterilized aquifer material and excluded other organisms, thus precluding any impact from other organisms such as predatory protozoa. In effect, protection offered by aquifer substrate may be the more important difference and its impact thus an indirect one to the complex issue of native microorganism effects on the survival of enterics. In terms of experimentation, saturated aquifer substrate would present an environment free from direct aeration, allowing a possibly rapid decline of dissolved oxygen due to bacterial metabolism depending on temperature and nutrient content of the water. The potential impacts of dissolved oxygen have been discussed above. This presents a potentially significant difference between laboratory conditions employing simple water microcosms and those in the environment, something that will be important to address more directly in future studies. For instance, mesoscale models of aquifers employing saturated aquifer material or even consolidated material may be much more helpful in recreating subsurface conditions if they can be engineered.

The inactivation rates pooled for analyses in this review were derived from many independently performed experiments, some which directly calculated rates in these terms and others which did not. The data from these reports were originally presented in disparate ways, and the number of observations for a given condition may not be large enough to draw conclusions. Furthermore, the data reviewed here were obtained from many studies in which quantifying survival was not a primary emphasis but secondary to other research objectives such as modeling transport. Thus, mechanistic insights were not enabled.

Many variables may come into play in bench-scale experiments, particularly the source and handling of organisms. For instance, some studies involved populations of bacteria or viruses derived from natural sources such as wastewater or animal feces, while others utilized pure strains maintained in laboratory conditions for many generations. Treatment of organisms prior to seeding survival experiments also varied, such as propagation and purification procedures. Evaluating the impact of these protocol variations among many studies is difficult. An additional point of concern is the extrapolation of experimental results from bench-scale studies to in situ behavior of these many types of organisms. Of the studies reviewed here, only one evaluated in situ decline of indicator organisms. From the results of Bitton et al. (*34*), inactivation rates of total coliform, fecal coliform, and fecal streptococci (enterococci) were approximately $0.02-0.03\ \log_{10}\ \text{day}^{-1}$. These values are below the slow side of the middle 50% of observed rates reviewed here for their respective organism type. However, these rates still extrapolate to T_{90} inactivation times in a reasonable time frame, on the order of 30−50 days.

It is also important to note for very low reported inactivation rates that these frequently are the result of little to no observed inactivation over the experimental time frame. These should not necessarily be construed to indicate that the rate would be constant over a period required for instance for a 3 log reduction. Most experiments reviewed here lasted on the order of 1 month, and none were in excess of 100 days. Thus, inactivation rates below 0.01 could not necessarily be measured accurately.

Studies following consistent experimental procedures need to be performed to hopefully reduce variability among investigators' findings. Standards for performing bench-scale survival studies should include protocols for the propagation and preparation of seeded organisms and should include controls such as American Type Culture Collection strains of MS-2 and *E. coli* to preclude differences in the organisms themselves. However, many differences among investigators' findings are due to the complexity of interactive factors controlling survival. To address these, some aquifer survival studies need to move into the "why" and not just the "what" of survival and factors controlling it. These would have to be concise, controlled experimentation involving possibly radiolabel tracing, the use of mutants such as phage-resistant bacterial strains, additional microbiological manipulation such as inhibitors of protozoa, or measurement of metabolic activity and nutrients (i.e., bacterial production). In addition, more mesoscale model and field studies are needed to better match conditions as found in the subsurface such as dissolved oxygen and mineral matrix effects. While the introduction of potentially harmful microorganisms into the environment is generally opposed, innovative studies of groundwater contamination by natural sources could prove helpful. If the proper safeguards could be ensured, controlled field studies involving seeded nonpathogenic microorganisms could prove even more beneficial if the results of such studies are expressed in quantitative terms and are published in peer-reviewed literature to enable wide dissemination of this information.

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There were footnote designation errors in the version published ASAP August 27, 2005; the corrected version was published ASAP August 30, 2005.

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