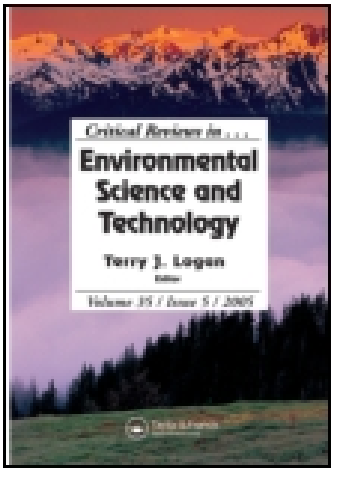

Critical Reviews in Environmental Control
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/best19

Modeling microbial fate in the subsurface environment

Marylynn V. Yates [a] , Scott R. Yates [b] & Charles P. Gerba [c]
[a] Subsurface Processes Branch , U.S. Environmental Protection Agency , Ada, Oklahoma
[b] U.S. Department of Agriculture/Agricultural Research Service , U.S. Salinity Laboratory , Riverside, California
[c] Department of Microbiology and Immunology , University of Arizona , Tucson, Arizona
Published online: 09 Jan 2009.

MODELING MICROBIAL FATE IN THE SUBSURFACE ENVIRONMENT

Authors: **Marylynn V. Yates**
Subsurface Processes Branch
U.S. Environmental Protection Agency
Ada, Oklahoma

Scott R. Yates
U.S. Department of Agriculture/
Agricultural Research Service
U.S. Salinity Laboratory
Riverside, California

Referee: Charles P. Gerba
Department of Microbiology and Immunology
University of Arizona
Tucson, Arizona

INTRODUCTION

Groundwater supplies over 100 million Americans with their drinking water; in rural areas, there is an even greater reliance on groundwater as it comprises up to 95% of the water used.[1] It has been assumed traditionally that groundwater is safe for consumption without treatment because the soil acts as a filter to remove pollutants. As a result, private wells generally do not receive treatment,[2] nor do a large number of public water supply systems.[3] However, the use of contaminated, untreated or inadequately treated groundwater has been the major cause of waterborne disease outbreaks in this country since 1920.[4,5]

Between 1920 and 1980, 1405 waterborne outbreaks were reported in the U.S. involving over 386,000 people and resulting in 1083 deaths.[4] From 1981 to 1983, there were 112 reported waterborne outbreaks and 28,791 cases of illness associated with drinking water.[5] Since 1971, the average annual number of reported outbreaks has increased: from 1971 to 1975, an average of 25 outbreaks was reported; from 1976 to 1983, this number increased to 40.[4,5] The increase in reported numbers of outbreaks may be due to an improved system for reporting implemented in 1971;[6] however, it is still believed that only a fraction of the total number of outbreaks is reported.[7]

Etiologic agents were determined in less than one half of the outbreaks that have occurred since 1971 (Table 1).[4,5] *Giardia lamblia* was the most frequently identified cause of disease, and caused over 20% of the illness associated with waterborne disease outbreaks. Enteric viruses were identified as the causative agents of disease in 8.7% of the outbreaks during this period.

When considering outbreaks that have occurred due to the consumption of contaminated, untreated or inadequately treated groundwater, from 1971 to 1982, the most commonly identified etiologic agents are Shigellae and hepatitis A virus (Table 2).[6] Hepatitis A virus was responsible for 7.8% of the reported groundwaterborne disease outbreaks; in all, viruses were identified as the causative agents in 11.2% of the outbreaks. In almost two thirds (64.7%) of the outbreaks, no etiologic agent could be identified, and the illness was simply listed as gastroenteritis of unknown etiology. However, recent retrospective serological studies of outbreaks of acute nonbacterial gastroenteritis from 1976 through 1980 indicated that 42% of these outbreaks (i.e., the 64.7% for which no etiologic agent was identified)

Table 1
ETIOLOGY OF WATERBORNE DISEASE OUTBREAKS IN THE UNITED STATES, 1971 to 1983[4,5]

	Outbreaks		Illnesses	
Disease	No.	% of total	No.	% of total
Gastroenteritis, unknown etiology	227	50.9	60,191	56.16
Giardiasis	81	18.16	22,721	21.2
Chemical poisoning	46	10.31	3,743	3.49
Shigellosis	31	6.95	5,727	5.34
Hepatitis A	22	4.93	730	0.68
Viral gastroenteritis	17	3.81	5,734	5.35
Salmonellosis	10	2.24	2,300	2.15
Campylobacter diarrhea	5	1.12	4,773	4.45
Typhoid	4	0.9	222	0.21
Escherichia coli diarrhea	1	0.22	1,000	0.93
Cholera	1	0.22	17	0.02
Yersiniosis	1	0.22	16	0.01
Totals	446	100	107,174	100

Table 2
ETIOLOGY OF WATERBORNE DISEASE OUTBREAKS IN UNTREATED OR INADEQUATELY TREATED GROUNDWATER SYSTEMS, 1971 to 1982[6]

	Outbreaks		Illnesses	
Disease	No.	% of total	No.	% of total
Gastroenteritis, unknown etiology	132	64.7	25,700	74.85
Shigellosis	20	9.8	4,938	14.38
Hepatitis A	16	7.8	493	1.44
Chemical poisoning	12	5.9	157	0.46
Viral gastroenteritis	7	3.4	1,363	3.97
Giardiasis	7	3.4	96	0.28
Typhoid fever	4	2	222	0.65
Salmonellosis	4	2	352	1.03
Yersiniosis	1	0.5	16	0.05
Escherichia coli diarrhea	1	0.5	1,000	2.91
Totals	204	100	34,337	100

were caused by the Norwalk virus.[8] Thus, it has been suggested that the Norwalk virus is responsible for approximately 23% of all reported waterborne outbreaks in the U.S.[9]

The difficulty in the isolation of many enteric viruses from clinical and environmental samples probably accounts for the limited number of viruses identified as causes of waterborne disease. As methods for the detection of enteric viruses have improved, so has the percentage of waterborne disease identified as having a viral etiology.[10]

Bacteria are microscopic organisms, ranging from approximately 0.2 to 10 μm in length. They are distributed ubiquitously in nature and have a wide variety of nutritional requirements. Many types of harmless bacteria colonize the human intestinal tract, and are routinely shed in the feces. One group of intestinal bacteria, the coliform bacteria, has historically

Table 3
SEPTIC TANKS AND WATERBORNE DISEASE

Disease	No. of cases	Source of contamination	Ref.
Gastroenteritis	1200	Septic tank 150 ft from city well	22
Hepatitis A	98	Septic tank near water supply for commercial ice pellet operation	23
	17	Septic tank 6 ft from a 100-ft-deep well	24
Typhoid	5	Septic tank 210 ft from well	25
Gastroenteritis	400	Septic tank 50 ft above spring	26
	—	Septic tank 100 ft from 40-ft-deep well	27

been used as an indication that an environment has been contaminated by human sewage. In addition, pathogenic bacteria, such as *Salmonella* and *Shigella*, are present in the feces of infected individuals. Thus, a wide variety of bacteria is introduced into septic tanks. Many of these bacteria can survive and grow in septic tanks, and are present in the effluent when it moves to the soil absorption field.

Viruses are obligate intracellular parasites, i.e., they are incapable of replication outside of a host organism. They are very small, ranging in size from approximately 20 to 200 nm. Viruses that replicate in the intestinal tract of man are referred to as human enteric viruses. These viruses are shed in the fecal material of individuals who are infected either purposely (i.e., by vaccination) or inadvertently by consumption of contaminated food or water, swimming in contaminated water, or person-to-person contact with an infected individual. More than 100 different enteric viruses may be excreted in human fecal material;[11] as many as 10^6 plaque-forming units (PFU) of enteroviruses (a subgroup of the enteric viruses) per gram and 10^{10} rotaviruses per gram may be present in the feces of an infected individual.[12] Thus, viruses are present in domestic sewage and, depending on the type of treatment process(es) used, between 50 and 99.999% of the viruses are inactivated during sewage treatment.[13]

Viruses and enteric bacteria may be introduced into the subsurface environment in a variety of ways. Goyal et al.[14] isolated viruses from the groundwater beneath cropland being irrigated with sewage effluent. Viruses have been detected in the groundwater at several sites practicing land treatment of wastewater; these cases were reviewed by Keswick and Gerba.[15] The burial of disposable diapers in sanitary landfills is a means by which pathogenic microorganisms in untreated human waste may be introduced into the subsurface. Vaughn et al.[16] detected viruses as far as 408 m downgradient of a landfill site in New York. Land application of treated sewage effluent for the purposes of groundwater recharge has also resulted in the introduction of viruses to the underlying groundwater.[17, 18]

Septic tank effluent may be the most significant source of pathogenic bacteria and viruses in the subsurface environment. Septic tanks are the source of approximately 1 trillion gal of waste disposed to the subsurface every year,[19] and are the most frequently reported sources of groundwater contamination.[20] The overflow or seepage of sewage, primarily from septic tanks and cesspools, was responsible for 43% of the reported outbreaks and 63% of the reported cases of illness caused by the use of untreated water.[6]

There have been several waterborne disease outbreaks documented to have been caused by the contamination of groundwater with septic tank effluent (Table 3); these have been reviewed recently.[21] In a town of 6500 people, 1200 developed acute gastroenteritis after

consuming tap water which had been contaminated by septic tank effluent.[22] A dye tracer was used to show that the source of contamination was a septic tank located 49 m (150 ft) from the city's drinking water well. Effluent from a septic tank serving a household which had recently had infectious hepatitis contaminated a well used to make commercial ice, resulting in a 98-person outbreak of hepatitis.[23] A drinking water spring contaminated with septic tank effluent was responsible for over 400 persons developing gastroenteritis caused by a Norwalk virus-like agent.[26]

II. MICROBIAL FATE IN THE SUBSURFACE

The facts that microorganisms remain infective long enough, and can travel far enough in the subsurface to contaminate drinking water and cause waterborne disease outbreaks, have led to attempts to develop predictive models of microbial fate in the subsurface. In order to model the survival and transport of microorganisms in the subsurface, it is necessary to determine the factors which influence them. In addition to identifying these factors, it is necessary to quantify these effects in some way so that they can be used in the development of predictive models. Once in the subsurface, there are two major factors which control microbial fate: survival and migration. The longer a microorganism persists, the greater the chance that it will still be capable of causing infection when it reaches the groundwater after migrating through the soil.

In general, both the survival and migration are controlled by the specific microorganism type, the nature of the soil, and the climate of the environment.[28] The susceptibility of microorganisms to different environmental factors varies considerably among different species as well as strains. The size and chemical composition of different microorganisms influence the extent to which they can travel in the subsurface. The soil properties play a major role in the survival and migration of bacteria and viruses. The texture of the soil, its pH, organic matter content, and moisture content all influence how long microorganisms can survive and how far they can travel in the subsurface. Two aspects of climate are particularly important in determining microbial fate: temperature and rainfall. Microorganisms can survive for extended periods of time at low temperatures. Rainfall is important in that it can mobilize adsorbed microorganisms and promote their migration to the groundwater.

More specifically, the factors that control the fate of bacteria and viruses in the subsurface are listed in Tables 4 and 5, respectively. The exact mechanism(s) whereby these factors influence the inactivation or protection of microorganisms is unknown in many instances. In some cases, it is difficult to consider the factors separately, as interactions between them undoubtedly occur. Each of the factors is discussed in more detail.

A. Factors Influencing Bacteria

1. Temperature

It has been known for many years that temperature has a profound influence on the length of time that bacteria remain viable in soil and water. In 1940, it was reported that the causative agent of typhoid fever, *Salmonella typhi*, survived for 2 years in moist soils and 1 year in fecal material at freezing temperatures.[32] An inverse relation between viability and water temperature was also observed for *S. typhi*: the survival decreased from 9 weeks at 0°C to 2 weeks at 37°C.[33] Several investigators have observed that the survival of fecal coliform bacteria is greater at lower temperatures.[34-36] Bacteria are also capable of surviving in soil under subfreezing conditions: dysentery bacilli could be isolated from −45°C soil for 135 days days after they were added.[37]

2. Microbial Activity

The influence of other microorganisms on the survival of enteric bacteria in soil and water

Table 4
FACTORS INFLUENCING BACTERIAL FATE IN THE SUBSURFACE[29,30]

Factor	Influence on Survival	Influence on Migration
Temperature	Bacteria survive longer at low temperatures	
Microbial activity	Increased survival time in sterile soil	
Moisture content	Greater survival time in moist soils and during times of high rainfall	Generally, migration increases under saturated flow conditions
pH	Increased survival time in alkaline soils (pH > 5) than in acid soils	Low pH enhances bacterial retention
Salt species and concentration		Generally, increasing the concentration of ionic salts and increasing cation valences enhance bacterial adsorption
Soil properties		Greater bacterial migration in coarse-textured soils; bacteria are retained by the clay fraction of soil
Bacterium type	Different bacteria vary in their susceptibility to inactivation by physical, chemical, and biological factors	Filtration and adsorption are affected by the physical and chemical characteristics of the bacterium
Organic matter	Increased survival and possible regrowth when sufficient amounts of organic matter are present	The accumulation of organic matter can aid in the filtration process
Hydraulic conditions		Generally, bacterial migration increases with increasing hydraulic loads and flow rates

has been studied by comparing results from experiments conducted under sterile and nonsterile conditions. Rudolfs et al.[38] discuss a study in which *S. typhi* survived 216 days in sterile soil compared with only 100 days in nonsterile soil. The survival of *S. typhi* has been shown to be four to seven times longer in sterile water than in nonsterile water.[38,39] Kligler[40] observed an antagonistic effect on *S. typhi* by *Proteus vulgaris* and *Pseudomonas fluorescens*, however, another investigator could not confirm this antagonism.[32]

The antagonistic effects of protozoa on enteric bacteria have also been described. Tate[41] noted a decrease in the number of *Escherichia coli* concomitant with a sixfold increase in the protozoan population size in an organic muck. The survival of *S. typhi* in groundwater was also found to be adversely affected by the presence of protozoa.[42] It has been suggested that competition for nutrients with the indigenous soil bacteria may be the reason for the eventual disappearance of enteric bacteria from soil.[29]

3. Moisture Content

The amount of moisture in the soil is important in determining both the survival and migration characteristics of bacteria. Several investigators have found that bacteria survive longer in moist soils than dry soils.[32,40,43-45] Young and Greenfield[43] could isolate *E. coli* from moist soil for 5 years after inoculation; they found that survival was optimum when the soil moisture content was between 10 and 40% of saturation. The relationship between survival of enteric bacteria and soil moisture has led to the suggestion that the thickness of soil used for removal of microorganisms in effluents may be defined as a function of the soil-moisture characteristic curve.[44] Tanner [45] described a study by Firth and Horrocks in which it was concluded that an excess or deficiency of soil moisture appears to be the dominant factor affecting the chances of survival of *Bacterium typhosum (S. typhi)* in soil. Kligler[40] found that *S. typhi* could be recovered from moist soil for 70 days, whereas in the same soil dry, they could be recovered for only 2 weeks.

Table 5
FACTORS INFLUENCING VIRUS FATE IN THE SUBSURFACE[31]

Factor	Influence on Survival	Influence on Migration
Temperature	Viruses survive longer at lower temperatures	Unknown
Microbial activity	Some viruses are inactivated more readily in the presence of certain microorganisms; however, adsorption to the surface of bacteria can be protective	Unknown
Moisture content	Some viruses persist longer in moist soils than dry soils	Generally, virus migration increases under saturated flow conditions
pH	Most enteric viruses are stable over a pH range of 3 to 9; survival may be prolonged at near-neutral pH values	Generally, low pH favors virus adsorption and high pH results in virus desorption from soil particles
Salt species and concentration	Some viruses are protected from inactivation by certain cations; the reverse is also true	Generally, increasing the concentration of ionic salts and increasing cation valencies enhance virus adsorption
Virus association with soil	In many cases, survival is prolonged by adsorption to soil; however, the opposite has also been observed	Virus movement through the soil is slowed or prevented by association with soil
Virus aggregation	Enhances survival	Retards movement
Soil properties	Effects on survival are probably related to the degree of virus adsorption	Greater virus migration in coarse-textured soils; there is a high degree of virus retention by the clay fraction of soil
Virus type	Different virus types vary in their susceptibility to inactivation by physical, chemical, and biological factors	Virus adsorption to soils is probably related to physicochemical differences in virus capsid surfaces
Organic matter	Presence of organic matter may protect viruses from inactivation; others have found that it may reversibly retard virus infectivity	Soluble organic matter competes with viruses for adsorption sites on soil particles
Hydraulic conditions	Unknown	Generally, virus migration increases with increasing hydraulic loads and flow rates

The importance of soil moisture in determining the extent of migration of bacteria through soil has long been known. Most states require that a minimum thickness of unsaturated soil, generally 2 to 3 m, be present beneath a septic tank soil adsorption field.[46] Unsaturated soil is necessary to achieve adequate treatment of septic tank effluent as it percolates through the soil.[47] In a study of the movement of microorganisms from a septic system in Canada, the limited depth of unsaturated soil (1.22 m) was implicated as the reason for high numbers of bacteria present in the groundwater was far as 15.25 m from the site.[48] A comparison of the migration of bacteria in several studies led Hagedorn et al.[49] to the conclusion that bacteria are capable of moving much greater distances under saturated conditions than unsaturated conditions. This is due to the fact that bacterial movement through unsaturated soils is influenced by local infiltration, while movement in the saturated zone is controlled by regional groundwater flow conditions.[50]

4. pH

Generally, acid pH values (3 to 4) have an adverse effect on bacterial survival in soils and water. Cohen[51] found that *S. typhi* and *E. coli* survival was optimal at pH 5 to 6.4, while Kligler[40] observed that slightly alkaline soils were most favorable for the survival of *S. typhi*. Cuthbert et al.[52] studied the survival of *E. coli* and *Streptococcus faecalis* in several

peat (pH 2.9 to 4.5) and limestone (pH 5.8 to 7.8) soils. They found that while both organisms survived for several weeks in the limestone soils, both were inactivated rapidly (within a few days) in the acid peat soils. These investigators suggested that the low pH affected the availability of nutrients and the activity of antimicrobial agents as well as the ability of the bacteria to survive.

In addition to being adversely affected by low pH conditions, some bacteria are also more susceptible to inactivation at highly alkaline pH values. Several enteric bacteria have been found to be rapidly inactivated in alkaline water, with an upper pH tolerance limit of 9.5 reported.[53] Rudolfs et al.[38] stated that the optimum pH range for *S. typhi* survival was 6 to 8, although 5 to 10 may be tolerated if other environmental conditions are favorable.

Adsorption to soil particles is one mechanism of bacterial removal in soil. Although both soil particles and bacteria are negatively charged under typical environmental conditions, adsorption can occur when the repulsive forces are overcome. This phenomenon is discussed more fully in the section describing the effects of pH on virus migration in soil. One factor that reduces the repulsion is a slightly acidic environment.[54] In a study of coliform movement through sand, Goldschmid et al.[55] found that the retention efficiency increased as the pH of the water decreased from 9.3 to 3.9.

5. Salt Species and Concentration

The presence of certain salts has an impact on the degree of retention of bacteria in soils.[54] Certain cations, such as Ca^{2+}, Mg^{2+}, and Na^{+}, can promote the adsorption of bacteria to soil by neutralizing the negative bacterial surface charge.[50] In a study of the removal efficiency of coliforms in sand, it was found that more removal occurred when the suspending medium was tap water than distilled water; no removal was observed using triple-distilled water.[54] The addition of cations, especially multiple valence cations, resulted in increased removal by promoting bacterial adsorption. These investigators demonstrated the reversibility of the adsorption by reducing the cationic strength of the medium and observing an increase in coliform numbers in the effluent.

6. Soil Properties

The major mechanism of bacterial removal is straining or filtration by the soil particles.[29] Therefore, soils with large pore sizes, such as sands and gravels, do not remove bacteria as efficiently as do clayey soils which have small openings between the grains. A summary of the reported distances of bacterial migration in various types of subsurface materials is shown in Table 6.

Butler et al.[67] studied the movement of coliform bacteria in a variety of soils with effective grain sizes ranging from 0.0056 to 0.015 mm. They found that the removal was appreciably less in soils with large effective grain sizes than for finer-grained soils. Hagedorn et al.[76] studied the movement of *E. coli* and *Strep. faecalis* when introduced into pits of two different depths, one of which had a higher clay content than the other. Both organisms traveled two to ten times farther in the silty loam soil than in the silty clay loam.

In a weathered limestone aquifer, bacteria have been observed to travel as far as 1000 m from the point of introduction.[68] In contrast, very limited movement has been noted in clayey soils, with reported distances generally less than 1 m.[69,87] Both removal mechanisms — adsorption and filtration — are probably active in clay soils. As discussed previously, bacteria can be removed by sorption to soil particles in the presence of cations or low pH. The clay fraction of the soil is the most active in adsorbing microorganisms due to its ability to adsorb and exchange ions at its surface. This results in a negatively charged surface at high pH values and a positively charged surface at low pH values.[90]

7. Bacterium Type

The susceptibility to inactivation by various chemical, physical, and biological means

Table 6
MIGRATION OF BACTERIA IN THE SUBSURFACE

Microorganism	Medium	Maximum distance traveled (m)		Ref.
		Vertical	Horizontal	
Bacillus stearothermophilus	Fractured rock		29	56
Bacteria	Fine sand		457	57
	Medium to coarse sand		21	58
	Alluvial gravel		90	59
	Pea gravel + sand		30	60
	Coarse gravels		457	61
	Gravel		920	62
	Sandy clay		15.25	48
	Fine to coarse sand		30.5	63
	Fine to medium sand		6.1	64
Clostridium welchii	Fine + medium sand		15.5	65
Coliforms	Loam + sandy loam			
	Sand + gravel	10 — 12	850	66
	Fine sandy loam	4	1.2	67
	Fine sand	4	2	68
	Pebbles		850	68
	Weathered limestone		1000	68
	Stony clay + sand	0.91		69
	Stone + clay	0.61		69
	Firm clay	0.3		69
	Coarse sand + gravel		55	70
	Sandy clay loam	2	6.1	71
	Sandy clay loam	4.3	13.5	71
	Sandy loam	0.64	28	71
Escherichia coli	Sand		3.1	72
	Fine + coarse sand	4	24.4	73
	Fine + medium sand	0.15		74
	Fine + medium sand		3.1	65
	Sand + sandy clay	1.5	10.7	75
	Silt loam		3	76
	Silty clay loam		1.5	76
	Medium sandy gravel		125	77
	Fine sandy gravel with cobbles		50	77
	Silty clay loam	1	15	78
	Fine sand		19.8	79
	Fine sand	0.3	70.7	80
Fecal coliforms	Fine loamy sand + gravel		9.1	81
	Stony silt loam		900	82
	Fine to medium sand		2.4	83
	Gravel with sand + clay		9	84
	Saturated gravels		42	84
	Sandy clay + clay	0.85		85
	Sandy clay	1.2		86
Salmonella enteriditis	Clay	0.1		87
S. typhi	Limestone		457	88
Streptococcus faecalis	Silty clay loam		0.5	76
	Silt loam		5	76
Strep. zymogenes	Sandy gravel	0.15	15.2	89

varies from one bacterium to another. Bacteria that produce spores, such as *Bacillus* sp., are more capable of surviving under adverse conditions than those that do not. When conditions are again favorable for growth, the spores can germinate and new bacteria flourish. Other bacteria may be more capable of competing for nutrients with the indigenous microflora, or may be less susceptible to antibiotics produced by the soil bacteria. Rudolfs et al.[38] concluded from a review of several studies that *E. coli* and *Enterobacter aerogenes* survive far longer than *S. typhi* under similar conditions in soil. In general, 2 to 3 months should be adequate to reduce the numbers of pathogens in soil to nonharmful levels;[29] however, survivals of up to 4 years have been reported.[91] This demonstrates the need to study the survival of the individual bacterium of interest in the desired environment.

The extent of migration in soil is also affected by the type of bacteria in question; this is apparent from Table 6. The size and shape of the bacteria dictate whether or not they are capable of moving through the soil pores, as well as how quickly they can travel. In the absence of adsorption, larger bacteria should move more rapidly through the soil than smaller cells.[29] This is due to the fact that large bacteria are excluded from the small pores, and thus must travel through the large pores which have higher velocities.

Caldwell[65] found that *Clostridium welchii* moved five times farther than *E. coli* in a sandy soil. In a study by Hagedorn et al.[76] *Strep. faecalis* traveled farther than *E. coli* in a silt loam, but the opposite was true for the silty clay loam. This may have been due to a stronger adsorption of the *Strep. faecalis* to the clay fraction of the soil in the latter case.

8. Organic Matter

The presence of organic matter can affect the survival of bacteria by providing nutrients required for growth. In a study reported by Rudolfs et al.,[38] it was found that *S. typhi*, added as a broth culture to soil, could survive for 10 months when frequent additions of nutrient broth were made. Without the additional nutrients, the bacteria survived for only 4 months. Several investigators have found that bacteria survive for longer periods of time in organic soils or soils amended with sewage than in mineral soils.[45,92-94] In contrast, Firth and Horrocks (as cited by Tanner[45]) found that survival of *S. typhi* was not affected by the presence of organic matter. They observed that the survival was the same in organically polluted or uncontaminated soil with or without the additional nutrients in the form of dilute sewage.

Organic material can also affect the migration of bacteria in the subsurface in the form of a mat composed of bacteria or extracellular polymers. Bouma et al.[44] found that the greatest decline in bacterial populations in a septic tank-soil absorption system occurred at the "biological mat" or "clogged zone". They stated that this zone was the primary barrier to the subsurface migration of bacteria, and emphasized its importance to the proper functioning of the absorption field. Butler et al.[67] also noted the importance of a mat in bacterial removal by soil. In addition to a surface mat, they described the formation of a second clogged zone deeper in the subsurface, usually 10 to 50 cm deep. This zone was formed by the accumulation of bacterial cells in the soil, which eventually form a filter in themselves. Krone et al.[95] demonstrated that this zone of accumulated bacteria enhanced the filtration efficiency of the soil by monitoring the movement of *E. coli* through sand columns.

9. Hydraulic Conditions

The amount of liquid applied to the soil surface and the rate at which it is applied will affect the extent of bacterial migration. High loading rates will cause the liquid to move rapidly through the soil, decreasing the time available for contact between the bacteria and soil, and thus decreasing the probability of adsorption.[50] Bouma[96] found that bacteria were removed more efficiently when effluent was applied at a rate of 5 rather than 10 cm/day. It has also been suggested that 3 ft of unsaturated soil may be adequate to remove pathogenic bacteria to nonharmful levels, provided that the soil is not overloaded.[47]

B. Factors Influencing Viruses

1. Temperature

Temperature is probably the most important factor influencing virus inactivation in the environment.[90] In reviews[97,98] of virus survival in all types of surface waters (i.e., marine, river, and lake), temperature was found to affect the length of time that viruses remained infective: at lower temperature, virus persistence was prolonged compared with that at higher temperatures. More recently, it has been determined that this trend is true for virus persistence in groundwater.[99] In a study using groundwater samples collected throughout the U.S. none of the measured water characteristics including pH, nitrate, ammonia, sulfate, iron, hardness, turbidity, and total dissolved solids, except temperature was significantly correlated ($p < 0.01$) with the inactivation rate of the viruses. Linear regression analysis of virus inactivation rate as a function of temperature produced a correlation coefficient, r, of 0.88. The coefficient of determination, r^2, was 0.775, indicating that 77.5% of the variation in virus inactivation rates may be explained by temperature effects.

Temperature also affects the persistence of viruses in soils. Lefler and Kott[100] found that it took 42 days for 99% inactivation of poliovirus in sand at 20 to 25°C, whereas more than 175 days were required at 1 to 8°C. Poliovirus was found to persist for more than 180 days in saturated sand and sandy loam soils at 4°C, whereas no viruses could be recovered from the soils incubated at 37°C after 12 days.[101] Hurst et al.[102] studied the survival of poliovirus at three temperatures: 1, 23, and 37°C in a loamy sand. They also found that the inactivation rate was significantly correlated ($p < 0.01$) with incubation temperature, noting faster inactivation rates at the higher temperatures.

The exact mechanism whereby temperature inactivates viruses in soils has not been determined, but several theories have been proposed. The inactivation may be due to denaturation of the viral capsid;[90] however, it has been shown that the RNA released during thermal denaturation remains infective as it is more resistant to heat inactivation.[103] Dimmock[104] suggested that different mechanisms may be at work, depending on the temperature. At low temperatures (less than 44°C), the rate of virus inactivation corresponds with the inactivation rate of the viral RNA. However, at high temperatures (greater than 44°C), the rate of virus inactivation exceeds that of the inactivation rate of the viral RNA, and is associated with structural changes in the viral capsid. Kapuscinski and Mitchell[105] have suggested that temperature itself may not be responsible for virus inactivation, but may merely control whether other inactivation mechanisms can occur.

2. Microbial Activity

There are conflicting reports regarding the role of microorganisms in virus inactivation; many of these have been reviewed.[98,105] Sobsey et al.[106] found that the inactivation rates of polio and reovirus in eight different soil suspensions were almost always greater under nonsterile conditions compared with sterile conditions. Hurst et al.[102] reported that virus inactivation rates were similar in nonsterile anaerobic soils and sterile soils at 1, 23 and 37°C. Under aerobic conditions, polio- and echovirus inactivation rates were more rapid in nonsterile soil preparations than sterile at 23 and 37°C. At very low temperatures, 1°C, inactivation rates were similar in both sterile and nonsterile soils.

Autoclaving or filtering seawater has been shown to reduce its antiviral activity.[107,108] Although specific bacteria have been isolated from some waters having antiviral activity, after subculture on artificial medium the antiviral activity could not be demonstrated.[109] Herrmann et al.[110] found that viruses were inactivated more rapidly in untreated lake water than in lake water that had been filtered.

The antiviral activity of marine microorganisms has been investigated by Fujioka et al.[111] These investigators observed that human enteroviruses persisted longer in seawater that had been boiled, autoclaved, or filtered through a 0.22-μm or 0.45-μm membrane than in raw

seawater or in seawater that had been passed through a 1.0-μm filter. The fact that the water that had been passed through a 1.0-μm filter possessed similar antiviral activity to the raw seawater led them to hypothesize that a marine microorganism was responsible. The antiviral activity could be stopped upon addition of antibiotics and restored upon inoculation of 0.25 mℓ raw seawater to the filtered water, lending support to this hypothesis.

In contrast to these studies, others[112,113] have found that neither filtration nor autoclaving affected the antiviral activity of seawater from the Gulf of Mexico and the Mediterranean Sea. Using Rio Grande River water, O'Brien and Newman[114] found that virucidal activity was comparable in raw river water and in filter-sterilized river water. Virus inactivation rates were lower in autoclaved water, leading them to suggest that there was a heat-labile or volatile inactivating factor in the water.

The influence of groundwater bacteria on the persistence of viruses has also been investigated. In a study using more than 30 groundwater samples, the presence or absence of the indigenous bacteria was not significantly correlated with the inactivation rate of the virus.[115] The bacteria had an inconsistent effect on virus persistence: in some sterile samples, viruses persisted longer than in the nonsterile counterparts, while in other samples, the opposite effect was observed.

Several mechanisms by which microorganisms may influence virus inactivation have been suggested.[11] Proteolytic enzymes released by certain bacteria were capable of destroying the capsid of coxsackievirus A9, but polioviruses were unaffected.[116] Some microorganisms produce substances which render viruses more susceptible to inactivation by photodynamic processes[117] or enzymes.[118] Other compounds produced by microorganisms such as humic acids, tannins, phenols, and ascorbic acid may act as oxidizing or reducing agents which lead to virus inactivation.[11]

3. Moisture Content

The moisture content of the soil also influences virus persistence in soils. Although some investigators have observed no difference in inactivation rates in dried vs. saturated sand,[100] the majority of the reports have indicated a difference. Bagdasaryan[119] observed that several enteroviruses, including poliovirus 1, coxsackievirus B3, and echoviruses 7 and 9, could survive for 60 to 90 days in soil with 10% moisture compared with only 15 to 25 days in air-dried soils. Ninety-nine percent inactivation of poliovirus occurred in 1 week as the soil moisture content was reduced from 13 to 0.6%; however, 7 to 8 and 10 to 11 weeks were required for the same amount of inactivation in soils with 25 and 15% moisture content, respectively.[120]

Hurst et al.[102] found that the moisture content affected the survival of poliovirus in a loamy sand. The inactivation rate increased as the moisture content was increased from 5 to 15%, then decreased as more liquid was added. It was noted that the fastest inactivation rate occurred near the saturation moisture content of the soil (15 to 25%). The slowest inactivation rates were observed at the lowest moisture contents, 5 and 10%.

In another study, it was observed that virus inactivation rates were greater in more rapidly drying soils. In a field study of virus inactivation during rapid infiltration of wastewater, it was shown that allowing the soils to periodically dry and become aerated enhances virus inactivation.[121] Using radiolabeled viruses,[101] it was shown that the viruses are inactivated during the drying process, rather than becoming irreversibly bound to the soil particles. Studies by Yeager and O'Brien[122] suggested that the mechanisms responsible for poliovirus inactivation are different in moist and drying soils. In moist soils, under both sterile and nonsterile conditions, the viral RNA was recovered in a degraded form, suggesting that the nucleic acid was degraded prior to its release from the capsid. In dried, nonsterile soils, complete loss of viral infectivity was observed, and the RNA was recovered in degraded form. In contrast, the nucleic acid was recovered intact from sterile, dry soils.

The moisture content also influences the movement of viruses through soil. In a column of loamy sand, poliovirus penetrated 40 cm under unsaturated flow conditions. However, virus could be recovered at a depth of 160 cm during saturated flow conditions.[123] Under saturated conditions, some of the soil pores are filled with gas rather than water, leaving a film of water around the particles. Thus, viruses can get closer to the soil than in saturated conditions when the pores are completely filled with water. This increases the opportunity for virus adsorption to soil under unsaturated conditions.[29]

4. pH

The effect of pH on virus survival in soil has not been extensively studied. It has been suggested that pH indirectly influences virus survival by controlling adsorption onto soil particles which, in turn, affects virus survival.[29] The direct effects of pH on virus persistence have been studied by a few investigators. Sobsey[31] reported the results of a study using simian adenovirus SV-11 in which it was shown that inactivation was more rapid at pH 5.0 and 6.0 than at pH 4.0 and 7.0. Salo and Cliver[118] found that, in the aqueous environment, pH affects different viruses in different ways. While poliovirus 1 was inactivated more rapidly at pH 3 and 9 than the near-neutral values of 5 and 7, coxsackievirus A9 was inactivated more rapidly at pH 5 than the extremes of 3 and 9. Murphy et al.[124] observed that mouse encephalomyelitis virus survival was longer in neutral soils than in soils with the pH adjusted to 3.7 or 8.5. Hurst et al.[102] studied the survival of poliovirus 1 and two bacteriophages, MS-2 and T2, in nine soils with pH values ranging from 4.5 to 8.2. They found that virus inactivation was significantly correlated ($p \leq 0.05$) with soil saturation pH, with longer survival at the lower pH values.

The exact mechanism whereby pH causes virus inactivation has not been fully elucidated, but one investigator[125] found that the inactivation of adenovirus 2 was accompanied by an increase in sensitivity to DNase and the loss of structural proteins from the virus capsid. Results obtained by Salo and Cliver[118] also suggested that virus inactivation involves alterations in the virus capsid. These investigators found that the RNA of the inactivated virus particles became sensitive to ribonuclease at all pH levels tested (pH 3 to 9), and at pH 5 and 7 the RNA was hydrolyzed in the absence of ribonuclease.

The effects of pH on the movement of viruses through soil or, more specifically, the retardation of virus movement by adsorption to soil particles has been extensively studied. Goyal and Gerba[126] measured the effects of 7 soil properties on the adsorption of 15 viruses to 9 soils. They found that, in general, soils with a saturation pH of less than 5 were good virus adsorbers. The adsorption of 5 of the viruses (four echoviruses and phage ϕx174) showed a significant negative correlation ($p \leq 0.05$) with soil pH. Subsequent analysis of these data using factor analysis[127] divided the viruses into two general groups based on their adsorption behavior. For viruses in group I (two coxsackie B4 viruses, four echo 1 viruses, ϕx174, and MS-2), pH was an important factor affecting virus adsorption to soil. However, for viruses in group II (polio 1, echo 7, coxsackie B3, and phages T4 and T2) none of the studied soil characteristics, including pH, was correlated with adsorption to soil.

Burge and Enkiri[128] found that the adsorption of bacteriophage ϕx174 to five soils showed a significant negative correlation with soil pH. The adsorption of poliovirus 1 and reovirus 3 to eight soils suspended in sewage at pH values from 3.5 to 7.5 was generally greater at the lower pH values.[106] The adsorption of encephalomyelitis virus to montmorillonite clay was greatest at pH 5.5.[129] At pH 9.5, the adsorption was less, but the poorest adsorption was observed at pH 3.5. In an extensive study of poliovirus adsorption by 34 minerals and soils, no significant correlation was found between substrate pH and virus adsorption.[130] The investigators reported that although adsorption by most of the neutral and acidic materials was strong, the variation in percentage of virus bound in the alkaline materials was so great that no significant correlation could be detected.

The mechanism(s) whereby pH affects virus adsorption to soil particles has been explained in terms of the electrochemical nature of the virus and soil surfaces.[29, 31, 131] The outer surface of the enteric viruses is made of protein; therefore, the surface charge is influenced by the ionization of the carboxyl and amino groups in the capsid. The isoelectric point (pI) of many enteric viruses is below 7;[131] thus, at neutral pH, most viruses are negatively charged. Most soils are also negatively charged at neutral pH, and virus adsorption is not favored due to the mutual repulsion. If the pH of the environment is decreased, the surface charge of the virus will become positive (or less negative) due to increased ionization of the amino groups and decreased ionization of the carboxyl groups. While soils also become less negatively charged at lower pH values, soil pI values are generally lower than those of viruses, thus they may still have a net negative charge at acidic pH levels. This results in an electrostatic attraction between the virus particle and the soil, which leads to adsorption. In reality, the effect of pH on virus adsorption is not so clear-cut. There are many complicating factors which can interfere with the mechanism discussed above. One is that a given virus may have more than one isoelectric point: poliovirus 1 (Brunhilde strain) has isoelectric points at pH 4.5 and 7.0.[131] The factors responsible for passage from one form to another are unknown at this time. Other soil factors such as cations and humic and fulvic acids may also influence the net surface charge of viruses.

5. Salt Species and Concentration

The presence of certain chemicals may render a virus more or less susceptible to inactivation. Burnet and McKie[132] found that bacteriophage inactivation at 60°C was partially prevented in the presence of 0.002 to 0.01 *M* $CaCl_2$, or $BaCl_2$. However, when the concentration was increased to 0.15 *M* or greater, thermal inactivation was increased. Enhanced stabilization of poliovirus at temperatures ranging from 4 to 50°C in the presence of high concentrations (1*M*) of $MgCl_2$ has been reported.[133] Echoviruses have also been found to possess this property.[134] Cords et al.[135] found that many type A coxsackieviruses were inactivated more rapidly in low ionic strength media than in high ionic strength media. Melnick and Gerba[11] suggested that the salts in seawater may exert some protection on poliovirus, as this virus has been found to be more stable at high temperatures in seawater than in distilled water. A role for heavy metals in virus inactivation in seawater has been suggested by Kapuscinski and Mitchell.[105]

The types and concentrations of salts in the environment have a profound influence on the extent of virus transport in the subsurface. In general, it has been found that transport is retarded in the presence of increasing concentrations of ionic salts and increasing cation valencies due to increased virus adsorption.[31] Taylor et al.[136] studied the effects of pH and electrolytes on the adsorption of poliovirus 2 to five soils, ranging from a sand to a montmorillonite clay. These investigators found that both the type of electrolyte and its concentration affected poliovirus 2 adsorption to the soils. A "critical" pH region was observed for each soil in which there was a rapid transition from weak to strong uptake. When the pH was at or above the critical region, the virus adsorption increased with electrolyte concentration. Sobsey et al.[106] found that viruses could be made to adsorb to some relatively poor adsorbents upon the addition of divalent cations, especially Mg^{2+}.

The role of the soil cation-exchange capacity (CEC) in virus adsorption has also been investigated. Burge and Enkiri[128] found that the CEC of 4 of 5 soils was correlated significantly ($p \leq 0.05$) with virus adsorption. In contrast, Goyal and Gerba[126] did not find a significant correlation between soil CEC and adsorption of 15 different viruses. Additionally, no correlation was found between virus adsorption and total phosphorus or total and exchangeable iron, calcium and magnesium.

A decrease in the salt concentration or ionic strength of the soil water, such as would occur during a rainfall event, can cause desorption of viruses from soil particles.[28] This has

been demonstrated in the laboratory by applying distilled water to simulate rainfall to soil columns containing viruses.[137-140] While some virus particles were readsorbed farther down the column, others were detected in the column effluent. This phenomenon has also been observed at a field site. Wellings et al.[141] detected viruses in wells which had previously been virus free at a land application site in Florida after a period of heavy rainfall.

6. Virus Association with Soil

The adsorption of viruses to soils and other surfaces may prolong or reduce survival, depending upon the properties of the sorbent. Gerba and Schaiberger[142] found that a certain type of suspended solid in seawater enhanced bacteriophage inactivation. It has also been found that poliovirus adsorption onto some inorganic substances, such as CuO, results in decreased infectivity of the virus.[143] These investigators suggested that van der Waal interactions between the virus and the particle surface induced spontaneous disassembly of the virus.

In the marine environment, it has been shown that bacteriophage adsorption to pure clays, bacterial colloids, and naturally occurring suspended matter exerts a protective effect.[142] Hurst et al.[102] found that adsorption to soil was one of the most important factors affecting virus survival. In contrast, the survival of polio and reovirus was not always prolonged in soil suspensions compared with soil-free controls in a study using eight different soil materials.[106]

The mechanisms whereby adsorption to a solid surface prolongs or reduces virus survival have not been elucidated. However, Gerba and Schaiberger[142] have suggested several possibilities, including interference with the action of virucides, increased stability of the viral protein capsid, prevention of aggregate formation, and adsorption of enzymes and other inactivating substances.

The transport of viruses through soil is retarded by association with the soil particulates. As discussed previously, however, an adsorbed virus is not necessarily permanently immobilized. A change in ionic strength or salt concentration of the surrounding medium, such as would be induced by a rainfall event, can cause virus desorption, allowing further migration in the soil. Virus adsorption acts to slow the apparent rate of virus movement in the soil.

7. Virus Aggregation

The formation of aggregates influences virus survival in natural waters. It has been suggested that this is because virus particles within the aggregates are highly resistant to destruction by enviromental factors.[90] It has been shown that aggregation renders virus particles more resistant to inactivation by chemical disinfectants such as bromine.[144] Although there are no definitive reports on the effects of aggregation on virus survival in soil, the results of studies in aqueous media would suggest that aggregates would survive longer in soils than would monodispersed viruses.[31]

Although virus adsorption to soil particles is the major mechanism of removal, as is discussed below, filtration may play a role in removing virus aggregates. Single virus particles are generally smaller than even the smallest soil pores; however, aggregated or particulate-associated viruses may be removed by straining through the small pores.

8. Soil Properties

The influence of soil type on virus survival is probably related to the degree of adsorption. Hurst et al.[102] suggested that the correlation between pH and virus survival was probably mediated through its influence on virus adsorption. A positive correlation between virus survival and soil exchangeable aluminum and a negative correlation with resin-extractable phorphorus were also attributed to influencing virus adsorption to the soil.

The soil properties have a profound influence on virus movement in the subsurface. Table

Table 7
MIGRATION OF VIRUSES IN THE SUBSURFACE

Microorganism	Medium	Maximum distance traveled (m)		Ref.
		Vertical	Horizontal	
Bacteriophage	Sand	45.7	400	145
	Sandy clay	1.2		60
	Clay	0.85		61
	Boulder clay		510	146
	Sandstone		570	146
Coliphage f2	Silty sand	29	183	147
Coliphage T4	Karst		1600	148
Coxsackievirus B3	Fine loamy sand	18.3		15
	Sand	22.8	408	17
Echovirus	Coarse sand + fine gravel	11.3	45.7	16
Enterovirus	Sandy loam	3.5	14.5	149
Poliovirus	Loamy sand	0.4		137
	Medium sand		0.6	150
	Loamy sand	1.6		151
	Sand	0.2		100
	Silt loam		46.2	152
	Medium to fine sand		9	152
	Loamy medium sand		6	152
	Sand	9.1		17
	Coarse sand + fine gravel	10.6	3.0	16
	Coarse sand + fine gravel	7.62		153
Viruses	Sand	6		154
	Sandy clay	3		141
	Sand	38		141
	Sand + coarse gravel	16.8	250	155

7 provides a list of some of the reported distances of virus migration, both horizontal and vertical, as well as the type of soil involved. Considerable vertical migration has been reported, up to 1600 m in one case.[148] Closer inspection of the table will reveal that, in general, virus migration is greater in coarse-textured and karstic terrain than in fine-textured soils.

As mentioned previously, adsorption to soil particles is the major mechanism of virus removal in soils.[29,31] As a result of its high surface area and high CEC, the clay fraction is the most active in terms of virus adsorption.[29] Thus, fine-textured soils which contain clays generally remove viruses more efficiently than do coarse-textured soils, which have little capacity for adsorption. The mechanisms responsible for virus adsorption to soil particles include van der Waal forces, double-layer interactions, and hydrophobic interactions. These have been discussed recently by Gerba.[131]

9. Virus Type

As is obvious from the previous discussions, different viruses vary in their susceptibility to inactivation in the subsurface environment. Sobsey et al.[106] found that the rates of inactivation of polio and reovirus in eight soils were different. Hurst et al.[102] also showed that the inactivation rates of seven different viruses varied even when incubated under the same conditions. In a comparative study of the survival of poliovirus, echovirus, and coliphage MS-2 in several different groundwater samples, no significant difference ($p \leq 0.01$) was found overall.[99] There were, however, differences in the inactivation rates of the viruses in individual water samples. In a recent report on the survival of hepatitis A virus (HAV), it was shown that this virus generally survived longer than polio and echovirus at 25°C.[156]

In addition to affecting the survival, the properties of the specific virus also affect how the virus is transported in the subsurface. The experiments by Goyal and Gerba[126] which studied the adsorption of 15 different viruses to soils found a high degree of variability not only among different virus types, but among different strains of the same virus type. Using factor analysis, they were able to place the viruses in three groups based on adsorption characteristics: good sorbers, poor sorbers, and f2, which did not seem to fit in the other two groups.[127] Sobsey et al.[156] found that the movement of HAV in soil was intermediate between polio and echovirus. Differences in the adsorption and elution behavior of several enteroviruses were also observed by Landry et al.[139]

In contrast, in column studies of polio and echovirus movement through soil columns, Lance et al.[157] found that the viruses behaved very similar to one another, suggesting that poliovirus would be an appropriate model of virus movement in soil.

10. Organic Matter

The influence of organic matter on virus survival has not been firmly defined. In some studies, it has been found that proteinaceous materials present in wastewater may have a protective effect on viruses; however, in others, no effect has been observed.[90] Lefler and Kott[100] found that poliovirus survived longer in sand columns saturated with oxidation pond effluent than distilled water. They suggested that the prolonged survival was probably due to the protective effect of proteinaceous materials in the effluent. Hurst et al.[102] did not find a significant correlation between virus survival and percent soil organic matter.

Humic and fulvic acids in soils may cause loss of virus infectivity and prevent adsorption.[158] The infectivity could be partially restored by treatment with 3% beef extract, pH 9. Sobsey[31] has observed a similar phenomenon: poliovirus infectivity was reduced in the presence of humic acid. At least some of the infectivity could be restored when the samples were filtered, probably by disruption of the humic acid-virus complexes.

Dissolved organic matter has generally been found to decrease virus adsorption by competing for adsorption sites on soil particles. In their study of poliovirus adsorption by 34 mineral and soils, Moore et al.[130] found that organic matter showed a strong negative correlation ($p \leq 0.001$) with virus adsorption. Bitton et al.[159] suggested that the organic material in secondary effluent interfered with virus adsorption to a sandy cypress dome soil. Several investigators have found that organic material can act not only as a competitor for virus adsorption sites, but also as an eluting agent, i.e., it can cause sorbed viruses to desorb from the soil.[131] Widespread use has been made of this property of organic matter in the area of removing viruses from filters in order to detect them in environmental samples. Mucks and other soils with high organic matter content are poor adsorbers of viruses[160] and may not be suitable for wastewater application sites.[31]

11. Hydraulic Conditions

The rate at which water or effluent is applied to the soil affects the amount of virus removal or adsorption to the soil particles. As shown in Table 8, the amount of virus removal increases as the application rate decreases. Lance and Gerba[123] found that increasing the application rate from 0.6 to 1.2 m/day caused an increase in the number of virus particles in the column effluent. Increasing the application rate above 1.2 up to 12 m/day did not, however, cause any further increase in virus movement through the columns. Vaughn et al.[153] also found that application rate had a large influence on the movement of poliovirus through a coarse sand-fine gravel soil. They speculated that the formation of a surface mat of sewage solids may have been responsible for the greater virus removals at the lower application rates.

Table 8
MICROORGANISM REMOVAL AS AFFECTED BY APPLICATION RATE

Microorganism	Application rate (cm/day)	Soil texture	Removal (log_{10} no./cm)	Ref.
Poliovirus 1	13,214	Sand	3.90×10^{-2}	161
Total coliforms	9	Fine sandy loam	$>6.99 \times 10^{-2}$	162
	6	Sandy loam	$>6.99 \times 10^{-2}$	162
	9	Sandy loam	4.00×10^{-2}	162
	3	Sand	5.10×10^{-2}	162
Bacteriophage T2	66	Loam	7.97×10^{-2}	163
	70	Sandy clay loam	4.37×10^{-2}	163
	50	Loam	5.41×10^{-2}	163
	18	Clay loam	$>7.86 \times 10^{-2}$	163
	53	Sandy loam	$>7.32 \times 10^{-2}$	163
Bacteriphage T1	57	Loam	8.18×10^{-2}	163
	62	Sandy clay loam	6.42×10^{-2}	163
	32	Loam	8.06×10^{-2}	163
	62	Sandy loam	8.21×10^{-2}	163
Poliovirus	5	Medium sand	1.59×10^{-1}	164
	50	Medium sand	4.83×10^{-2}	164
	55	Loamy sand	2.78×10^{-2}	138
	15	Loamy sand	2.90×10^{-2}	138
Poliovirus 1	60	Coarse sand	1.56×10^{-2}	150
	120	Coarse sand	7.1×10^{-3}	150
	240	Coarse sand	5.47×10^{-3}	150
	400	Coarse sand	9.08×10^{-3}	150
	1,200	Coarse sand	8.09×10^{-3}	150
Poliovirus	115	Sand	1.26×10^{-3}	100
	229	Sand	6.83×10^{-3}	100
Poliovirus 1	86	Unsaturated sand	4.10×10^{-2}	165
	17	Unsaturated sand	7.35×10^{-2}	165
	11,745	Coarse sand	1.81×10^{-3}	165
	5,872	Coarse sand	1.41×10^{-3}	165
	367	Coarse sand	1.08×10^{-2}	165
	184	Coarse sand	$>8.48 \times 10^{-3}$	165
	92	Coarse sand	2.07×10^{-2}	165
	46	Coarse sand	$>2.63 \times 10^{-2}$	165
	11,745	Fine sand	6.18×10^{-3}	165
	5,872	Fine sand	1.96×10^{-3}	165
	267	Fine sand	5.93×10^{-3}	165
	184	Fine sand	8.67×10^{-3}	165
	92	Fine sand	6.80×10^{-3}	165
	46	Fine sand	2.63×10^{-2}	165
Poliovirus	1	Sand	1.70×10^{-1}	106
	1	Sand	2.52×10^{-1}	106
	1	Organic	1.82×10^{-1}	106
	1	Sandy clay loam	4.40×10^{-1}	106
Fecal coliforms	1	Sand	4.40×10^{-1}	106
	1	Organic	5.00×10^{-1}	106
	1	Sandy clay loam	5.70×10^{-1}	106
	1	Sand	4.30×10^{-1}	106
Poliovirus 2	1,800—2,400	Fine gravel and coarse sand	4.41×10^{-3}	154
	144	Fine gravel and coarse sand	7.87×10^{-2}	154
	24	Fine gravel and coarse sand	8.92×10^{-3}	154
	12	Fine gravel and coarse sand	1.22×10^{-2}	154

Table 8 (continued)
MICROORGANISM REMOVAL AS AFFECTED BY APPLICATION RATE

Microorganism	Application rate (cm/day)	Soil texture	Removal (log_{10} no./cm)	Ref.
Echovirus 1	76	Sand	2.70×10^{-2}	166
	88	Sand	2.60×10^{-2}	166
	118	Sand	2.20×10^{-2}	166
	222	Sand	1.90×10^{-2}	166
	282	Sand	7.00×10^{-3}	166
Poliovirus 1	33	Sandy loam	4.00×10^{-2}	166
	75	Sand	2.70×10^{-2}	166
	153	Sand	2.00×10^{-2}	166
	194	Sand	1.90×10^{-2}	166
	204	Sand	1.60×10^{-2}	166
	314	Sand	9.00×10^{-3}	166
	1,352	Sand	7.00×10^{-3}	166
Bacteriophage T4	559	Low humic latasol	$>6.93 \times 10^{-1}$	167
	571	Low humic latasol	$>6.93 \times 10^{-1}$	167
	144,000	Cinder	1.26×10^{-2}	167
Poliovirus 2	559	Low humic latasol	1.65×10^{-1}	167
	571	Low humic latasol	1.65×10^{-1}	167

III. MODELING MICROBIAL FATE IN THE SUBSURFACE

The previous discussion focused on describing the factors that influence the survival and transport of viruses and bacteria in the subsurface environment. In order to try to reduce the probability of groundwater contamination by microorganisms, this information must be incorporated into predictive models of microbial survival and transport. In the remaining sections of the paper, we discuss the efforts that have been made to develop these models. The majority of the models are concerned with virus fate rather than bacterial fate, possibly because, in general, viruses survive for longer periods of time and migrate greater distances than bacteria.

A. Process Modeling

In many of the experiments that were discussed previously, the data obtained were used to describe the process in a qualitative rather than a quantitative sense. In some cases, too few data were generated to describe the results mathematically. In others, the results are so microorganism or soil specific that they could not easily be generalized to describe all situations. Another reason for the difficulty encountered in mathematically expressing experimental results is that, in many cases, modeling is not the driving force behind the basic process research. Thus, experiments are not designed in such as way as to derive information in a form that is easily incorporated into mathematical models. A few investigators have, however, developed models from experimental data to describe processes affecting microbial fate in the subsurface. These models are described in the following sections.

1. Inactivation

The inactivation of microorganisms in water and soil has usually been described as a first-order reaction.[99,102,168-170] Nonlinear survival curves may result if viral aggregates are present or a significant variation exists in sensitivities among the microbial population to the factors causing inactivation. The inactivation rate described by a first-order reaction rate expression is

$$\text{Inactivation rate} = \frac{dC}{dt} = KC \quad (1)$$

where C is the infective microorganism concentration at time t and K is the first-order inactivation constant (time^{-1}). Here K would be an expression of the sum total of all factors which influence microorganism survival. Measurement of virus inactivation has been conducted on a wide variety of surface waters, but such information on soils and groundwater has been limited until recently. Values used by Reddy et al.[168] were developed from virus inactivation studies during anaerobic digestion as well as from studies with soil columns flooded with sewage. In their model calculations, Matthess and Pekdeger[171] used values as presented for surface waters by Akin et al.[97] Grosser[172] reviewed virus inactivation observed in a number of environments, and used a variety of values for virus inactivation in his model calculations. Vilker[169] discussed in detail virus inactivation observed in various environments, but values for groundwater had not been determined at the time. In general, most of the previous work has lacked experimentally determined values for K in groundwater and soil.

Only three literature reports exist on inactivation rates for viruses in groundwater. Keswick et al.[173] reported inactivation rates of 0.19 $\log_{10}$ day^{-1} for coxsackievirus B3 and 0.21 $\log_{10}$ day^{-1} for poliovirus in water from an 84-m-deep well with water temperature ranging from 3 to 15°C in Houston, Tex. In well water from Florida, Bitton et al.[174] observed a 0.0456 $\log_{10}$ day^{-1} inactivation rate for poliovirus 1. In the most extensive study to date, Yates et al.[99] found a mean inactivation rate of 0.1615 $\log_{10}$ day^{-1} in 11 groundwater samples collected from around the U.S. In surface waters, typical virus inactivation rates are 0.1 $\log_{10}$ day^{-1} at 4 to 6°C and 0.5 $\log_{10}$ day^{-1} at 20 to 25°C.[97]

Such information, while providing an idea of inactivation rates for a particular virus which could be used in the development of a model, does not provide information which can be applied generally to specific locations where environmental factors may dictate widely varying inactivation rates from those already reported. To avoid testing each site in question, information is needed on the relative importance of factors which can be used to predict virus or bacterial inactivation. With this in mind, Yates et al.[99] studied the influence of various factors likely to be useful in predicting virus inactivation in groundwater. They found that groundwater temperature was the single most important predictor of virus inactivation. Linear regression analysis gave a correlation coefficient of 0.88, which was significant at the 0.01 level. The coefficient of determination, R^2, was 0.775, meaning that 77.5% of the variation in inactivation rates among samples could be explained by temperature. The inactivation rate for coliphage MS-2 as a function of temperature was expressed by the following equation:

$$\text{Inactivation rate } (\log_{10} \text{day}^{-1}) = -0.181 + 0.0214 \times \text{temperature (°C)} \quad (2)$$

The finding is not unexpected as temperature has been found to be an important factor controlling virus persistence in all types of water.[97] However, viruses persisted for longer periods of time in well water samples than had been found in experiments using surface waters incubated at similar temperatures.[99] For example, Hejkal and Gerba[170] analyzed all the published literature (143 cases) on the effect of temperature on virus inactivation in seawater and found that it varied with temperature according to the following equation:

$$\text{Inactivation rate } (\log_{10} \text{day}^{-1}) = -0.184 + 0.0335 \times \text{temperature (°C)} \quad (3)$$

It is also important to note that examination of the equation developed by Yates et al.[99] indicates that, as groundwater temperatures approach about 8°C, virus inactivation becomes negligible, compared with 5°C for seawater. It is probable that virus inactivation occurs at

these temperatures but over much greater periods of time than could be observed in laboratory experiments convering a time period of 3 to 4 months. Recent field studies by Stramer[152] on the persistence of viruses leached into groundwater from septic tanks appear to confirm prolonged virus survival at groundwater temperatures of 9 to 20°C. She observed persistence of poliovirus type 1 in a contaminated aquifer for over 105 days after being released from the septic tank into the aquifer.

Hurst et al.[102] studied the survival of seven viruses (poliovirus 1, echovirus 1, coxsackieviruses A9 and B3, rotavirus SA11 and bacteriophages T2 and MS2) in nine different soils to determine what soil characteristics affect virus inactivation rate. They found that soil temperature and virus adsorption to soil were the most important factors affecting virus survival. An equation was developed using stepwise multiple regression to predict virus survival in soil:

$$y = 0.1005 + 0.0025\,x_1 - 0.0008\,x_2 - 0.0007\,x_3 - 0.0510\,x_4 \qquad (4)$$

where y is the average of the survival rate values for the three viruses under the conditions of the experiment; x_1 is the average percent adsorption of all three viruses to the soil; x_2 is the resin-extractable phosphorus value (ppm) for the given soil; x_3 is the exchangeable aluminum value (ppm) for the given soil; and x_4 is the saturation pH value for the given soil. The authors acknowledged that it would be difficult to use this equation to predict virus survival under natural field conditions of constantly changing temperature and soil moisture. They suggest that the equation might best be used to estimate relative virus survival in different soils based on their known physical properties.

Reddy et al.[168] also attempted to model microbial survival in soil. They stated that the concentration of microorganisms in the soil at time t could be described using this first-order rate expression:

$$M_t = M_o \exp[(K_B - K_D)t] \qquad (5)$$

where M_t is the microbial concentration at time t, M_o is the initial microbial concentration after the waste application; K_B is the rate coefficient for the rate of division of the microorganism; and K_D is the rate coefficient for the die-off rate of the microorganism. This equation was simplified to

$$M_t = M_o \exp(-Kt) \qquad (6)$$

by defining $-K = K_B - K_D$.

These investigators felt that temperature, pH, moisture, and the method of waste application were the most important factors controlling microbial inactivation. They then reviewed the literature to find data which could be used to develop equations quantifying the influence of these factors on inactivation. Functional relationships were developed for each of the four factors, which were then incorporated into the following expression for the die-off rate constant, K:

$$K_2 = K_1 \cdot F_T \cdot F_M \cdot F_{pH} \cdot F_{ma} \qquad t_{1/2} = 0.693/K_2 \qquad (7)$$

where the correction coefficients are F_T = temperature; F_M − = soil moisture content; F_{pH} = soil pH; F_{ma} = method of application, and $t_{1/2}$ = the half-life for the survival of the organisms. A table is presented which lists the $t_{1/2}$ values for fecal coliforms under various environmental conditions. Caution should be taken in generalizing the results to other systems, as most of the equations used by these investigators were developed from one set or a limited set of experimental data, usually involving only one or a few microorganisms.

2. Physical Filtration

Another factor which affects the transport of microorganisms through porous media is filtration. Acording to Cookson,[175] the filtration mechanism includes straining, sedimentation, inertial impingement, and diffusion. The straining mechanism occurs where the particle in suspension in the porous matrix cannot pass through a smaller pore opening or constriction (i.e., the wedge between two soil particles), and thus its transport is halted.[176,177] The relative magnitude of the effect of this process depends on many soil, water, and microbial factors.[178] For small microbial particles (i.e., viruses) in coarse-grained material, filtration is probably negligible.[29,31,90,176,177] For the large bacteria, on the other hand, physical straining may be an important consideration.[29,176,178,179] In general, under high flow velocities, the amount of bacteria filtered is less than for low flow velocities.[96,162,178,180] This is probably due to the fact that a larger amount of the total flow quantity is derived from the larger pores, which will transmit a greater portion of the total number of bacteria present.

Smith et al.[179] found that more bacteria (*E. coli*) were filtered by disturbed soil than by undisturbed soils. It was postulated that this was a result of the fact that there was a larger component of flow through macropores in the undisturbed soils than in the disturbed soils. In particular, it was found that 21 to 78% of the bacteria applied to three undisturbed soils was retained, whereas for disturbed samples of the same three soils, 93 to 99.8% of the bacteria was retained. This suggests that if one desires to filter bacteria from contaminated water, the soil should be disturbed to close any macropores or preferential paths that may be present. Although undisturbed samples, in general, filter fewer bacteria than disturbed samples, the difference between two undisturbed samples can also be large.[180]

Sedimentation in the pores occurs where there is a density difference between the microorganism and water. If the microorganism is more dense than water and the flow properties are such that the tendency for gravitational settling is greater than the tendency to be resuspended into the flow stream, the bacteria may settle into quiescent parts of the porous matrix. Corapcioglu and Haridas[176,177] analyze the gravitational settling using Stokes law.[181] A disadvantage of using this method is that Stokes law was derived for fluids at rest, which is generally not the case in groundwater systems.

3. Adsorption

Numerous studies have demonstrated that soils can effectively remove viruses from water. Filtration is not believed to be a significant mechanism of virus removal in coarse-textured soils such as sands, but may be of some importance in fine-textured soils where the pore sizes of the soil matrix are of the same magnitude as the virus (approximately 20 to 200 nm).[171] One of the most important physicochemical processes for virus removal in soils is adsorption of viruses on solid surfaces. This mechanism is also important in the removal of bacteria in soils.

Suspended virus particles can be treated as dispersions of colloidal particles, and interactions between the suspended viruses and solid surfaces often can be described using physical equilibrium adsorption isotherms. Gerba[131] has summarized both the theoretical and applied aspects of virus adsorption to surfaces. For the purposes of developing a quantitative relationship for this removal process, Langmuir and Freundlich isotherms and kinetic adsorption models have been used.

The Langmuir equilibrium isotherm is based on the assumption that every adsorption site is of equal strength, that there is no interaction between adsorbed molecules on the surface, and that maximum adsorption corresponds to a saturated monolayer of molecules on the adsorbent surface. It can be written as[182-184]

$$C_s = \frac{K_L C C_{sm}}{1 + K_L C} \tag{8}$$

where C_s is the concentration of adsorbed virus on the solid phase; C_{sm} is the maximum concentration when all of the active surface sites are occupied; and C is the concentration of viruses in suspension. The term K_L is a constant related to the bonding energy.

Moore et al.[130] found that a Langmuir isotherm fit their data for poliovirus type 2 adsorption to minerals and soils under near-saturation conditions quite well. Vilker et al.[185] also used a Langmuir isotherm to describe the Cookson and North[186] batch equilibrium measurements of T4 phage adsorption on activated carbon.

As Vilker[169] points out, when the adsorbed state is only weakly favored and the liquid-phase concentration is small so that $K_L C << 1$, the Langmuir isotherm reduces to

$$C_s = K_L C C_{sm} \tag{9}$$

which is essentially a linear isotherm. Whether the assumption that the adsorbed state is only weakly favored is valid is highly dependent upon the soil properties and the specific virus of concern, as has been discussed previously. Under such environmental conditions where these assumptions are valid, the equations which describe the virus transport can be simplified (i.e., linearized) and allow the solution to be written in analytical form for situations with simplified geometries and initial and boundary conditions.

Freundlich isotherms have also been used quite successfully to describe the adsorption of viruses in a variety of virus-soil-water systems.[131] The isotherm, which does not assume homogeneity among active sites for adsorption, is expressed as[182,187,188]

$$C_s = K_F C^n \tag{10}$$

where K_F and n are constants. For many systems, the empirical "constant" n is not significantly different from unity, and the Freundlich isotherm reduces to a linear form:

$$C_s = K_F C \tag{11}$$

which is indistinguishable from the linear form of the Langmuir isotherm (Equation 9).

For the purposes of quantifying adsorption data, the choice of an equilibrium isotherm may simply be one of convenience in fitting the isotherm constants, particularly when the isotherm appears linear. For a fluid of arbitrary concentration, however, the use of the Langmuir type of isotherm (Equation 8) connotes saturated-limited adsorption. Adsorption data can indicate a linear relationship if virus adsorption is characterized by a large number of active sites and an equilibrium which strongly favors the suspended phase over the adsorbed phase.[169] Laboratory experiments have shown that even when high concentrations of viruses were applied to soils, less than 1% of the soil particle surface was covered with viruses.[130] These investigators suggested that it is unlikely that even coarse-textured materials would become saturated under natural conditions.

For the description of virus movement through soils, the choice of an isotherm may have important consequences. The isotherm parameters K_L and C_{sm}, or K_F and n, are macroscopic variables which reflect the integrated effects of molecular or ionic interactions between viruses and adsorption sites on the soil matrix. This interaction is electrostatic in nature. Thus, divalent cations can be particularly effective in increasing adsorption by compressing the Gouy layers around both virus and soil particles. This phenomenon could explain the desorption and enhanced movement of viruses observed by Duboise et al.[189] and Lance et al.[138] when deionized water was cycled with secondarily treated wastewater percolates in sandy soil columns. Thus, both the adsorption and desorption isotherms may be important in predicting virus transport.

4. Kinetic Adsorption

Another method for characterizing the adsorption-desorption process is with nonequilibrium adsorption models. A fairly detailed discussion of nonequilibrium adsorption is given by Rao and Jessup[188] along with a list of review articles concerning the adsorption process. Conceptually, two types of sorption are described by Rao and Jessup:[188] chemically and physically controlled adsorption. In the chemically controlled adsorption process, the soil is viewed as being composed of two types of adsorption sites: one type which behaves in an equilibrium-adsorption manner, the other in a first-order reversible manner. For the physically controlled adsorption, the adsorption process is viewed as a two-step transport process whereby a contaminant is transported by diffusion through a quiescent layer, followed by instantaneous adsorption to the soil surface on the soil side of the water film (quiescent layer). Since this latter conceptualization has been used to describe the adsorption process in the transport of microorganisms, it is described in more detail.

Using the notation of Vilker,[169] the nonequilibrium rate of adsorption is described mathematically as

$$\rho_b \frac{\partial q}{\partial t} = k_{f,c}(C-C^*) \tag{12}$$

where ρ_b is the bulk density of the soil; $k_{f,c}$ is an adsorption rate coefficient which describes the diffusive transport of a virus particle from the bulk solution across a quiescent water film towards the soil surface; q is the mass of virus adsorbed to the soil surface; C is the concentration of viruses; and C* is the equilibrium concentration in the fluid in immediate contact with the soil particles. In this case, compared to equilibrium sorption, the sorption process is time dependent.

Filmer et al.[190] investigated the rate of virus adsorption using a diffusion-controlled adsorption model. They investigated two boundary conditions. First, an infinite boundary model (model I) where it is assumed that there are no interactions between adjacent soil surfaces and that all the particles that come into contact with the soil surface are adsorbed. As stated by the authors, this model produces unrealistic results except at early times. A more realistic model is the finite boundary model (model II) where it is assumed that a concentration gradient develops between the soil surface and the fluid.

Comparing experimental data to each model demonstrates that the overall behavior predicted by model I (i.e., square root of time profile) is observed and suggests that model I is valid for early times. At later times, however, the adsorbed-phase concentration profiles with respect to time resulting from model I do not fit the experimental data. Model II, on the other hand, fits the data reasonably well for all times, which suggests that it is a more realistic formulation for the adsorption process.

B. Modeling Microbial Transport

At the present time, the physical processes related to the movement of water through saturated and unsaturated soils are fairly well understood. A variety of methods and models are available to simulate the flow of water in soils, especially saturated soils. Within the context of predicting the movement and attenuation of microorganisms in the subsurface, the flow models currently available are probably adequate for predicting the movement of water. The major exceptions are in fractured rocks and karst systems which are difficult to characterize, both structurally and hydrogeologically. For the most part, current modeling techniques are probably as reliable as — if not more reliable than — estimates of the physical and hydraulic properties required to describe the flow system. The major impediments to predicting concentrations of microorganisms in groundwater lie in quantifying the transport processes, not in predicting the movement of water.

In general, modeling the fate and transport of microorganisms in the subsurface requires various conservation (i.e., mass or energy balance) equations as well as other equations which describe the mass or energy flux due to a potential field, reactions, interactions, etc., for the various constituents which are believed to be important to the problem.

Typically, efforts to model the fate and transport of contaminants in the subsurface incorporate the water flow and contaminant transport processes into a mathematical framework. An extensive review concerning the movement of contaminants in the groundwater is given by Anderson.[191] Under suitably simplifying assumptions, such as for saturated flow and when the average pore water velocity is a constant, it may be possible to describe the transport process using one equation without having to solve the flow equation as well.

For the general case, the flow equation for saturated-unsaturated conditions where the fluid density is assumed constant is generally written as[192,193]

$$\frac{\partial\theta}{\partial\phi}\frac{\partial\phi}{\partial t} + \frac{\theta S_e}{n}\frac{\partial\phi}{\partial t} = \nabla \cdot [K(\theta) \cdot \nabla\phi] + Q \tag{13}$$

where θ is the volumetric water content ($V\ V^{-1}$); n is the porosity ($V\ V^{-1}$); S_e is the specific storage (L^{-1}); ϕ is the hydraulic potential, $\phi = \psi + 1z$, ψ is the pressure head (L); $K(\theta)$ is the hydraulic conductivity tensor (L); and Q is a source/sink term (T^{-1}).

The two terms on the right-hand side describe the changes in potential, respectively, due to changes in water content (i.e., wetting or drying) and compressibility effects. The terms on the left-hand side describe changes in potential due to the flux and from sources and sinks. For saturated conditions, $K(\theta)$ is a constant, i.e., K_s, and is called the saturated hydraulic conductivity. For an unconfined aquifer where the medium and fluid compressibility are negligible, $S_e \approx 0$.

The mathematical model which describes the transport of a solute in an incompressible medium[188, 189] is usually referred to as the advection-dispersion equation

$$\frac{\partial(\theta_a \rho_a C_a + \theta_s \rho_s C_s)}{\partial t} = \nabla \cdot [\theta_a D_h \cdot \nabla(\rho_a C_a) - \rho_a C_a q_a] - \theta_a \rho_a \Gamma_a - \theta_s \rho_s \Gamma_s \tag{14}$$

where D_h is the hydrodynamic dispersion and is a tensor quantity which describes the effects of dispersion and diffusion; q_a is the specific discharge[188] (i.e., the Darcian flux density; L/T); θ_a is the volumetric fraction for phase "a" (i.e., porosity); C_s is the concentration of the adsorbed constituent (M/M); C_a is the concentration of the aqueous phase; and Γ_a is the net loss or production of the "a" phase (i.e., a source/sink term).

Each term in Equation 14 describes a part of the transport process. The first and second terms of the left-hand side describe, respectively, the time rate of change in the concentration in the aqueous and adsorbed phases. On the right-hand side, the first two terms describe the diffusion/dispersion and advection processes, while the remaining terms describe the addition or loss mechanisms.

As described earlier, the inactivation of microorganisms depends on many factors. An example for viruses is temperature. To accurately model the fate and transport of viruses in temperature-dependent subsurface systems, an additional governing equation is necessary which describes the energy transport. In general, this equation will be coupled to the water flow and contaminant transport equations, and thus the complexity of the solution increases. To our knowledge, no research efforts are underway which incorporate effects of temperature (or pH, moisture content, organic matter, etc.) into modeling the transport and fate of microorganisms. This appears to be an open area of research.

Jury,[194,196] Jury et al.,[195,197] and Sposito et al.[198] describe an alternate method for modeling the solute transport process which has an advantage in that it is unnecessary to describe in detail all the factors which affect transport, yet includes these factors implicitly. This approach has an advantage, in an operational sense, since it does not require all the input data necessary for models which account for these factors explicitly. In particular, dispersion is not accounted for explicitly but a "dispersive-like" concentration profile is obtained which results from the variations in the travel times accounted for by the method.

The basis of the method is the probability function:[194]

$$P_L(I) = \int_o^I f_L(I')\, dI' \tag{15}$$

where P(I) is the probability that a tracer injected at the surface will arrive at a depth L after a quantity I of water has infiltrated into the soil; $f_L(I')$ is the probability density function; and I is the cumulative infiltration.

The transport of a solute from the entrance surface to a depth L is determined as an average concentration, $C_L(I)$, of the spatially variable input concentrations, $C_{in}(I - I')$, multiplied by the probability density function for the travel times:[194,196]

$$C_L(I) = \int_o^\infty C_{in}(I - I')\, f_L(I')\, dI' \tag{16}$$

If the probability density function, $f_L(I')$, has a log-normal distribution and a step change in concentration occurs at the input boundary (i.e., $C_{in} = C_o$ for $I > 0$, and zero otherwise) the concentration at depth z and time t is

$$C_L(z, t) = \frac{Co}{2}\left[1 + \text{erf}\left(\frac{\ln(itL/z) - \mu}{\sqrt{2}\sigma}\right)\right] \tag{17}$$

where $I = it$ and i is the infiltration rate at the surface and $C_L(z,t)$ indicates that the concentration is based on a calibration at depth L. It should be noted that if the statistical properties of the soil change markedly (such as for different soil horizons), it may be necessary to use either different calibrations for different regions of the soil profile or a calibration depth that is below the region of interest.[194] Also, Jury[196] has shown that the transfer function model is equivalent to the convection-dispersion model when the solutions to each are fitted to the same data. However, if different depths and input fluxes are used, the "calibrated" models no longer produce identical results. This result demonstrates that the convective-dispersion model is a special case of the more general transfer function model.

C. Solution Methods

If the effects due to temperature can be ignored, then Equations 13 and 14 represent a system of equations describing the transport of contaminants coupled to a flow field. To solve a problem, the solution for the concentration of contaminants as a function of the spatial coordinates and time is sought. There are a variety of techniques and approaches to solving the transport problem, including analytical and numerical methods.

Many analytical solutions to Equation 13 exist and usually rely on simplifying assumptions. In general, it is assumed that the aquifer properties are homogeneous and isotropic. Solutions to Equation 14 have been derived for uniform flow fields (i.e., the velocity is constant) and one-dimensional,[199-205] two dimensional,[205,206] and three-dimensional [205,207,208] transport. One-dimensional transport in aggregated or structured soils has been modeled by van Genuchten

and Wierenga,[209] van Genuchten et al.,[210] and van Genuchten.[211] Warrick et al.[212] have described simultaneous solute and water transfer in an unsaturated soil.

When simplified assumptions cannot be imposed, more comprehensive numerical methods such as finite-difference,[212-215] finite element,[212-215] method of characteristic,[212,214] and boundary integral techniques[192,212,214] may be necessary.

D. Models Applied to Virus Transport

Grosser[172] used a one-dimensional form of Equation 14 to model virus transport between septic tanks and private water supply wells. An implicit assumption of Grosser's model is the adequacy of using a "solute transport" model when modeling a colloidal entity such as a virus. It was assumed that the adsorption process could be described by a linear adsorption isotherm (a linearized Freundlich or Langmuir isotherm, i.e., Equation 9 or 11) and that adsorption occurs instantaneously.

The finite-difference solution method was used by Grosser[172] to simultaneously solve the flow equation for a one-dimensional flow field and was coupled to the transport equation. From his simulations, Grosser[172] found that there was an 80% removal of virus within the first 1 m of flow and that about 15 m was required before the virus concentration was below prescribed limits of 1 PFU/100 gal or 1 PFU/1000 gal. A sensitivity analysis demonstrated that the inactivation rate was the most important parameter affecting the concentration profile at steady state.

Another example of modeling the transport of viruses is given by Vilker et al.,[185] Vilker and Burge,[217] and Vilker,[169,218] who introduced an "adsorption-mass transfer" model. The objective of these studies was to determine the adsorption behavior of viruses on soil particles.

As an alternative to linear equilibrium adsorption models, Vilker[169,218] proposed a rate-controlled adsorption model where the virus particles diffuse through a quiescent layer of fluid to the adsorption site on the soil particles.

From analyzing several virus-soil type combinations it was found that viruses are weakly adsorbed to soil particles. This affinity for the soil or solution phase is characterized by a separation factor r:

$$r = 1/(1 + K_L C_e) \tag{18}$$

where K_L is a Langmuir isotherm coefficient and C_e is the influent virus concentration. When r is small (i.e., $r << 1$) the affinity of virus for the soil is strong and therefore adsorption dominates. When $r \approx 1$, the adsorption is weak and the virus prefers the liquid phase.

The transport behavior of viruses in columns differs depending on whether adsorption is weak or strong. The case where adsorption is strong is described by Vilker et al.,[185] and for weak adsorption by Vilker and Burge[217] and Vilker.[169] Several simplifications are incorporated into their modeling approach: (1) the flow is steady and continuous through a homogeneous soil column; (2) dispersion and diffusion in the bulk fluid are assumed to be negligible, (3) the soil column is initially saturated with solution which is devoid of virus; (4) virus inactivation is assumed to be negligible; and (5) the viruses are single particles (i.e., not aggregated). The fourth assumption has serious implications on the presented results since the times involved are long enough for appreciable inactivation to occur. The authors indicated that inactivation should be included in the model.

Using these assumptions, Equation 14 can be written as

$$\frac{\partial C}{\partial t} + v\frac{\partial C}{\partial z} + \frac{\rho_b}{\theta}\frac{\partial C_s}{\partial t} = 0 \tag{19}$$

The approach used by Vilker et al.[185] to characterize the adsorption differs markedly from that of Grosser[172] where the rate of adsorption of viruses onto the soil particles is described by the rate equation:

$$\rho_b \frac{\partial C_s}{\partial t} = K_{f,c}(C - C^*) \tag{20}$$

where $K_{f,c}$ is an adsorption rate, or interfacial mass transfer coefficient; C* is an adsorption isotherm relationship; and $C - C^*$ is the driving force. The last term ($\partial C_s/\partial t$) in Equation 19 was eliminated by using Equation 20 to describe the rate of adsorption, along with a Langmuir isotherm to compute C*.

Two simple initial and boundary conditions were considered by Vilker et al.[185] The adsorption boundary condition corresponds to a solid phase with no adsorbed virus initially and a column influent of constant virus concentration. Also considered was the elution boundary condition which corresponds to a solid phase which initially contains an amount of adsorbed virus in equilibrium with an initial liquid-phase concentration and an influent devoid of virus.

The general analytical solutions were obtained and show that complex solutions result even when simplifying assumptions are employed. Vilker et al.[185] found that for a case where the adsorbed state is only weakly favored and liquid-phase concentrations are small, the Langmuir isotherm reduces to a linear form and their solution simplified considerably.

Using the solutions supplied by Vilker et al.[185] and Vilker and Burge,[217] one can estimate the column breakthrough curves and adsorbed virus concentrations under these idealized conditions. Applications are restricted to short laboratory columns with short retention times, since virus inactivation is neglected and the effects of dispersion were neglected. The greater travel distances, and therefore retention times, encountered under field conditions would require the incorporation of these (and possibly other) processes.

To use the solution presented by Vilker,[169,218] values for $K_{f,c}$, K_L and C_{sm} (see Equations 8 and 19) must be obtained. Vilker used batch experiments to obtain values for these parameters which were then incorporated into the mathematical model to simulate the concentration profiles for adsorption and elution in a laboratory column. The model was not verified using experimental data.

An important question that arises in modeling the transport and fate of viruses is whether the solute transport equation can be used to model the transport behavior of a colloidal entity such as viruses. A laboratory study has been undertaken by Grondin and Gerba[219] to investigate this question. The goal of this study was to answer the following questions: (1) will viruses disperse producing a concentration distribution which can be described using a dispersion coefficient and solute transport theory? and (2) will available analytical solutions adequately describe the virus concentration in laboratory column experiments?

The laboratory experiment was conducted using a column 1.15 m in length and 5.04 cm in diameter, containing a saturated gravelly sand soil. MS-2 bacteriophage (10^3 to 10^4 PFU/mℓ) was introduced at a constant flow rate.

By comparing analytical solutions,[192,220,221] to the experimental concentration profiles, Grondin and Gerba[219] found that dispersion of MS-2 did occur and could be modeled using existing analytical solutions.

Another interesting result of this study was that the average velocity of the viruses was found to be 1.6 to 1.9 times that of the average velocity for the water. To account for this behavior, a value for the retardation factor, R, less than unity was used in the analytical solution. A possible alternative to reducing R to a value less than unity (which implies that the viruses are subjected to anion exclusion) would be to use the average virus velocity instead of the average pore water velocity. One possible explanation for the behavior is

pore-size exclusion. This conceptualization states that because of the size of the viruses (and aggregates of viruses), they are restricted to the larger pores which have a pore water velocity that is larger than the average pore water velocity of all the pores taken together. Similar results have been demonstrated using macromolecules by Enfield and Bengtsson[222] and *Streptomycete* conidia by Wollum and Cassel.[178] Other results of this study were that viruses were found not to adsorb to the gravelly sandy soil and there was no appreciable inactivation over the time period of the experiments.

In a laboratory column study, Wollum and Cassel[178] investigated the transport of *Streptomycete* conidia and an unidentified bacterium in a saturated sand. Two column lengths (20.3 and 152 cm) and several mean pore water velocities (14.4 to 131 cm/hr) were used. It was found that at higher pore water velocities, the maximum concentration of conidia in the effluent occurred at about 0.85 pore volumes. It was postulated that for high flow rates, the conidia were preferentially transported in the interconnected velocity pores. This same reasoning was used to explain the apparent increased maximum concentration found for the higher pore water velocities, since the varied velocities would help prevent conidia from becoming entrapped by the soil particles. The entrapment of microorganisms by the smaller pores was also demonstrated by comparing the concentration profiles for two columns of different length and water content. It was found that a larger quantity of conidia was retained for the longer column which had a lower water content, since for this case there is a reduced likelihood of continuous pathways.

A final comparison was made between the *Streptomycete* conidia and the bacterium. It was found that a greater quantity of the bacterium was retained than conidia. The investigators suggested that this was due to the fact that the bacterium produced a sticky extracellular polysaccharide.

Wollum and Cassel[178] suggest that the pore water velocity, the number of organisms, the morphology of the organisms, as well as soil water properties such as the water content, bulk density, clay type, and quantity affect the entrainment of microorganisms. They also found that more microorganisms were filtered out near the surface of the soil.

The transport of *E. coli* has been investigated in the laboratory using soil columns by Smith et al.[179] The experiment included various application rates (5 to 40 mm/hr) of a suspension of *E. coli* (10^7 cells/mℓ in 0.005 *M* $CaCl_2$ solution onto disturbed and undisturbed soils. It was found that a large percentage (approximately 33%) of the *E. coli* applied to the columns was recovered, and that the concentration profile vs. pore volumes of *E. coli* was approximately a constant. For a column containing Huntington soil, it was found that macropore flow accounted for most of the transport of both *E. coli* and Cl^-. For this soil, the breakthrough occurred before 0.1 pore volumes. It was also found that the concentration of *E. coli* in the effluent was greater for the undisturbed soil columns compared to the disturbed columns. This was attributed to the increased efficiency of filtration for a disturbed soil due to the absence of preferential flow paths (i.e., macropores).

The application rate was found to be very important in describing the transport of *E. coli*. Smith et al.[179] found that increasing the application rate from 5 to 40 mm/hr caused a 6-fold increase in the concentration in the column effluent. It is unfortunate that this investigation did not include determining the profile of *E. coli* in the columns at the end of the experiment. If this had been done, it would have given some insight into the ability of the soil to filter *E. coli*. Also, the possibility exists that the concentration front for *E. coli* was moving within the porous zone toward the exit point, but did not have a chance to reach the exit in the time frame of experiment. Future experiments should address these points.

White[180] and White et al.[223] set up a laboratory experiment to investigate the transport of *E. coli* in an undisturbed soil which was constructed in a similar manner to Smith et al.[179] In this investigation, however, a solute transport model[194,195,197,198] was used in an attempt to describe the movement of *E. coli* in the soil column. Using the transfer function model,

White et al.[223] found that the probability of the *E. coli* reaching the exit point changes very little with time after the initial breakthrough. Although the model assumes that a breakthrough for *E. coli* occurs [i.e., P(0) is required to be 0], the experimental data were obtained at a frequency which missed the breakthrough part of the concentration curves. This experiment found that for the Maury silt loam, *E. coli* was restricted to pores less than 5 to 10 μm in diameter, comprising about 4% of the undisturbed soil volume.

In recent review articles, Matthess and Pekdeger[171] and Corapcioglu and Haridas[176,177] describe the factors which affect the transport and fate of bacteria and viruses in the subsurface, including advection-dispersion, diffusion, adsorption, growth, decay (i.e., death), interception, sedimentation, chemotaxis, and tumbling. Matthess and Pekdeger[171] describe conceptually the processes which are important in describing the fate and transport of microorganisms in the subsurface. They provide equations which describe many of the transport processes, but do not incorporate the process descriptions into an overall governing equation.

Corapcioglu and Haridas[176,177] provide a mathematical representation for each of these processes and incorporate them into governing equations for microbial transport. This results in a set of three coupled equations, where two of the equations describe the changes in the concentrations of microbial mass and substrate in the liquid and solid phases and the third describes the substrate utilization by bacterial growth.

To use the governing equations developed by Corapcioglu and Haridas,[176] some solution technique must be employed to solve the highly nonlinear equations. Corapcioglu and Haridas[177] use two approaches to solve the system of equations they[176] initially developed. The first is an analytical solution for a situation where the substrate concentration remains constant. This reduces the problem to advection, dispersion, and adsorption where the adsorption is rate controlled. The finite element method is used to calculate a complete solution to the governing equations in one and two dimensions. A simulation of a soil column was undertaken using each solution. Given the input data used, it was found that at steady state the bacterial concentration was negligible for depths larger than 7 cm and that there was a 3% loss in porosity due to microbial deposition and adsorption. Below about 6 cm, there was little microbial deposition. The substrate was found at a depth of up to 9 cm.

The modeling approach of Corapcioglu and Haridas[176, 177] is noteworthy in that many of the factors which affect the transport and fate of microorganisms are considered. The main difficulty in using this model (as well as other transport models) is in obtaining the input parameters which are appropriate for the field site being investigated as well as capturing the spatial and temporal variability of these parameters. In particular, including the spatial and temporal variability of the dispersion coefficient and the fluid velocity vector would probably affect the results of the model more than accounting for chemotaxis and assuming that advection and dispersion can be considered constant. Also, the gravitational velocity, V_g,[177] which is based on Stokes law, is probably not a valid approach to determine thesettling velocity in a moving flow field.[181] Before some of these other factors which affect the transport process (i.e., chemotaxis, sedimentation, and tumbling) can be used with confidence, more research in both the field and laboratory is needed.

IV. CONCLUSIONS

We have discussed in detail the biological, chemical, and physical factors which are known to influence virus and bacterial survival and transport in the subsurface (i.e., temperature, pH, and microbial activity). Upon examination of the models that have been developed to predict the fate of microorganisms, one notices that these factors are not explicitly addressed in the equations used in the models. This is most likely due to the fact that much of the known information is qualitative in nature, and that it is difficult, and

sometimes impossible, to generalize the results of one or several experiments to all microorganisms of concern under all environmental conditions which may be encountered. This problem has been encountered in modeling the transport of chemical contaminants as well. As pointed out by Anderson[191]

> To date, only relatively simple chemical processes such as adsorption and radioactive decay have been incorporated into most contaminant transport models. This can be attributed to the difficulties encountered when solving transport models for conservative constituents and to the problems of incorporating geochemical data into contaminant transport models. Although the types of possible reactions may be known in a general way and numerous case histories of contamination problems have been documented, the reactions are difficult to quantify for purposes of contaminant transport modeling.

Our ability to model the flow of water has greatly surpassed our knowledge of the factors affecting the behavior of contaminants which is necessary to model their transport in the subsurface. Well-designed and -conducted field studies are needed to determine how closely laboratory studies represent what is occurring in the field. In addition, much of the available data has been determined in laboratory studies using a few "model" microorganisms. It has become apparent that it is unlikely that there is a good bacterial indicator of virus behavior, and "model viruses" such as poliovirus may not mimic the behavior of other viruses such as hepatitis A virus. As methods are developed for the cultivation and detection of other viruses known to cause waterborne disease outbreaks, such as Norwalk and Norwalk-like viruses, these viruses will also have to be evaluated in terms of their ability to survive and be transported in the subsurface environment.

DISCLAIMER

Although the research described in this article has been funded by the U.S. Environmental Protection Agency through in-house research efforts, it has not been subjected to agency review and therefore does not necessarily reflect the views of the agency and no offical endorsement should be inferred.

REFERENCES

1. **Bitton, G. and Gerba, C. P.,** Groundwater pollution microbiology: the emerging issue, in *Groundwater Pollution Microbiology,* Bitton, G. and Gerba, C. P., Eds., John Wiley & Sons, New York, 1984, 1.
2. **DiNovo, F. and Jaffe, M.,** *Local Groundwater Protection, Midwest Region,* American Planning Association, Chicago, 1984, 18.
3. U.S. Public Health Service, Statistical Summary of Municipal Water Facilities in the United States, January 1, 1963, Publ. No. 1039, Washington, D.C., 1965.
4. **Craun, G. F.,** Statistics of waterborne outbreaks in the U.S. (1920 to 1980), in *Waterborne Diseases in the United States,* Craun, G. F., Ed., CRC Press, Boca Raton, Fla., 1986, chap. 5.
5. **Craun, G. F.,** Recent statistics of waterborne disease outbreaks (1981 to 1983), in *Waterborne Diseases in the United States,* Craun, G. F., Ed., CRC Press, Boca Raton, Fla., 1986, chap. 6.
6. **Craun, G. F.,** A summary of waterborne illness transmitted through contaminated groundwater, *J. Environ. Health,* 48, 122, 1985.
7. **Lippy, E. C. and Waltrip, S. C.,** Waterborne disease outbreaks — 1946 to 1980: a thirty-five-year perspective, *J. Am. Water Works Assoc.*, 76, 60, 1984.
8. **Kaplan, J. E., Gary, G. W., Baron, R. C., Singh, W., Schonberger, L. B., Feldman, R., and Greenberg, H.,** Epidemiology of Norwalk gastroenteritis and the role of Norwalk virus in outbreaks of acute nonbacterial gastroenteritis, *Ann. Intern. Med.*, 96, 756, 1982.
9. **Keswick, B. H., Satterwhite, T. K., Johnson, P. C., DuPont, H. L., Secor, S. L., Bitsura, J. A., Gary, G. W., and Hoff, J. C.** Inactivation of Norwalk virus in drinking water by chlorine, *Appl. Environ. Microbiol.*, 50, 261, 1985.

10. **Gerba, C. P.,** Strategies for the Control of Viruses in Drinking Water, Rep. Am. Assoc. Adv. Sci., Washington, D.C., 1984.
11. **Melnick, J. L. and Gerba, C. P.,** The ecology of enteroviruses in natural waters, *CRC Crit. Rev. Environ. Control,* 10, 65, 1980.
12. **Tyrrell, D. A. and Kapikian, A. Z., Eds.,** *Virus Infections of the Gastro-intestinal Tract,* Marcel Dekker, New York, 1982.
13. **Gerba, C. P.,** Virus survival in wastewater treatment, in *Viruses and Wastewater Treatment,* Goddard, M. and Butler, M., Eds., Pergamon Press, Elmsford, N.Y., 1981, 39.
14. **Goyal, S. M., Keswick, B. H., and Gerba, C. P.,** Viruses in groundwater beneath sewage irrigated cropland, *Water Res.,* 18, 299, 1984.
15. **Keswick, B. H. and Gerba, C. P.,** Viruses in groundwater, *Environ. Sci. Technol.,* 14, 1290, 1980.
16. **Vaughn, J. M., Landry, E. F., Baranosky, L. J., Beckwith, C. A., Dahl, M. C., and Delihas, N. C.,** Survey of human virus occurrence in wastewater-recharged groundwater on Long Island, *Appl. Environ. Microbiol.,* 36, 47, 1978.
17. **Vaughn, J. M. and Landry, E. F.,** *Data report: An Assessment of the Occurrence of Human Viruses in Long Island Aquatic Systems,* Department of Energy and Environment, Brookhaven National Laboratory, Upton, N.Y., 1977.
18. **Vaughn, J. M. and Landry, E. F.,** The occurrence of human enteroviruses in a Long Island groundwater aquifer recharged with tertiary wastewater effluents, in *State of Knowledge in Land Treatment of Wastewater,* Vol. 2 U.S. Government Printing Office, Washington, D.C., 1978, 233.
19. Office of Technology Assessment, *Protecting the Nation's Groundwater from Contamination,* Vol. 1, U.S. Congress, Office of Technology Assessment, Washington, D.C., 1984.
20. U.S. Environmental Protection Agency, *The Report to Congress, Waste Disposal Practices and Their Effects on Ground Water,* Washington, D.C., 1977.
21. **Yates, M. V.,** Septic tank density and ground water contamination, *Ground Water,* 23, 586, 1985.
22. **Craun, G. F.,** Outbreaks of waterborne disease in the United States: 1971 to 1978, *J. Am. Water Works Assoc.,* 73, 360, 1981.
23. **Craun, G. F.,** Waterborne disease — status report emphasizing outbreaks in ground-water systems, *Ground Water,* 17, 183, 1979.
24. **Vogt, J.,** Infectious hepatitis epidemic at Posen, Michigan, *J. Am. Water Works Assoc.,* 53, 1238, 1961.
25. **McGinnis, J. A. and DeWalle, F.,** The movement of typhoid organisms in saturated, permeable soil, *J. Am. Water Works Assoc.,* 75, 266, 1983.
26. **Craun, G. F.,** Health aspects of groundwater pollution, in *Groundwater Pollution Microbiology,* Bitton, G. and Gerba, C. P., Eds., John Wiley & Sons, New York, 1984, chap. 7.
27. **Wellings, F. M., Mountain, C. W., and Lewis, A. L.,** Virus in ground water, in *Proc. 2nd Natl. Conf. Individual Onsite Waste-water Systems,* McClelland, N. I., Ed., Ann Arbor Science Publishers, Ann Arbor, Mich., 1977.
28. **Gerba, C. P.,** Virus survival and transport in groundwater, *Dev. Ind. Microbiol.,* 24, 247, 1983.
29. **Gerba, C. P. and Bitton, G.,** Microbial pollutants: their survival and transport pattern to groundwater, in *Groundwater Pollution Microbiology,* Bitton, G. and Gerba, C. P., Eds., John Wiley & Sons, New York, 1984, chap.4.
30. **Gerba, C. P., Wallis, C., and Melnick, J. L.,** Fate of wastewater bacteria and viruses in soil, *J. Irrig. Drain. Div. ASCE,* 101, 157, 1975.
31. **Sobsey, M. D.,** Transport and fate of viruses in soils, in *Microbial Health Considerations of Soil Disposal of Domestic Wastewaters,* U.S. Environmental Protection Agency, Cincinnati, 1983.
32. **Beard, P. J.,** Longevity of *Eberthella typhosus* in various soils, *Am. J. Public Health,* 30, 1077, 1940.
33. **Houston, A. C.,** *Studies in Water Supply,* Macmillan, London, 1913.
34. **Jordan, E. O.,** The changes in bacterial content of stored normal and typhoid feces, *J. Infect. Dis.,* 38, 306, 1926.
35. **Ostrolenk, M., Kramer, N., and Cleverdon, R. C.,** Comparative studies of enterococci and *Escherichia coli* as indices of pollution, *J. Bacteriol.,* 53, 197, 1947.
36. **Van Donsel, D. J., Geldreich, E. E., and Clarke, N. A.,** Seasonal variations in survival of indicator bacteria in soil and their contribution to storm-water pollution, *Appl. Microbiol.,* 15, 1362, 1967.
37. **Mirzoev, G. G.,** Extent of survival of dysentery bacilli at low temperatures and self-disinfection of soil and water in the far-north, *Hyg. Sanit.,* 31, 437, 1968.
38. **Rudolfs, W., Falk, L. L., and Ragotzkie, R. A.,** Literature review on the occurrence and survival of enteric, pathogenic, and relative organisms in soil, water, and sludges, and on vegetation, *Sewage Ind. Wastes.* 22, 1261, 1950.
39. **Jordan, E. O., Russell, J. L., and Zeit, F. R.,** The longevity of the typhoid bacillus in water, *J. Infect. Dis.,* 1, 641, 1904.

40. **Kligler, I. J.,** Investigations of Soil Pollution and the Relation of the Various Types of Privies to the Spread of Intestinal Infections, Monogr. No. 15, International Health Board, Rockefeller Inst. Med. Res., 1921.
41. **Tate, R. L.,** Cultural and environmental factors affecting the longevity of *Escherichia coli* in Histosols, *Appl. Environ. Microbiol.*, 35, 925, 1978.
42. **Wibaut, N. L. and Moens, S.,** The disappearance of typhoid bacteria in water, *Verslag.*, 36, 129, 1927.
43. **Young, C. C. and Greenfield, H.,** Observations on the viability of the *Bacterium coli* group under natural and artificial conditions, *Am. J. Public Health*, 13, 270, 1923.
44. **Bouma, J., Ziebell, W. A., Walker, W. G., Olcott, P. G., McCoy, E., and Hole, F. D.,** Soil Adsorption of Septic Tank Effluent, Inf. Circular No. 20, University of Wisconsin Extension, Madison, 1972.
45. **Tanner, F. W.,** Public health significance of sewage sludge when used as a fertilizer, *Sewage Works J.*, 7, 611, 1935.
46. **Plews, G.,** Management guidelines for conventional and alternative onsite sewage systems — Washington state, in *Proc. 3rd Natl. Conf. Individual Onsite Wastewater Systems*, McClelland, N. I., Ed., Ann Arbor Science Publishers, Ann Arbor, Mich., 1976.
47. **Bouma, J.,** Unsaturated flow during soil treatment of septic tank effluent, *J. Environ. Eng. Div. Am. Soc. Civ. Eng.*, EE6, 967, 1975.
48. **Viraraghavan, T.,** Travel of microorganisms from a septic tile, *Water Air Soil Pollut.*, 9, 355, 1978.
49. **Hagedorn, C., McCoy, E. L., and Rahe, T. M.,** The potential for ground water contamination from septic effluents, *J. Environ. Qual.*, 10, 1, 1981.
50. **Canter, L. and Knox, R. C.,** Evaluation of Septic System Effects on Ground Water Quality, U.S. EPA Publ. No. EPA-600/2-84-107, Washington, D.C., 1984.
50. **Cohen, B.,** Disinfection studies, *J. Bacteriol.*, 7, 183, 1922.
52. **Cuthbert, W. A., Panes, J. J., and Hill, E. C.,** Survival of *Bacterium coli* type 1 and *Streptococcus faecalis* in soil, *J. Appl. Bacteriol.*, 18, 408, 1950.
53. **Scott, R. D. and McClure, G. M.,** The hydrogen-ion concentration of lime treated water and its effect on bacteria of the colon-typhoid group, *J. Am. Water Works Assoc.*, 11, 578, 1924.
54. **Gilbert, R. G., Gerba, C. P., Rice, R. C., Bouwer, H., Wallis, C., and Melnick, J. L.,** Virus and bacteria removal from wastewater by land treatment, *Appl. Environ. Microbiol.*, 32, 333, 1976.
55. **Goldschmid, J., Zohar, D., Argamon, Y., and Kott, Y.,** Effect of dissolved salts on the filtration of coliform bacteria in sand dunes, in *Advances in Water Pollution Research*, Jenkins, S. H., Ed., Pergamon Press, New York, 1973, 147.
56. **Allen, M. J. and Morrison, S. M.,** Bacterial movement through fractured bedrock, *Ground Water*, 11, 6, 1973.
57. **Dappert, A. F.,** Tracing the travel and changes in composition of underground pollution, *Water Works Sewerage*, 79, 265, 1932.
58. **Ditthorn, F. and Luersson, A.,** Experiments on the passage of bacteria through soil, *Eng. Rec.*, 60, 642, 1909.
59. **Marti, F., Valle, G. D., Krech, V., Gees, R. A., and Baumgartner, E.,** Tracing tests in ground-water with dyes, bacteria, and viruses, *Alimenta*, 18, 135, 1979.
60. **McGauhey, P. H. and Krone, R. B.,** Report on the Investigation of Travel of Pollution, Publ. No. 11, State Water Pollution Control Board, State of California, 1954.
61. **Merrell, J. C., Jr.,** The Santee Recreaton Project, Santee, Calif., Water Pollut. Res. Ser. Publ. No. WP-20-7, Fed. Water Pollut. Control Admin., Cincinnati, OH, 1967.
62. **Sinton, L. W.,** Investigations into the use of the bacterial species *Bacillus stearothermophilus* and *Escherichia coli* (H_2S positive) as tracers of ground water movement, Water and Soil Tech. Publ. No. 17, Water Soil Div., Ministry of Works and Development, Wellington, N.Z., 1980.
63. **Wesner, G. M. and Baier, D. C.,** Injection of reclaimed wastewater into confined aquifers, *J. Am. Water Works Assoc.*, 62, 203, 1970.
64. **Young, R. H. F.,** Effects on ground water, *J. Water Pollut. Control Fed.*, 46, 1296, 1973.
65. **Caldwell, E. L.,** Pollution flow from a pit latrine when permeable soils of considerable depth exist below the pit, *J. Infect. Dis.*, 62, 225, 1938.
66. **Anan'ev, N. I. and Demin, N. D.,** On the spread of pollutants in subsurface waters, *Hyg. Sanit.*, 36, 292, 1971.
67. **Butler, R. G., Orlob, G. T., and McGauhey, P. H.,** Underground movement of bacterial and chemical pollutants, *J. Am. Water Works Assoc.*, 46, 97, 1954.
68. **Kudryavtseva, B. M.,** An experimental approach to the establishment of zones of hygienic protection of underground water sources on the basis of sanitary-bacteriological indices, *J. Hyg. Epidemiol. Microbiol. Immunol.*, 16, 503, 1972.
69. **Malin, A. and Snellgrove, J.,** The examination of sewage effluent and affected soils, *Lab. Pract.*, 7, 219, 1958.

70. **Randall, A. D.,** Movement of bacteria from a river to a municipal well — a case history, *J. Am. Water Works Assoc.*, 62, 716, 1970.
71. **Reneau, R. B. and Pettry, D. E.,** Movement of coliform bacteria from septic tank effluent through selected coastal plain soils of Virginia, *J. Environ. Qual.*, 4, 41, 1975.
72. **Baars, J. K.,** Travel of pollution, and purification en route, in sandy soils, *Bull. World Health Org.*, 16, 727, 1957
73. **Caldwell, E. L.,** Pollution flow from pit latrines when an impervious stratum closely underlies the flow, *J. Infect. Dis.*, 62, 270, 1937.
74. **Caldwell, E. L.,** Studies of subsoil pollution in relation to possible contamination of the ground water from human excreta deposited in experimental latrines, *J. Infect. Dis.*, 62, 272, 1938.
75. **Caldwell, E. L. and Parr, L. W.,** Ground water pollution and the bored hole latrine, *J. Infect. Dis.*, 61, 148, 1937
76. **Hagedorn, C., Hansen, D. T., and Simonson, G. H.,** Survival and movement of fecal indicator bacteria in soil under conditions of saturated flow, *J. Environ. Qual.*, 7, 55, 1978
77. **Pyle, B. H. and Thorpe, H. R.,** Evaluation of the potential for microbiological contamination of an aquifer using a bacterial tracer, in *Proc. Groundwater Pollut. Conf. Perth, West Australia,* Australian Water Resources Council, Australian Government Publication Service, Canberra, 1981, 213.
78. **Rahe, T. M., Hagedorn, C., and McCoy, E. L.** A comparison of flourescein dye and antibiotic-resistant *Escherichia coli* as indicators of pollution in groundwater, *Water Air Soil Pollut.* 11, 93, 1979
79. **Stiles, C. W. and Crohurst, H. R.,** Principles underlying the movement of *E. coli* in groundwater with the resultant pollution of wells, *Public Health Rep.*, 38, 1350, 1923.
80. **Warrick, L. F. and Muegge, O. J.,** Safeguarding Wisconsin's water supplies, *J. Am. Water Works Assoc.*, 22, 516, 1930.
81. **Bouwer, H., Lance, J. C., and Riggs, M. S.,** High-rate land treatment. II. Water quality and economic aspects of Flushing Meadows project, *J. Water Pollut. Control Fed.*, 46, 844, 1974.
82. **Martin, G. N. and Noonon, M. J.,** Effects of domestic wastewater disposal by land irrigation on groundwater quality of central Canterbury plains. Water Soil Tech. Publ. No. 7, Water and Soil Division, Ministry of Work and Development, Wellington, N.Z., 1977.
83. **McMichael, F. C. and McKee, J. E.,** Wastewater Reclamation at Whittier Narrows, Pub. No. 33, State Water Quality Control Board, State of California, 1965.
84. **Sinton, L. W.,** Microbial contamination of alluvial gravel aquifers by septic tank effluent, *Water Air Soil Pollut.*, 28, 407, 1986
85. **Brown, K. W., Slowey, J. F., and Wolf, H. W.,** The movement of salts, nutrients, fecal coliform, and virus below septic leach fields in three soils, in *Pro. 2nd Natl. Home Sewage Treatment Symp.*, American Society of Agricultural Engineers, 1978.
86. **Brown, K. W., Wolf, H. W., Donnelly, K. C., and Slowey, J. F.,** The movement of fecal coliforms and coliphages below septic lines, *J. Environ. Qual.*, 8, 121, 1979.
87. **Weaver, R. W.,** Transport and fate — bacterial pathogens in soil, in *Microbial Health Considerations of Soil Disposal of Domestic Wastewaters,* Pub. No. EPA-600/9-83-017, U.S. Environmental Protection Agency, Washington, D.C., 1983, 123.
88. **Kingston, S. P.,** Contamination of water supplies in limestone formation, *J. Am. Water Works Assoc.*, 35, 1450, 1943.
89. **Fournelle, H. J., Day, E. K., and Page, W. B.,** Experimental ground water pollution at Anchorage, Alaska, *Public Health Rep.*, 72, 203, 1957
90. **Bitton, G.,** *Introduction to Environmental Virology,* John Wiley & Sons, New York, 1980
91. **Krone, R. B.,** The movement of disease producing organisms through soil, in *Municipal Sewage Effluent for Irrigation,* Wilson, C. W. and Beckett, F. E., Eds., The Louisiana Tech Alumni Foundation, Ruston, La., 1968, chap. 8.
92. **Melick, C. O.,** The possibility of typhoid infection through vegetables, *J. Infect. Dis.*, 21, 28, 1917.
93. **Heukelekian, H. and Schulhoff, H. B.,** Studies on the survival of *B. typhosus* in surface waters and sewage, *N.J. Agric. Exp. Stn. Bull.*, 1935.
94. **Winslow, E. E. A. and Brooke, O. R.,** The viability of various species of bacteria in aqueous suspensions, *J. Bacteriol.*, 13, 235, 1927
95. **Krone, R. B., Orlob, G. T., and Hodgkinson, C.,** Movement of coliform bacteria through porous media, *Sewage Ind. Wastes,* 30, 1, 1958.
96. **Bouma, J.,** Subsurface applications of sewage effluent, in *Planning the Uses and Management of Land,* Am. Soc. Agron., Madison, Wis., 1979, 665.
97. **Akin, E. W., Benton, W. H., and Hill, W. F.,** Enteric viruses in ground and surface waters: a review of their occurrence and survival, in *Proc. 13th Water Qual. Conf. Virus Water Qual. Occurrence Control,* Snoeyink, V., Ed., The University of Illinois, Urbana, 1971, 59.
98. **Sattar, S. A.,** Virus survival in receiving waters, in *Viruses and Wastewater Treatment,* Goddard, M. and Butler, M., Eds., Pergamon Press, New York, 1981, 91.

99. **Yates, M. V., Gerba, C. P., and Kelley, L. M.,** Virus persistence in groundwater, *Appl. Environ. Microbiol.*, 49, 778, 1985.
100. **Lefler, E. and Kott, Y.,** Virus retention and survival in sand, in *Virus Survival in Water and Wastewater Systems,* Malina, J. F. and Sagik, B. P., Eds., Center for Research in Water Resources, Austin, Tex., 1974, 84.
101. **Yeager, J. G. and O'Brien, R. T.,** Enterovirus inactivation in soil, *Appl. Environ. Microbiol.*, 38, 694, 1979.
102. **Hurst, C. J., Gerba, C. P., and Cech, I.,** Effects of environmental variables and soil characteristics on virus survival in soil, *Appl. Environ. Microbiol.*, 40, 1067, 1980.
103. **Larkin, E. P. and Fassolitis, A. C.,** Viral heat resistance and infectious nucleic acid, *Appl. Environ. Microbiol.*, 38, 650, 1967.
104. **Dimmock, N. J.,** Differences between the thermal inactivation of picornaviruses at "high" and "low" temperatures, *Virology,* 31, 338, 1967.
105. **Kapuscinski, R. B. and Mitchell, R.,** Processes controlling virus inactivation in coastal waters, *Water Res.*, 14, 363, 1980.
106. **Sobsey, M. D., Dean, C. H., Knuckles, M. E., and Wagner, R. A.,** Interactions and survival of enteric viruses in soil materials, *Appl. Environ. Microbiol.*, 40, 92, 1980.
107. **Lo, S., Gilbert, J., and Hetrick, F.,** Stability of human enteroviruses in estuarine and marine waters, *Appl. Environ. Microbiol.*, 32, 245, 1976.
108. **Shuval, H. I., Thompson, A., Fattal, B., Cumbalista, S. and Wiener, Y.,** Natural virus inactivation processes in seawater, *J. Sanit. Eng. Div. Am. Soc. Civil Eng.*, 97, 587, 1971
109. **Hetrick, F. M.,** Survival of human pathogenic viruses in estuarine and marine waters, *ASM News,* 44, 300, 1978.
110. **Herrmann, J. E., Kostenbader, K. D., Jr., and Cliver, D. O.,** Persistence of enteroviruses in lake water, *Appl. Microbiol.*, 28, 895, 1974.
111. **Fujioka, R. S., Loh, P. C., and Lau, L. S.,** Survival of human enteroviruses in the Hawaiian ocean environment: evidence for virus-inactivating microorganisms, *Appl. Environ. Microbiol.*, 39, 1105, 1980.
112. **Akin, E. W., Hill, W. F., Jr., Cline, G. B., and Benton, W. H.,** The loss of poliovirus 1 infectivity in marine waters, *Water Res.*, 10, 59, 1976.
113. **Matossian, A. M. and Garabedian, G. A.,** Virucidal action of sea water, *Am. J. Epidemiol.*, 85, 1, 1967
114. **O'Brien, R. T. and Newman, J. S.,** Inactivation of polioviruses and coxsackieviruses in surface water, *Appl. Environ. Microbiol.*, 33, 334, 1977.
115. **Yates, M. V.,** Virus Persistence in Ground Water, Ph.D. dissertation, University of Arizona, Tucson, 1984.
116. **Cliver, D. O. and Herrmann, J. E.,** Proteolytic and microbial inactivation of enteroviruses, *Water Res.*, 6, 797, 1972.
117. **Fukada, T., Hoshino, M., Endo H., Mutai, M., and Shirota, M.** Photodynamic antiviral substance extracted from *Chlorella* cells, *Appl. Environ. Microbiol.*, 16, 1809, 1968.
118. **Salo, R. J. and Cliver, D. O.,** Inactivation of enteroviruses by ascorbic acid and sodium bisulfite, *Appl. Environ. Microbiol.*, 36, 68, 1978.
119. **Bagdasaryan, G.,** Survival of viruses of the enterovirus group (poliomyelitis, echo, coxsackie) in soil and on vegetables. *J. Hyg. Epidemiol. Microbiol. Immunol.*, 8, 497, 1964.
120. **Sagik, B. P., Moore, B. E., and Sorber, G. A.,** Infectious disease potential of land application of wastewater, in *State of Knowledge of Land Treatment of Wastewater,* McKim, H. L., Ed., U.S. Army Corps of Engineers Cold Regions Research and Engineering Laboratory, Hanover, N.H., 1978, 35.
121. **Hurst, C. J., Gerba, C. P., Lance, J. C., and Rice, R. C.,** Survival of enteroviruses in rapid-infiltration basins during the land application of wastewater, *Appl. Environ. Microbiol.*, 40, 192, 1980.
122. **Yeager, J. G. and O'Brien, R. T.,** Structural changes associated with poliovirus inactivation in soil, *Appl. Environ. Microbiol.*, 38, 702, 1979.
123. **Lance, J. C. and Gerba, C. P.,** Virus movement in soil during saturated and unsaturated flow, *Appl. Environ. Microbiol.*, 47, 335, 1984.
124. **Murphy, W. J., Jr., Eylaz, O. R., Schmidt, E. L., and Sylverton, J. T.,** Adsorption and translocation of mammalian viruses by plants. I. Survival of mouse encephalomyelitis and poliomyelitis viruses in soil and plant root environment, *Virology,* 6, 612, 1958.
125. **Prage, L., Patterson, V., Hoglund, S., Lonberg-Holm, K., and Philipson, L.,** Structural proteins of adenoviruses. IV. Sequential degradation of the adenovirus type 2 virion, *Virology,* 42, 341, 1970.
126. **Goyal, S. M. and Gerba, C. P.,** Comparative adsorption of human enteroviruses, simian rotavirus, and selected bacteriophages to soils, *Appl. Environ. Microbiol.*, 38, 241, 1979.
127. **Gerba, C. P., Goyal, S. M., Cech, I., and Bogdan, G. F.,** Quantitative assessment of the adsorptive behavior of viruses to soils, *Environ. Sci. Technol.*, 15, 940, 1981.
128. **Burge, W. D. and Enkiri, N. K.,** Virus adsorption by five soils, *J. Environ. Qual.*, 7, 73, 1978.

129. **Schaub, S. A. and Sagik, B. P.,** Association of enteroviruses with natural and artifically introduced colloidal solids in water and infectivity of solids-associated virions, *Appl. Environ. Microbiol.*, 30, 212, 1975.
130. **Moore, R. S., Taylor, D. H., Sturman, L. S., Reddy, M. M., and Fuhs, G. W.,** Poliovirus adsorption by 34 minerals and soils, *Appl. Environ. Microbiol.*, 42, 963, 1981.
131. **Gerba, C. P.,** Applied and theoretical aspects of virus adsorption to surfaces, *Adv. Appl. Microbiol.*, 30, 133, 1984.
132. **Burnet, F. M. and McKie, M.,** Balanced salt action as manifested in bacteriophage phenomena, *Aust. J. Exp. Biol. Med. Sci.*, 7, 183, 1930.
133. **Wallis, C. and Melnick, J. L.,** Stabilization of poliovirus by cations, *Tex. Rep. Biol. Med.*, 19, 683, 1961.
134. **Dulbecco, R. and Ginsberg, H. S.,** *Virology*, Harper & Row, Philadelphia, 1980, 1112.
135. **Cords, C. E., James, C. G., and McLaren, L. C.,** Alteration of capsid proteins of coxsackievirus A13 by low ionic conditions, *J. Virol.*, 15, 244, 1975.
136. **Taylor, D. H., Moore, R. S., and Sturman, L. S.,** Influence of pH and electrolyte composition on adsorption of poliovirus by soils and minerals, *Appl. Environ. Microbiol.*, 42, 976, 1981.
137. **Gerba, C. P. and Lance, J. C.,** Poliovirus removal from primary and secondary sewage effluent by soil filtration, *Appl. Environ. Microbiol.*, 36, 247, 1978.
138. **Lance, J. C., Gerba, C. P., and Melnick, J. L.,** Virus movement in soil columns flooded with secondary sewage effluent, *Appl. Environ. Microbiol.*, 32, 520, 1976.
139. **Landry, E. F., Vaughn, J. M., Thomas, M. Z., and Beckwith, C. A.,** Adsorption of enteroviruses to soil cores and their subsequent elution by artificial rainwater, *Appl. Environ. Microbiol.*, 38, 680, 1979.
140. **Landry, E. F., Vaughn, J. M., and Penello, W. F.,** Poliovirus retention in 75-cm soil cores after sewage and rainwater application, *Appl. Environ. Microbiol.*, 40, 1032, 1980.
141. **Wellings, F. M., Lewis, A. C., Mountain, C. W., and Pierce, L. V.,** Demonstration of viruses in groundwater after effluent discharge onto soil, *Appl. Environ. Microbiol.*, 29, 751, 1975.
142. **Gerba, C. P. and Schaiberger, G. E.,** Effect of particulates on virus survival in seawater, *J. Water Pollut. Control Fed.*, 47, 93, 1975.
143. **Murray, J. P. and Laband, S. J.,** Degradation of poliovirus by adsorption onto inorganic surfaces, *Appl. Environ. Microbiol.*, 37, 480, 1979.
144. **Young, D. C. and Sharp, D. G.,** Poliovirus aggregates and their survival in water, *Appl. Environ. Microbiol.*, 33, 168, 1977.
145. **Aulenbach, D. B.,** Long-Term Recharge of Trickling Filter Effluent into Sand, Pub. No. EPA-600/2-79-068, U.S. Environmental Protection Agency, Washington, D.C., 1979.
146. **Martin, R. and Thomas, A.,** An example of the use of bacteriophage as a ground water tracer, *J. Hydrol.*, 23, 73, 1974.
147. **Shaub, S. A. and Sorber, C. A.,** Virus and bacteria removal from wastewater by rapid infiltration through soil, *Appl. Environ. Microbiol.*, 33, 609, 1977.
148. **Fletcher, M. W. and Myers, R. L.,** Ground water tracing in karst terrain using phage T-4, *Abstr. Annu. Mtg. Am. Soc. Microbiol.*, 74, 52, 1974.
149. **Hain, K. E. and O'Brien, R. T.,** The Survival of Enteric Viruses in Septic Tanks and Septic Tank Drainfields, Rep. No. 108, New Mexico Water Resources Research Institute, Las Cruces, N.M., 1979.
150. **Green, K. M. and Cliver, D. O.,** Removal of virus from septic tank effluent by sand columns, in *Home Sewage Disposal, Proc. Natl. Home Sewage Disposal Symp.*, ASAE, St. Joseph, Mich., 1974, 137.
151. **Lance, J. C. and Gerga, C. P.,** Poliovirus movement during high rate land filtration of sewage water, *J. Environ. Qual.*, 9, 31, 1980.
152. **Stramer, S. L.,** Fates of Poliovirus and Enteric Indicator Bacteria during Treatment in a Septic Tank System Including Septage Disinfection, Ph.D. Dissertation, University of Wisconsin, Madison, 1984.
153. **Vaughn, J. M., Landry, E. F., Beckwith, C. A., and Thomas, M. Z.,** Virus removal during groundwater recharge: effects of infiltration rate on adsorption of poliovirus to soil, *Appl. Environ. Microbiol.*, 41, 139, 1981.
154. **Wellings, F. M., Lewis, A. L., and Mountain, C. W.,** Virus survival following wastewater spray irrigation of sandy soils, in *Virus Survival in Water and Wastewater Systems*, Malina J. F. and Sagik, B. P., Eds., Center for Research in Water Resources, Austin, Tex., 1974, 253.
155. **Koerner, E. L. and Haws, D. A.,** Long-Term Effects of Land Application of Domestic Wastewater: Vineland, N.J., Rapid Infiltration Site, Pub. No. EPA-600/2-79-072, U.S. Environmental Protection Agency, Washington, D.C., 1979.
156. **Sobsey, M. D., Shields, P. A., Hauchman, F. H., Hazard, R. L., and Caton, L. W.,** Survival and transport of hepatitis A virus in soils, groundwater, and wastewater, *Water Sci. Technol.*, in press.
157. **Lance, J. C., Gerba, C. P., and Wang, D.-S.,** Comparative movement of different enteroviruses in soil columns, *J. Environ. Qual.*, 11, 347, 1982.

158. **Bixby, R. L. and O'Brien, D. J.,** Influence of fulvic acid on bacteriophage adsorption and complexation in soil, *Appl. Environ. Microbiol.*, 38, 840, 1979.
159. **Bitton, G., Masterson, N., and Gifford, G. E.,** Effect of a secondary treated effluent on the movement of viruses through a cypress dome soil, *J. Environ. Qual.*, 5, 370, 1976.
160. **Scheuerman, P. R., Bitton, G., Overman, A. R., and Gifford, G. E.,** Transport of viruses through organic soils and sediments, *J. Environ. Eng. Div. Am. Soc. Civil Eng.*, 105, 629, 1979.
161. **Berg, G., Dean, R. B., and Dahling, D. R.,** Removal of poliovirus-1 from secondary effleunts by lime flocculation and rapid sand filtration, *J. Am. Water Works Assoc.*, 60, 193, 1968.
162. **Butler, R. G., Orlob, G. T., and McGauhey, P. H.,** Underground movement of bacterial and chemical pollutants, *J. Am. Water Works Assoc.*, 46, 97, 1954.
163. **Drewry, W. A. and Eliasson, R.,** Virus movement in groundwater, *J. Water Pollut. Control Fed.*, 40, R257, 1968.
164. **Kreissl, J. F.,** Current practices — subsurface disposal, in Microbial Health Considerations of Soil Disposal of Domestic Wastewaters, Pub. No. EPA-600/9-83-017, U.S. Environmental Protection Agency, Washington, D.C., 1983, 27.
165. **Robeck, G. G., Clarke, N. A., and Dostal, K. A.,** Effectiveness of water treatment processes in virus removal, *J. Am. Water Works Assoc.*, 54, 1275, 1962.
166. **Wang, D. S., Gerba, C. P., and Lance, J. C.,** Effect of soil permeability on virus removal through soil columns, *Appl. Environ. Microbiol.*, 42, 83, 1981.
167. **Young, R. H. F. and Burbank, N. C.,** Virus removal in Hawaiian soils, *J. Am. Water Works Assoc.*, 65, 598, 1973.
168. **Reddy, K. R., Khaleel, R., and Overcash, M. R.,** Behavior and transport of microbial pathogens in soils treated with organic wastes, *J. Environ. Qual.*, 10, 255, 1981.
169. **Vilker, V. L.,** Simulating virus movement in soils in *Modeling Wastewater Renovation: Land Treatment*, Iskandar, I. K., Ed., John Wiley & Sons, New York, 1981.
170. **Hejkal, T. W. and Gerba, C. P.,** *A Model of Virus Die-Off in Sea Water*, NOAA Office of Marine Pollution Assessment, Rockville, Md., 1982.
171. **Matthess, G. and Pekdeger, A.,** Concepts of a survival and transport model of pathogenic bacteria and viruses in groundwater, *Sci. Total Environ.*, 21, 149, 1981.
172. **Grosser, P. W.,** A one-dimensional mathematical model of virus transport, *Paper 2nd Int. Conf. Ground-Water Qual. Res.*, Tulsa, Okla., 1984.
173. **Keswick, B. H., Gerba, C. P., Secor, S. L., and Cech, I.,** Survival of enteric viruses and indicator bacteria in groundwater, *J. Environ. Sci. Health*, A17, 903, 1982.
174. **Bitton, G., Farrah, S. R., Ruskin, R. H., Butner, J., and Chou, Y. J.,** Survival of pathogenic and indicator organisms in ground water, *Ground Water*, 21, 405, 1983.
175. **Cookson, J. T.,** Removal of submicron particles in packed beds, *Environ. Sci. Technol.*, 4, 128, 1970.
176. **Corapcioglu, M. Y. and Haridas, A.,** Transport and fate of microorganisms in porous media: a theoretical investigation, *J. Hydrol.*, 72, 149, 1984.
177. **Corapcioglu, M. Y. and Haridas, A.,** Microbial transport in soils and groundwater: a numerical model, *Adv. Water Resour.*, 8, 188, 1985.
178. **Wollum, A. C. and Cassel, D. K.,** Transport of microorganisms in sand columns, *Soil Sci. Soc. Am. J.*, 42, 72, 1978.
179. **Smith, M. S., Thomas, G. W., White, R. E., and Ritonga, D.,** Transport of *Escherichia coli* through intact and disturbed soil columns, *J. Environ. Qual.*, 14, 87, 1985.
180. **White, R. E.,** Transport of chloride and non-diffusible solutes through soil, *Irrig. Sci.*, 6, 3, 1985.
181. **Sears, F. W. and Zemansky, M. W.,** *University Physics*, Addison-Wesley, Reading, Mass., 1955.
182. **Bohn, H., McNeal, B., and O'Connor, G.,** *Soil Chemistry*, Wiley-Interscience, New York, 1979.
183. **Hendricks, D. W.,** Sorption in flow-through porous media, in *Developments in Soil Science, Vol. 2, Fundamentals of Transport Phenomena in Porous Media*, Elsevier, New York, 1972, 384.
184. **Enfield, C., Phan, T., and Walters, D.,** Kinetic model for phosphate transport and transformation in calcareous soils. II. Laboratory and field transport, *Soil Sci. Soc. Am. J.*, 45, 1064, 1981.
185. **Vilker, V. L., Frommhagen, L. H., Kamdar, R., and Sundarum, S.,** Applications of ion exchange/adsorption models to virus transport in percolating beds, *AIChE Symp. Ser.*, 74, 84, 1978.
186. **Cookson, J. T. and North, W. J.,** Adsorption of viruses on activated carbon, *Environ. Sci. Technol.*, 1, 46, 1967.
187. **Freeze, R. A. and Cherry, J. A.,** *Groundwater*, Prentice-Hall, Englewood Cliffs, N.J., 1979.
188. **Rao, P. S. C. and Jessup, R. E.,** Sorption and movement of pesticides and other toxic substances in soil, in *Chemical Mobility and Reactivity in Soil Systems*, Am. Soc. Agron., Madison, Wis., 1983, 183.
189. **Duboise, S. M., Sagik, B. P., and Malina, J. F., Jr.,** Virus migration through soils, in *Virus Survival in Water and Wastewater Systems*, Malina, J. F. and Sagik, B. P., Eds., Center for Research in Water Resources, Austin, Tex., 1974, 233.

190. **Filmer, R. W., Felton, M., and Yamamoto, T.,** Virus-sized adsorption on soil. I. Rate of adsorption, in *Proc. 13th Water Qual. Conf. Virus Water Qual. Occurrence Control,* Snoeyink, V., Ed., The University of Illinois, Urbana, 1971, 171.
191. **Anderson, M. P.,** Using models to simulate the movement of contaminants through groundwater flow systems, *CRC Crit. Rev. Environ. Control,* 9, 97, 1979.
192. **Bear, J.,** *Hydraulics of Groundwater,* McGraw-Hill, New York, 1979.
193. **Liggett, J. A. and Liu, P. L-F.,** *The Boundary Integral Equation Method for Porous Media Flow,* George Allen & Unwin, Herts, U.K., 1983.
194. **Jury, W. A.,** Simulation of solute transport using a transfer function model, *Water Resour. Res.,* 18, 363, 1982.
195. **Jury, W. A., Stolzy, L. H., and Shouse, P.,** A field test of the transfer function model for predicting solute transport, *Water Resour. Res.,* 18, 369, 1982.
196. **Jury, W. A.,** Chemical transport modeling: current approaches and unresolved problems, in *Chemical Mobility and Reactivity in Soil Systems,* Nielson, D. W., Elrick, D. E., and Tanji, K. K., Eds., Am. Soc. Agron., Madison, Wis., 1983, 49.
197. **Jury, W. A., Sposito, G., and White, R. E.,** A transfer function model of solute transport through soil. I. Fundamental concepts, *Water Resour. Res.,* 22, 243, 1986.
198. **Sposito, G., White, R. E., Darrah, P. R., and Jury, W. A.,** A transfer function model of solute transport through soil. III. The convection-dispersion equation, *Water Resour. Res.,* 22, 255, 1986.
199. **van Genuchten, M. Th. and Alves, W. J.,** Analytical Solutions of the One-Dimensional Convective-Dispersion Solute Transport Equation, USDA/ARS Tech. Bull. No. 1661, Riverside, Calif., 1982.
200. **van Genuchten, M. Th.,** Determining Transport Parameters from Solute Displacement Experiments, USDA Res. Rep. No. 118, Riverside, Calif., 1980.
201. **van Genuchten, M. Th.,** Non-Equilibrium Transport Parameters from Miscible Displacement Experiments, USDA Res. Rep. No. 119, Riverside, Calif., 1981.
202. **Lindstrom, F. T., Hague, R., Freed, V. H., and Boersma, L.,** Theory on the movement of some herbicides in soils. Linear diffusion and convection of chemicals in soils, *Environ. Sci. Technol.,* 1, 561, 1967.
203. **Marino, M. A.,** Distribution of contaminants in porous media flow, *Water Resour. Res.,* 10, 1013, 1974.
204. **Lapidus, L. and Amundson, N. R.,** Mathematics of adsorption in beds. IV. The effect of longitudinal dispersion in ion exchange and chromatographic columns, *J. Phys. Chem.,* 56, 984. 1952.
205. **Hunt, B.,** Dispersive sources in uniform ground-water flow, *Am. Soc. Civil Eng. J. Hydrol. Div.,* HY4, 104, 75, 1978.
206. **Wilson, J. L. and Miller, P. J.,** Two-dimensional plume in uniform ground-water flow, *Am. Soc. Civil Eng. J. Hydrol. Div.,* HY4, 104, 503, 1978.
207. **Yeh, G. T. and Tsai, Y. J.,** Analytical three-dimensional transient modeling of effluent discharges, *Water Resour. Res.,* 12, 533, 1976.
208. **Kuo, E. Y. T.,** Analytical model for 3-D diffusion model, *J. Environ. Eng. Div. Am. Soc. Civil Eng.,* EE4, 102, 805, 1976.
209. **van Genuchten, M. Th. and Wierenga, P. J.,** Mass transfer studies in sorbing porous media. I. Analytical solutions, *Soil Sci. Soc. Am. J.,* 40, 473, 1976.
210. **van Genuchten, M. Th., Tang, D. H., and Guehnelon, R.,** Some exact solutions for solute transport through soils containing large cylindrical macropores, *Water. Resour. Res.,* 20, 335, 1984.
211. **van Genuchten, M. Th.,** A general approach for modeling solute transport in structured soils, in *Proc. 17th Int. Congr. Int. Assoc. Hydrogeol. Hydrol. Rocks Low Permeability,* Tucson, Ariz., 1985.
212. **Warrick, A. W., Biggar, J. W., and Nielson, D. R.,** Simultaneous solute and water transfer in an unsaturated soil, *Water Resour. Res.,* 7, 1216, 1971.
213. **Mercer, J. W. and Faust, C. R.,** *Groundwater Modeling,* National Water Well Association, Dublin, Ohio, 1981, 60.
214. **Wang, H. F. and Anderson, M. A.,** *Introduction to Groundwater Modeling, Finite Difference and Finite Element Methods,* W. H. Freeman & Co., San Francisco, 1982, 237.
215. **Huyakorn, P. S. and Pinder, G. F.,** *Computational Methods in Subsurface Flow,* Academic Press, New York, 1983, 473.
216. **Remson, I., Hornberger, G. M., and Molz, F. J.,** *Numerical Methods in Subsurface Hydrology,* Wiley-Interscience, New York, 1971, 389.
217. **Vilker, V. L. and Burge, W. D.,** Adsorption mass transfer model for virus transport in soils, *Water Res.,* 14, 783, 1980.
218. **Vilker, V. L.,** Virus Transport through Percolating Beds, Technical Completion Report, Calif. Office Water Res. Technol. Proj. No. B-184-CAL and Calif. Water Resour. Center Proj. UCAL-WRC-W-523, NTIS PB81-21240-9, Los Angeles, Calif., 1981.
219. **Grondin, G. H. and Gerba, C. P.,** Virus dispersion in a coarse porous medium, *Hydrol. Water Resour. Ariz. Southwest,* 16, 1986.

220. **Rifai, M. N. E., Kaufman, W. J., and Todd, D. K.,** Dispersion phenomena in laminar flow-through porous media, *Univ. Calif. I.E.R. Ser.*, 90, 3, 1956.
221. **Ogata, A. and Banks, R. B.,** A Solution of the Differential Equation of Longitudinal Dispersion in Porous Media, Prof. Paper 411-A, U.S.G.S., Washington, D.C., 1961.
222. **Enfield, C. G. and Bengtsson, G.,** Size exclusion chromatography in soils, *Ground Water*, submitted.
223. **White, R. E., Dyson, J. S., Haigh, R. A., Jury, W. A., and Sposito, G.,** A transfer function model of solute transport through soil. II. Illustrative examples, *Water Resour. Res.*, 22, 248, 1986.