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Comparative Adsorption of Human Enteroviruses, Simian Rotavirus, and Selected Bacteriophages to Soils

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Virus adsorption to soils is considered to be the most important factor in removing viruses after land treatment of wastewater. Most of the studies on virus adsorption to soils have utilized poliovirus as the model system. In the present study, comparative adsorption of a number of different types and strains of human enteroviruses and bacteriophages to nine different soil types was studied. Under the experimental conditions of this study, greater than 90% of all viruses adsorbed to a sandy loam soil except echovirus types 1, 12, and 29 and a simian rotavirus (SA-11), which adsorbed to a considerably lower degree. A great deal of variability was observed between adsorption of different strains of echovirus type 1, indicating that viral adsorption to soils is highly strain dependent. Of the five phages studied, f2 and ϕX174 adsorbed the least. In addition to being dependent on type and strain of virus, adsorption was found to be influenced also by type of soil. Thus, soils having a saturated pH of less than 5 were generally good adsorbers. From these results, it appears that no one enterovirus or coliphage can be used as the sole model for determining the adsorptive behavior of viruses to soils and that no single soil can be used as the model for determining viral adsorptive capacity of all soil types.

Land application of sewage sludge and effluent for recycling of resources as well as abatement of pollution is being practiced in about 1,000 communities in the United States today (17). This kind of use is expected to increase after the passage of Public Laws 92-500 and 95-217. Since secondary treatment of sewage does not remove all viruses present in domestic sewage (12, 15), it is necessary to determine the fate of viruses after the application of wastewater on land. Factors affecting virus removal during land treatment of wastewater, such as adsorption to soils, have not been studied extensively, and little is known about the fate of human viruses applied to soil. If viruses are not retained by the soil, they may migrate vertically, resulting in groundwater contamination. Enteroviruses have, in fact, been found in groundwater after land treatment of wastewater (26-29).

Virus removal by soil is believed largely due to adsorption, in contrast to bacteria which are removed by a combination of filtration, sedimentation, and adsorption (4, 11). Few studies which have been done on viral adsorption to soil have used poliovirus and/or certain bacteriophages as the indicator viruses for predicting the adsorptive behavior of all enteric viruses (2, 4, 5, 11, 16, 18, 30). It has been found, however, that coliphages do not necessarily behave in the same manner as poliovirus in their adsorption to soils (30). The present study was undertaken to compare the adsorption of a number of different types and strains of human enteroviruses, a simian rotavirus, and bacteriophages to different types of soils.

MATERIALS AND METHODS

Soils. Nine soils from different sources were used in this study. The major physicochemical properties and sources of these soils are shown in Table 1. Additional information on other soil properties may be found in Enfield et al. (6).

Viruses. The following enteroviruses were obtained from the Research Resources Branch, National Institutes of Health, Bethesda, Md.: echovirus types 1 to 8, 11 to 13, 22, 24 to 28, 29, and 31; poliovirus types 1 to 3; and coxsackievirus types B1 to B6. Several enteroviruses isolated from groundwater beneath a wastewater land disposal site were also studied. These included: five strains of echovirus type 1; two strains of coxsackievirus type B4; three strains of poliovirus type 2; and four strains of poliovirus type 3. In addition, one strain each of echovirus type 1 and poliovirus type 2 which had been isolated from estuarine water were used in adsorption studies. These viruses were concentrated from groundwater and seawater by membrane adsorption-elution methodology (8, 9) and were identified by the use of combination antiserum pools as described by Melnick et al. (19). A simian rotavirus (SA-11) was also used in this study and was obtained from H. H. Malherbe.

Bacteriophages. Five different coliphages were

TABLE 1. *Major characteristics of soils*[a]

Sample code	Family	Surface area (m^2/g)	% Clay	% Silt	% Sand	Cation-exchange capacity (meq/100 g)	% Organic matter	Saturated pH
B	Fine, mixed, thermic	84	39	13	48	32	0.30	4.5
C	Fine, montmorillonite, thermic	203	54	20	26	71	4.20	7.1
D	Fine, mixed, thermic	155	53	16	13	53	1.40	4.9
F	Fine, mixed, thermic	52	28	13	59	23	1.40	8.0
H	Not classified	105	36	24	40	30	0.78	8.0
K	Sandy, siliceous, hyperthermic		3	8	89	6.5	3.64	7.1
T	Coarse-loamy, mixed (calcareous), thermic	38	13	10	77	4.2	0.27	8.2
X	Sandy, mixed, frigid	18	4	4	92	5.6	0.40	5.5
FM	Sandy loam		3	8	89		0.88	7.8

[a] Data taken from Enfield et al. (6).

used. MS-2 (host, *Escherichia coli* B ATCC 15597) and ϕX174 (host, *E. coli* B ATCC 13706) were obtained from the American Type Culture Collection. T2 and T4 (host, *E. coli*) were obtained from G. Schaiberger (University of Miami, Miami, Fla.), and phage f2 (host, HI, [Hfr *E. coli* Hayes]) was obtained from Barbara Moore (University of Texas, San Antonio, Tex.).

Virus assay. Enteroviruses were grown and assayed in the BGM cell line by the plaque assay technique (20). SA-11 was grown and assayed in the MA-104 cell line (E. M. Smith, M. K. Estes, D. Y. Graham, and C. P. Gerba, J. Gen. Virol., in press). Bacteriophage concentrations were determined by the plaque-forming unit method. Culture and assay procedures were similar to those described by Adams (1) and Rovozzo and Burke (24).

Adsorption studies. The virus being studied was suspended in either deionized water, effluent from an activated sludge sewage treatment plant, solutions of calcium chloride in deionized water, or soil extract in deionized water. The soil extract was prepared by addition of 50 ml of deionized water to 50 g of dry soil. The mixture was shaken for 30 min, the soil was removed by centrifugation, and the supernatant was filtered through an HA membrane filter (Millipore Corp.) of 0.45-μm pore size. All batch experiments were performed with a 1:1 ratio of soil to liquid (wt/vol). Similarly, for studying the effect of soil concentration on virus adsorption, the soil-to-water ratio was always kept constant, i.e., 1 g of soil to each 1 ml of deionized water.

Adsorption of virus to soil was determined by addition of 2 g of test soil to a 2-ml suspension of virus containing 10^6 to 10^7 plaque-forming units in one of the solutions in a test tube. The test tube was stoppered, hand shaken, and then placed on a rotary shaker at 200 rpm for 30 min. The soil was then removed from suspension by centrifugation for 5 min at 2,500 $\times$ *g*, and the supernatant was assayed. A control suspension of virus without soil was treated in the same manner. The difference in titer between the control and the sample containing soil was used to determine the quantity of virus adsorbed to soil. The 30-min contact time was selected after time-rate tests indicated that, for all practical purposes, equilibrium was attained during that period. All experiments were performed with deionized water, unless otherwise indicated. The results reported are the average of two to five experiments.

Statistical analysis. Product-moment correlation coefficients were calculated for soil characteristics and the percent adsorption of different viruses (21). Significant statistics were calculated for each of the coefficients.

RESULTS

Nine different soil types were studied, and their major characteristics are given in Table 1.

The effects of virus concentration, soil concentration, and time on virus adsorption were initially evaluated with Flushing Meadows (FM) soil and polio 1. The results indicated that only 1 g of FM soil was necessary for adsorption of more than 99% of 10^6 plaque-forming units of this virus. From these results, it was decided to use 2-g amounts of soil in the remaining experiments.

With FM soil and deionized water, the rates of adsorption of echo 1 and polio 1 were determined. Most of the polio 1 was found to adsorb within 1 min after contact with the soil, whereas adsorption of the echo 1 leveled off after a 4-min contact period. Although more than 99% of the polio 1 adsorbed to the soil, only about 50% of the echo 1 adsorbed to the FM soil during the 1-h contact period. No significant inactivation of the viruses in control samples without soil occurred during this period of time. From these results, it was decided to use a 30-min contact period for adsorption studies to ensure that the greatest amount of viral adsorption had occurred.

The adsorption of varying quantities of polio 1 to FM soil was compared. The results indicated that the soil was not saturated with virus, even after addition of 4.5×10^{10} plaque-forming units of virus and that the percent adsorption was

similar for all the concentrations of virus studied.

The adsorption of 27 different reference enteroviruses and a simian rotavirus (SA-11) to FM soil is shown in Table 2. The enteroviruses used were supplied by the National Institutes of Health as standard reference material for each viral serotype. Most of the viruses adsorbed very well to the FM soil, with more than 90% of the added virus adsorbing to the soil. Exceptions were echo 1, 12, and 29 and SA-11, of which only 55.0, 78.0, 14.0, and 51.6% adsorbed to the soil, respectively.

Various strains of naturally occurring enteroviruses isolated from groundwater and polluted seawater were also compared for their ability to adsorb to FM soil; the results are shown in Table 3. No significant difference was observed between poliovirus isolates in their adsorption to FM soil, but a great deal of variability was observed among the echo 1 and coxsackie B4 isolates. The adsorption of echo 1 isolates varied from 99 to 0%. These results indicated that adsorption of virus to a given soil is very strain dependent.

In Table 4 is shown the adsorption of different enterovirus types and of strains of echo 1 and coxsackie B4 to FM soil in deionized water, treated sewage, soil extract, and solutions of $CaCl_2$. In general, strains of echo 1 adsorbed less in the presence of sewage and soil extract than in deionized water. Little difference in adsorption was seen among the other viral groups in these solutions. Adsorption of all of the viruses appeared to be enhanced in the presence of 0.01 M $CaCl_2$ but not in the presence of 0.001 M $CaCl_2$.

The adsorption of these viruses to nine differ-

TABLE 2. *Adsorption of different enterovirus types and rotavirus to FM soil*[a]

Virus type (strain)	% Adsorption	Virus type (strain)	% Adsorption
Echovirus		Poliovirus	
1 (Farouk)	55.0	1 (LSc)	99.9
2 (Cornelius)	99.4	2 (P-712)	98.0
3 (Morrisey)	98.8	3 (Leon)	99.6
4 (Pesascek)	96.0		
5 (Noyce)	99.8	Coxsackievirus	
6 (D'Amori)	99.99	B1 (Conn.-S)	99.99
7 (Wallace)	99.9	B2 (Ohio-1)	99.2
8 (Bryson)	96.0	B3 (Nancy)	96.0
11 (Gregory)	99.9	B4 (JVB)	99.99
12 (Travis)	78.0	B5 (Faulkner)	99.9
13 (Del Carmen)	91.0	B6 (Schmitt)	98.0
22 (Harris)	99.99		
24 (DeCamp)	94.0	Rotavirus SA-11	51.6
25 (JV-4)	95.0		
26 (Coronel)	99.99		
27 (Bacon)	99.99		
29 (JV-10)	14.0		
31 (Caldwell)	91.0		

[a] The virus suspension was made in deionized water, and 2 ml of it was mixed with 2 g of sandy loam soil. The mixture was shaken at 200 rpm for 30 min. The supernatant was assayed after light centrifugation. Controls were treated in a similar manner except that deionized water was substituted for the soil. The difference between control plaque-forming units and supernatant plaque-forming units was taken to be the amount of virus adsorbed to soil.

TABLE 3. *Adsorption of different enterovirus strains to FM soil*

Virus type	Strain no.[a]	% Adsorption[b]
Polio 2	R109	99.8
	R111	99.5
	R113	98.0
	9CH-1[c]	99.6
Polio 3	R201	99.9
	R202	99.9
	R203	99.9
	R204	99.9
Echo 1	4CH-1[c]	96.6
	R115	99.7
	V212	46.0
	V239	0
	V248	30.0
	V249	35.0
Coxsackie B4	V216	30.0
	V240	0

[a] Except as otherwise indicated, the viruses were isolated from groundwater beneath a wastewater land treatment site.

[b] A 2-g amount of soil was mixed with 2 ml of virus suspension made in deionized water.

[c] Viruses isolated from polluted estuarine water.

TABLE 4. *Effect of suspending medium on virus adsorption to FM soil*[a]

Virus	% Virus adsorption with:				
	Deionized water	Treated sewage	Soil extract	0.001 M $CaCl_2$	0.01 M $CaCl_2$
Reference strains					
Polio 1 (LSc)	99.9	99.3	96	96	99.0
Echo 1	55	0	38	4	82
Echo 7	99.9	94	97	96	97
Echo 12	78	84	97	14	99.0
Echo 29	14	12	54	23	99.2
Coxsackie B3	99.4	93	87	72	99.0
Recent isolates:					
Echo 1 (V212)	46	0	0	0	78
Echo 1 (V239)	0	14	0	7	80
Echo 1 (V248)	30	12	0	7	75
Echo 1 (V249)	35	6	0	34	70
Coxsackie B4 (V216)	30	27	6	15	85
Coxsackie B4 (V240)	0	17	37	0	80

[a] Virus suspensions were made in the indicated fluid, and 2 ml of the virus suspension was mixed with 2 g of soil for adsorption purposes.

ent soils was compared (Table 5). Again, a great deal of variability was found among the types and strains of virus in their adsorption to different soils. The exception was soil B, to which all of the viruses adsorbed very well. In general, all viruses adsorbed poorly to soil K. Poliovirus generally adsorbed better than most of the other viruses to all of the soils studied. Low soil pH appeared to be the only obvious factor influencing virus adsorption to the soils studied. All of the viruses exhibited the greatest amount of adsorption to soils B and D, both of which had a saturated pH below 5.0.

Adsorption of five bacteriophages to the nine different soils is also shown in Table 5. As with enteroviruses, soil B was the best virus adsorbent. It adsorbed more than 99% of all bacteriophages tested. Soil D adsorbed more viruses than all the other soil types studied except soil B. Soil K, as usual, was a very poor adsorbent except for phage MS-2. Of the five phages, f2 was the poorest adsorber, followed by ϕX174. The other three phages, i.e., MS-2, T2, and T4, were comparatively better adsorbers.

Factors determining virus adsorption. Product-moment correlation coefficients between all the soil parameters and percent adsorption of all viruses were computed. Partial results are shown in Table 6. Several soil characteristics apparently affected the adsorption of one or more viruses, but soil pH was found to be the most significant. A negative correlation was observed ($P < 0.05$) between soil pH and as many as five different viruses (echo 1, V212, V248, V216, and ϕX174). Four other viruses (V239, V240, MS-2, and f2) had a relatively higher correlation with soil pH, although not significant at <0.05. Other soil parameters which

TABLE 5. *Adsorption of animal viruses and selected bacteriophages to different soil types*[a]

Soil type	% Virus adsorption														
	Rotavirus SA-11	Reference enterovirus strain				Recent enterovirus isolate					Bacteriophage				
		Polio 1 (LSc)	Echo 1	Echo 7	Coxsackie B3	Echo 1			Coxsackie B4		MS-2	T2	T4	ϕX174	f2
						V212	V239	V248	V216	V240					
B	99.99	99.9	99.7	99.0	99.6	99.7	98	99.8	99.3	98.7	99.9	99.99	99.7	99.98	99
C	97.98	95	21	98	92	23	10	25	2	0	17	90	98	0.5	26
D	99.2	94	90	68	83	70	65	99.9	67	92	47	99.6	99.7	97	6
F	95.6	99.0	11	99.0	97	19	53	33	32	26	39	86	99.7	0	16
H	93.6	99.6	12	99.5	97	38	49	25	34	24	56	95	99.2	12	0
K	19.4	42	28	20	56	44	5	0	12	56	69	8	0	18	0
T	52.4	82	13	22	35	1	52	33	34	35	30	70	94	0	0.3
X	76.2	56	78	8	73	79	68	82	44	73	34	64	86	83	0
FM	51.6	99.9	60	99.9	97	32	22	16	19	0	17	36	72	0	0

[a] A 2-g amount of the indicated soil was mixed with 2 ml of the appropriate virus suspension in deionized water.

TABLE 6. *Product-moment correlation coefficients between soil characteristics and percent virus adsorption*

Virus type	% Clay	% Sand	% Organic matter	Saturated pH	Resin-extractable phosphorus (μg/g)	Total aluminum (%)	Exchangeable aluminum (μg/g)
Polio 1	0.6129	−0.5990	−0.2476	0.0863	−0.1217	0.7779[a]	0.3028
Echo 1	0.3256	−0.3366	−0.1829	−0.7418[a]	−0.4058	−0.0618	0.7430
Echo 7	0.5830	−0.5543	0.0898	0.0944	−0.4470	0.6372	0.3176
Coxsackie B3	0.4725	−0.4403	0.0268	−0.1944	−0.8517[a]	0.2465	0.3355
Echo 1 (V212)	0.1493	−0.1316	−0.2932	−0.9238[a]	−0.6644	−0.4028	0.6650
Echo 1 (V239)	0.2311	−0.2034	−0.8702[a]	−0.6104	−0.1737	−0.3501	0.7111
Echo 1 (V248)	0.3899	−0.3736	−0.5652	−0.8809[a]	−0.4569	−0.5028	0.5786
Coxsackie B4 (V216)	0.2974	−0.2826	−0.6901	−0.7579[a]	−0.2619	−0.2023	0.8124[a]
Coxsackie B4 (V240)	−0.2404	0.2037	−0.4502	−0.6401	−0.3163	−0.4390	0.6076
MS-2	0.1304	−0.1103	−0.2486	−0.5223	−0.3007	0.0857	0.8952[a]
T2	0.8247[a]	−0.7929[a]	−0.4023	−0.3169	−0.4704	0.6194	0.4556
T4	0.5968	−0.5614	−0.5083	−0.1375	−0.2017	0.7375	0.2893
ϕX174	0.2463	−0.2464	−0.4152	−0.9660[a]	−0.5164	−0.4659	0.5651
f2	0.4143	−0.2998	−0.1566	−0.5660	−0.3524	−0.0853	0.9571[a]

[a] Significant at $P < 0.05$.

were tested but not found to be significant were surface area, percent silt, cation exchange capacity, conductivity, total phosphorus, total and exchangeable iron, total and exchangeable calcium, and total and exchangeable magnesium.

DISCUSSION

An understanding of the mechanisms of virus removal by soil systems is critical in evaluation and management of land treatment systems of wastewater disposal. Land filtration of secondarily treated domestic sewage is considered to be an effective means for removal of most of the nitrogen, phosphorous, organic matter, and pathogenic microorganisms present in sewage (10). However, information on virus-removing capacity of soils is meager and is almost entirely based on studies with poliovirus type 1 and bacteriophages (11).

Results with these viruses generally indicate excellent removals of virus in soil columns and adsorption in batch studies. Only the nature of the soil was recognized as a major factor influencing the adsorption of the virus, and it was concluded that viruses were largely removed within a few centimeters of the soil surface during infiltration of virus-laden wastewater (4, 11, 18). With the development of methods for the concentration of enteroviruses from large volumes of water, it became possible to look for naturally occurring viruses in groundwater beneath sites used for the land treatment of sewage (13). Results of field studies indicate that virus-contaminated water may travel anywhere from a few inches to a few hundred feet through the soil to enter groundwater (22, 23, 26, 29). Both field and laboratory studies have indicated that environmental factors such as rainfall could result in the elution of viruses from soil and further subsurface migration (5, 16, 29). These results illustrated the need for additional work on understanding factors influencing the subsurface travel of enteric viruses in groundwater.

The purpose of this study was to determine whether poliovirus adsorption to soil truly reflected the behavior of other members of the enterovirus group, including recently isolated strains. It was also intended to study the adsorption of bacteriophages to soil as compared to animal viruses and to compare different soils as to their overall adsorptive behavior for these viruses. It quickly became evident that although poliovirus to a large extent reflected the behavior of most reference laboratory strains of enteroviruses in adsorption to a given soil (Table 2), it was not reflective of many strains recently isolated from sewage-polluted waters. In the initial screening of enteroviruses, the adsorption of laboratory strains to FM soil was evaluated (Table 2). Of the 27 different enterovirus types, only echo 1, 12, and 29, as well as the simian rotavirus, adsorbed significantly less than the other reference enteroviruses. In addition, the rate of echo 1 adsorption was found to be less than that of polio 1. No difference in adsorption was observed between the laboratory and natural isolates of poliovirus (Table 3), but a great deal of variability was observed between the natural isolates of echo 1 and coxsackie B4. Adsorption of echo 1 strains to FM soil ranged from 99 to 0%. Polioviruses are the enteroviruses most commonly isolated from sewage in the United States because of the widespread use of oral poliovirus vaccine. Thus, all of the poliovirus isolates are probably vaccine progeny strains and not true wild-virus variants.

From this work it is apparent that virus adsorption to soil is highly dependent on the strain of virus. Differences in adsorption between different strains of the same virus type might result from variability in the configuration of proteins in the outer capsid of the virus, since this will influence the net charge on the virus (11). The net charge on the virus would affect the electrostatic potential between virus and soil, and thereby could influence the degree of interaction between the two particles.

Soluble organics have been shown to compete with viruses for adsorption sites on mineral surfaces (11), and it is not surprising that the viruses studied, in general, adsorbed less to FM soil in the presence of secondarily treated sewage (Table 4). Adsorption of all the viruses was enhanced in the presence of 0.01 M $CaCl_2$, again indicating that the surface charge of the virus is involved in the adsorption of viruses to soil (11).

A great deal of variability was found to occur among different viruses in their adsorption to soils (Table 5). Overall, polio 1 and coliphage T4 adsorbed best of all the viruses tested to the nine different soils studied. Echo 1 and coliphage f2 were the poorest adsorbers. Schaub et al. (25) have also shown f2 to be a very poor adsorber. Although f2 is very close to poliovirus in its morphological properties and has been advocated as an indicator to evaluate the virological quality of water (3), it appears from this study that it is a very poor model to study the adsorptive behavior of enteroviruses to soils.

It is now clear that not all viruses, even strains of the same type, will behave the same under identical conditions in soil systems. Preliminary studies on the removal of different enteroviruses during migration through soil columns indicate that viruses which adsorb poorly in batch studies may also have a lower removal rate during soil

filtration. Thus, batch studies may reflect to some extent the degree of virus migration into the subsurface environment during the land treatment of wastewater, but additional studies are necessary.

In addition to being influenced by virus type, viral adsorption was found to depend also on the soil type (Table 5). Thus, soil B adsorbed 98% or more of all the animal viruses and bacteriophages studied. This soil and soil D, which was also a good virus adsorber, were characterized by having a saturated pH below 5.0. Soil X, which had a saturated pH of 5.5, was not an overall good adsorber of virus. Thus, a low soil pH (apparently below 5) appeared to favor virus adsorption of all the viruses studied. It has previously been reported that bacteriophage adsorption to soils correlates with cation-exchange capacity and soil surface area (2), but no clear overall linear correlation was evident between these factors and virus adsorption.

It may not be surprising that all of the naturally isolated viruses adsorbed well to soils of low pH, since the technique used to concentrate them from field samples involved adsorption to membrane filters at low pH (7, 9). Thus, viruses that did not have this characteristic would not have been isolated.

Results of product-moment correlation coefficients between soil characteristics and virus adsorption (data taken from Tables 1 and 5) are shown in Table 6. This table shows that soil pH is the single most important factor influencing viral adsorption to soil. Thus, with increasing pH, adsorption of many viruses to soil decreased, whereas a drop in pH resulted in an overall increase in viral adsorption. Although not all the viruses tested were related to soil pH at a high level of confidence, the general trend was apparent. Another explanation for this observation might be that soil pH and virus adsorption are linearly correlated until a certain pH, at which virus adsorption is significantly enhanced. A similar observation has been noted for poliovirus adsorption to activated carbon (12). Thus, lack of a linear correlation does not preclude the possibility of some other type of relationship between the virus and a soil factor. It is evident from Table 5 that soils having a saturated pH of <5 were very good adsorbers. In a study by Greaves and Wilson (14), the adsorption of calf thymus deoxyribonucleic acid and yeast ribonucleic acid to montmorillonite was found to be largely dependent on the pH of the system. Thus, a minimal adsorption was found to occur at pH's above 5, but it increased progressively with decreasing pH. The addition of electrolytes resulted in increased adsorption at all pH values tested (14).

Other factors influencing virus adsorption were percent clay, percent sand, percent organic matter, total phosphorus, resin-extractable phosphorus, total iron, total aluminum, exchangeable aluminum, and exchangeable magnesium. These soil characteristics influenced the adsorption of three viruses at the most, but no consistent pattern was observed for all of the viruses studied. However, exchangeable aluminum content of soil had a positive correlation with five of the viruses studied. From these results it appears that no one enterovirus or coliphage can be used as a model for determining viral adsorptive behavior to soils.

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