APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Oct. 1982, p. 852–859
0099-2240/82/100852-08$02.00/0

Vol. 44, No. 4

Adsorption of Reovirus by Minerals and Soils

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Received 30 December 1981/Accepted 17 June 1982

Adsorption of [^{35}S]methionine-labeled reovirus by 30 dry soils, minerals, and finely ground rocks suspended in synthetic freshwater at pH 7 was investigated to determine the conditions necessary for optimum virus removal during land application of wastewaters. All of the minerals and soils studied were excellent adsorbents of reovirus, with greater than 99% of the virus adsorbed after 1 h at 4°C. Thereafter, virus remaining in suspension was significantly inactivated, and within 24 h a three to five $\log_{10}$ reduction in titer occurred. The presence of divalent cations, i.e., Ca^{2+} and Mg^{2+}, in synthetic freshwater enhanced removal, whereas soluble organic matter decreased the amount of virus adsorbed in secondary effluent. The amount of virus adsorbed by these substrates was inversely correlated with the amount of organic matter, capacity to adsorb cationic polyelectrolyte, and electrophoretic mobility. Adsorption increased with increasing available surface area, as suspended infectivity was reduced further by the more finely divided substrates. However, the organic content of the soils reduced the level of infectious virus adsorbed below that expected from surface area measurements alone. The inverse correlation between virus adsorption and substrate capacity for cationic polyelectrolyte indicates that the adsorption of infectious reovirus particles is predominately a charged colloidal particle-charged surface interaction. Thus, adsorption of polyelectrolyte may be useful in predicting the fate of viruses during land application of sewage effluents and sludges.

Virus removal by soil systems is critical in the evaluation and management of land treatment sites for wastewater disposal. Both field and laboratory studies have indicated that environmental factors such as rainfall result in the elution of viruses from soil and their subsurface migration through the soil to enter groundwater, posing public health risks (8, 17, 28). Most studies on the adsorption of viruses to soil have used poliovirus or certain bacteriophages as an indicator virus for predicting the behavior of all enteric viruses. Recently, Goyal and Gerba (13), after studying nine soil types, indicated that no one enterovirus or coliphage can be used as a model for determining the adsorptive capacity of soil. However, little information is available about the adsorption of different viruses by a variety of mineral and soil types.

Viruses belonging to the family Reoviridae are common pathogens of enteric origin and are routinely isolated from wastewater sludge. Relatively few studies have focused on their survival in the soil environment. The purpose of this study was to determine the behavior of reovirus type 3, as a prototype of viruses in this family, and the conditions affecting its survival. It was also intended to examine the relation of virus removal to measurable properties of the mineral or soil substrate. The goal of our effort is to develop the ability to predict the adsorption of human pathogenic viruses in water recharge beds.

MATERIALS AND METHODS

Substrates. Ottawa sand (0.6 and 0.8 mm) meeting American Society for Testing and Materials C190 specifications was obtained from VWR Scientific, Rochester, N.Y. The quartz used in this study was a gift from Robert M. Buyce. Other soils and soil components were obtained from the Wards Natural Scientific Establishment, Rochester, N.Y. Characteristics of the 30 soils, minerals, and other substrates are given in Table 1. Additional information on other soil properties may be found in R. S. Moore et al. (20). When necessary, these substrates were ground with either a steel disc grinder or a tungsten carbide ringmill, and the portion which passed through a 0.15-mm sieve was used in the adsorption studies.

Substrate characterization. Surface areas of the substrates were determined by a BET-N_2 gas adsorption

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TABLE 1. Major characteristics of minerals and soils[a] and their adsorption of reovirus[b]

Substrate	Sample source (state)	Surface area (m^2/g)	Protein content (mg/g)	Capacity to adsorb PDADMA (mg/m^2)	pH in distilled water	% Virus adsorbed[c] determined by:	
						Infectivity	Radioactivity
Soils							
Muck	New York	1.18	34.00	14.00	7.2	97.80	99.0
Genesee silt loam	New York	4.18	9.50	1.10	7.6	98.90	97.5
Colonie sandy loam	New York	5.32	2.30	0.38	8.0	99.87	99.0
Genesee silt	New York	10.90	0.95	0.17	7.5	99.83	99.4
Loess	Kansas	25.10	0.29	0.10	7.7	99.89	98.9
Adobe	New Mexico	33.80	1.70	0.10	7.9	99.91	99.4
Sands							
Beach	New York	0.73	1.10	1.90	8.5	98.77	99.2
Dune	Michigan	1.21	0.06	0.58	8.7	98.79	99.3
Dune	New York	4.09	2.30	0.16	8.4	99.35	98.3
Magnetite	California	5.74	0.06	0.17	8.9	99.98	99.7
Sedimentary rocks							
Kame conglomerate	New York	3.06	0.00	0.07	8.8	99.52	99.5
Arenaceous shale	New York	3.52	0.07	0.36	8.8	99.95	99.6
Bituminous shale	New York	3.78	0.10	0.29	8.9	99.988	99.6
Fossiliferous-limy shale	New York	4.53	0.09	0.22	8.5	99.98	99.7
Brown sandstone	New York	5.56	0.54	0.18	7.6	99.99	99.1
Calcareous shale	New York	9.39	0.25	0.27	8.2	99.96	99.4
Argillaceous shale	New York	28.90	1.65	0.17	8.5	99.955	99.4
Illite-bearing shale	New York	55.00	1.70	0.19	7.1	99.965	99.7
Minerals							
Quartz	New York	0.54	0.15	0.48	8.0	99.41	98.0
Dolomite	New York	0.85	0.09	0.26	8.9	99.85	99.4
Quartz conglomerate	New York	3.19	0.24	0.36	8.9	99.27	98.8
Calcite	Illinois	4.08	0.10	0.45	8.5	99.71	99.7
Clay minerals[d]							
Dickite (API no. 16)	Utah	2.78	0.53	0.60	8.0	99.986	99.8
Kaolinite (API no. 4)	Georgia	12.00	0.25	0.23	8.0	99.96	99.7
Kaolinite (API no. 5)	South Carolina	16.40	0.30	0.14	5.0	99.79	99.8
Montmorillonite (API no. 26)	Wyoming	31.73	0.88	2.10	9.5	99.76	96.7
Montmorillonite (API no. 31)	Arizona	41.50	0.15	0.80	8.8	99.14	99.1
Halloysite (API no. 13)	Utah	42.00	1.35	0.08	6.0	99.998	99.8
Glauconite	New Jersey	53.50	0.45	0.16	6.6	99.56	99.2
Attapulgite (API no. 64)	Georgia	118.00	0.53	0.13	8.1	99.994	99.8

[a] Reference 21.

[b] A 500-mg amount of substrate was mixed with 2 ml of virus suspension containing 10^8 to 10^9 PFU (10^3 to 10^4 cpm) in synthetic freshwater for 1 h at 4°C.

[c] Based on analysis of the diluted stock suspension on the day of the experiment. Values are means from two to six determinations with each substrate.

[d] American Petroleum Institute reference clay minerals numbers are given in parentheses.

procedure with a Monosorb Surface Area Analyzer (Quantachome Corp., Greenville, N.Y.). Samples were outgassed at 200°C for 30 min. Calibration was checked between samples. The pH of the substrate suspensions was determined in a manner similar to the method described by Peech (22). After a slurry of the substrate in distilled water (1:1, wt/vol) was produced, the pH was measured with a Radiometer PHM 4 pH meter and a combination electrode (no. 1207; Markson Science, Inc., Del Mar, Calif.). Soil organic content was determined as follows. A 500-mg sample of each substrate was extracted twice with 2 ml of 0.5 M NaOH for 15 min in a boiling water bath, and the supernatant was analyzed for apparent protein content by the method of Lowry et al. (19). Organic content was also estimated by determining the carbon remaining in each sample after acid treatment with 2 M H_3PO_4 with a Perkin-Elmer model 240 elemental analyzer operating at 900°C (E. Canelli and M. M. Reddy, manuscript in preparation). Naturally occurring organic films and coatings were oxidatively destroyed by low-temperature ashing with an LFE LTA-302 low-temperature asher operated at 75°C and 133.3 Pa (1 mm Hg) for 4 h. Nitric acid-extractable metals were determined by the method of Krishnamurty et al. (16) and analyzed by atomic adsorption spectroscopy. The

capacity of the substrates to adsorb the cationic polyelectrolyte polydiallydimethylammonium chloride (PDADMA: Magnifloc 585 C, American Cyanamid Co., Pearl River, N.Y.) is a measure of the negative charge at the substrate surface and was determined by mixing 20 ml of a 300-mg/liter PDADMA solution with 500 ml of substrate in 20 ml of distilled water for 1 h. After the mixture was centrifuged at 3,000 rpm for 10 min (or longer if necessary), 10-ml portions were removed and titrated against potassium polyvinyl sulfate (Eastman Kodak, Rochester, N.Y.) with 0.1% toluidine blue 0 used as indicator to determine PDADMA concentration (14). The concentrations of Na^+, K^+, Mg^{2+}, and Ca^{2+} in the suspending medium after interaction with the substrates in a simulated adsorption experiment were determined by atomic adsorption spectroscopy. Electrophoretic mobility was determined by suspending approximately 2 mg of soil or clay in 20 ml of 10 mM NaCl and dispersing the mixture ultrasonically. The pH was adjusted with dilute NaOH or HCl to the value determined in the virus adsorption experiments, and the mixture was stirred for 30 min. The bulk pH was redetermined; NaOH or HCl was added to regain the desired value if excessive pH changes had occurred. The suspension was then transferred to a van Gils electrophoresis cell mounted in a Rank Brothers Mark II microelectrophoresis apparatus (Bottisham, Cambridge, England), and the mobility was measured at 25°C (23).

Virus and cell line. Reovirus type 3, Dearing strain, was produced and assayed in monolayer cultures of L2 cells grown in Dulbecco modified Eagle medium supplemented with 10% fetal bovine serum.

Virus production and purification. The L2 cell monolayers, grown in roller bottles, were washed with Dulbecco phosphate-buffered saline without Ca^{2+} and Mg^{2+}. Virus was produced at 37°C in cells infected at a multiplicity of 10 to 20 PFU per cell. After 2 h of adsorption at 37°C in Dulbecco modified Eagle medium supplemented with 5% fetal bovine serum, 0.2 mCi of [^{35}S]methionine in the same medium was added to each roller bottle. The virus was harvested 48 h later. The cells were scraped and collected by centrifugation at 650 × *g* for 30 min at 4°C. The cell pellet was suspended in 10 ml of phosphate-buffered saline without Ca^{2+} and Mg^{2+}.

The virus was purified by a modification of the method described by Floyd et al. (10). Virus was extracted from the cells by adding one-half volume of Genetron 113 (Allied Chemical, New York, N.Y.), and the cells were homogenized at one-half maximum speed in a Sorvall Omni-Mixer for 2 min at 4°C. The phases were separated at 12,000 × *g* for 10 min at 4°C, the lower phase was reextracted with one-half volume of phosphate-buffered saline without Ca^{2+} and Mg^{2+}, and the phases were again separated. The aqueous phase was pooled with the first and reextracted with one-half volume of Genetron 113. This aqueous phase was then layered onto preformed cesium chloride gradients (ρ = 1.2 to 1.4 g/cm^3) and centrifuged at 110,000 × *g* for 18 h at 4°C. Virus bands were collected, and a second isopycnic sedimentation on preformed cesium chloride gradients was performed. The purified virus was dialyzed against Ca^{2+} and Mg^{2+}. Stocks were stored at −70°C.

Virus assays. Reovirus infectivity was analyzed by plaque titration with a modification of the procedure described by Fields and Joklik (9). Confluent L2 monolayers in 60-mm tissue culture dishes were washed with phosphate-buffered saline without Ca^{2+} and Mg^{2+}; 0.5 ml of virus suspension was added, and the virus was allowed to adsorb for 2 h at 37°C. The cells were overlaid with 10 ml of a mixture composed of equal parts of 1.9% Noble agar (Difco Laboratories, Detroit, Mich.) and Eagle minimal essential medium supplemented with 10% fetal bovine serum. The plates were incubated at 37°C in a CO_2 incubator for 6 days. A second agar overlay containing neutral red (0.03%) was then added, and the plates were incubated for an additional 24 h at 37°C. Plaques were then counted. Radioactivity was measured by mixing samples of virus suspension with 10 ml of Aquasol (New England Nuclear Corp., Boston, Mass.) and counting in a Beckman LS 250 liquid scintillation spectrometer.

Suspending media. The synthetic freshwater, designed to be representative of secondary treated municipal wastewater effluents of New York State in major ion content, included 1.25 mM $NaHCO_3$, 0.375 mM $MgSO_4$, 0.10 mM KCl, and 1.0 mM $CaCl_2$. It had an ionic strength of 0.006 and was adjusted to pH 7 for the adsorption studies. The secondary effluent obtained from a sewage treatment plant (Ravena, N.Y.) was clarifed by filtration through a 0.22-μm membrane filter and then stored at 4°C. This effluent had a higher ionic strength than the synthetic freshwater. Its composition was 1.7 mM Na^+, 0.6 mM Mg^{2+}, 0.15 mM K^+, 1.6 mM Ca^{2+}, 1.5 mM Cl^-, and 0.65 mM SO_4^{2-}, with a total organic carbon content of 12 mg/liter. Solutions of NaCl, $MgSO_4$, and $CaCl_2$ were made in deionized water.

Virus electrophoretic mobility. The mobility of reovirus in synthetic freshwater was measured by laser-illuminated whole-particle microelectrophoresis (26). A suspension containing 10^8 to 10^9 PFU was adjusted to pH values between 3.0 and 10.5 with dilute NaOH or HCl, and the pH of the suspension was recorded after it was stirred for 10 min. The suspension was transferred to a cylindrical van Gils cell mounted in a Rank Mark II microelectrophoresis apparatus thermostatically maintained at 25°C. The particles were illuminated in the dark-field mode with a 3 mW HeNe laser (Scientific and Cook Electronics Ltd., London, England) focused at either stationary layer. A known electric potential gradient was applied through the cell, causing charged particles to migrate. The mobilities and standard deviations were calculated from the time required for 30 particles to travel 30 μm. The field polarity was reversed as needed so that electrode polarization was minimized, and equal numbers of particles were measured in each direction.

Adsorption studies. The batch-type method which we used to study virus uptake has been described previously (20). Briefly, 10^8 to 10^9 PFU of virus, corresponding to 10^3 to 10^4 cpm, was added to a slurry containing 500 mg of substrate in 2.0 ml of suspending medium within a 50-ml Oak Ridge-type polypropylene centrifuge tube. The mixture was shaken at 300 rpm on a Gyrotory shaker (New Brunswick Scientific) at 4°C for 1 h. Substrate and suspending medium were separated by centrifugation at 27,000 × *g* for 10 min at 4°C. Samples were removed for measurement of virus infectivity and radioactivity. Previous work in other laboratories (1) revealed that reovirus could be removed from suspension by the experimental container

itself. Similary, in our laboratory, we found progressive removal of the virus by the container, but the amount varied according to the type of container used. The Nalgene Oak Ridge-type 50-ml polypropylene container (no. 3119-0050) exhibited the least adsorption and was used in these studies. Ca^{2+} (1.0 mM) and Mg^{2+} (0.375 mM) at the concentrations employed in the synthetic freshwater significantly increased adsorption to the experimental container. However, kinetic studies with magnetite sand indicated that in less than 1 h, only 50 mg of the sand was necessary for adsorption of more than 99% of 10^8 PFU of reovirus, and that the affinity of the substrate surface for adsorption was much greater than that of the container. Therefore, virus adsorbed was determined as the difference between the amount of virus added, based on the titer of the diluted stock suspension on the day of the experiment, and the amount of virus recovered from suspension.

Statistical analysis. Correlation coefficients were calculated for soil characteristics and percent virus adsorbed in the synthetic freshwater after 1-h adsorption at 4°C. Analysis was performed with the BMDP BMOP-77 statistical programs (Biomedical Computer Programs, University of California Press, Berkeley, Calif.) and a PDP 11/45 computer.

RESULTS

Characterization of the minerals and soils. For aid in the determination of the relationship between soil properties and virus adsorption, the soils and soil components represented by sand, mineral, sedimentary rock, and clay mineral substrates were extensively characterized. The major physicochemical properties and sources of the 30 soils and soil components are listed in Table 1. They are arranged by surface area within each substrate type. These substrates were characterized previously (20) and had surface areas from 0.54 to 118 m^2/g, apparent protein contents from 0 to 34 mg/g, pH values from 5 to 9.5, low to high negative surface charges as shown by interaction with cationic polyelectrolyte and electrophoresis, and particle sizes from very fine to relatively coarse.

Adsorption of reovirus by minerals and soils. The results from batch adsorption experiments with reovirus and the 30 soils and soil components are also shown in Table 1. Each substrate was tested two to six times. All of the substrates studied were excellent virus adsorbents. Virus adsorbed varied from 97.8 to 99.998% as measured by infectivity and 96.7 to 99.8% as measured by radioactivity. In agreement with the results of other investigators (4, 6), the adsorption of viruses by minerals and soils occurs rapidly. With most of the substrates, more than 99% of the input virus was adsorbed after 1 h at 4°C. Muck, the substrate with the highest content of organic matter, exhibited the least adsorption as measured by infectivity. Radioactivity measurements indicated that Wyoming montmorillonite was a relatively poor adsorbent of virus particles, with only 96.7% adsorbed. When grouped according to soil type, the substrates adsorbed virus in the following order: sedimentary rocks ≥ clay minerals ≥ minerals ≥ sands ≥ soils. In addition, when compared on an equivalent basis of specific surface area, the soils showed a smaller uptake of infectious virus than did the soil components. Since the soils have a higher organic content, this soil constituent may have reduced virus adsorption.

Influence of suspending medium on adsorption. Five substrates were chosen to determine the effect of the suspending medium composition on adsorption. Adsorption of radiolabeled reovirus in synthetic freshwater was compared with adsorption in secondary effluent, deionized water, and solutions of NaCl, $CaCl_2$, $MgSO_4$. The results of one experiment are shown in Table 2. The interaction was found to be affected only moderately by the type of suspending medium. No significant difference was noted for beach sand and Genesee silt. With montmorillonite, quartz, and magnetite sand, more virus was adsorbed in synthetic freshwater and secondary effluent than in deionized water. Adsorption from 1 mM $CaCl_2$ was substantially greater than from the same concentration of NaCl. Adsorption from 1 mM $MgSO_4$ was similar to that from $CaCl_2$. Thus, the greater adsorption of reovirus

TABLE 2. Effects of suspending medium on reovirus adsorption to selected substrates[a]

Substrate	% Virus adsorbed[b] in indicated medium					
	Deonized water	NaCl (1 mM)	$CaCl_2$ (1 mM)	$MgSO_4$ (1 mM)	Synthetic freshwater	Secondary effluent
Montmorillonite (Wyoming)	92.2	94.2	96.8	96.7	99.1	98.8
Quartz	94.8	90.5	97.8	96.8	98.0	98.9
Beach sand (Lake Ontario)	99.0	97.4	98.4	99.5	99.2	97.5
Magnetite sand	96.0	95.9	99.9	99.5	99.7	99.6
Genesee silt	98.9	97.9	98.3	97.4	99.4	97.9

[a] Virus containing 10^3 to 10^4 cpm was added to 500 mg of substrate suspended in 2 ml of each medium and adsorbed at 4°C for 1 h. Values are based on a single experiment except those for synthetic freshwater, which are mean values from two to six experiments.

[b] Adsorption was determined by measuring radioactivity.

in synthetic freshwater is probably a consequence of the higher ionic strength of the medium and high concentration of divalent cations. The lower virus uptake from secondary effluent, which has a higher ionic strength than synthetic freshwater, may have been due to the presence of soluble organic matter.

Electrophoretic mobility of reovirus in synthetic freshwater. The electrophoretic mobility of reovirus in synthetic freshwater was determined between pHs 4.3 and 10.2 (Fig. 1). The virus was negatively charged over the entire range, and the apparent charge increased slowly with increasing addition of NaOH. In dilute NaCl alone, reovirus exhibited a pI of 3.8 (26) which was similar to the value obtained from isoelectric focusing (11; data not shown).

Inactivation of reovirus by minerals and soils. Initial investigation of the kinetics of reovirus adsorption by Ottawa sand revealed that the specific infectivity (ratio of PFU to counts per minute) of suspended virus recovered after 24 h was considerably lower than the value for the input virus (R. S. Moore, D. H. Taylor, M. Chen, and L. S. Sturman, Abstr. Annu. Meet. Am. Soc. Microbiol. 1980, Q98, p. 210). As the sedimentation behavior of the recovered virus was indistinguishable from that of the input virus, the loss of infectivity was not attributable to the formation of the virus aggregates or to disruption of virions.

Adsorption experiments with other substrates showed a similar decrease in the specific infectivity of reovirus. The data from five experiments with 28 substrates and four preparations of virus are summarized in Table 3. The initial specific infectivity ranged from 7×10^3 to 22×10^3 PFU/cpm. After 1 h, wide variability was evident in the specific infectivity of the virus recovered. After a 24-h incubation with most of the substrates, virus infectivity declined 3 to 5 $\log_{10}$, whereas there was little decrease in recoverable radiolabeled virions beyond 1 h. This observation illustrates the drawback in measuring suspended infectivity alone when virus uptake is being studied. From a practical standpoint, however, it is important to continue to measure the infectivity of recoverable virus.

TABLE 3. Alteration of the specific infectivity of reovirus recovered from suspension[a]

Substrate	Specific infectivity (PFU/cpm)[b] after incubation for:	
	1 h	24 h
Muck	8,000	10,000
Genesee silt loam	29,000	570
Colonie sandy loam	2,500	67
Genesee silt	1,100	700
Loess	4,000	134
Adobe	2,000	134
Beach sand (Lake Ontario)	400	84
Dune sand (Lake Superior)	3,400	420
Dune sand (Lake Ontario)	28	<10
Kame conglomerate	3,100	<10
Arenaceous shale	5,700	186
Bituminous shale	140	<10
Fossiliferous-limy shale	1,000	<10
Brown sandstone	98	<10
Calcerous shale	320	<10
Argilaceous shale	4,500	410
Illite-bearing shale	3,300	26
Quartz	1,300	<10
Quartz conglomerate	12,000	5,600
Calcite	4,500	<10
Dickite	<10	<10
Kaolinite (Georgia)	64,000	<10
Kaolinite (South Carolina)	9,600	<10
Montmorillonite (Wyoming)	7,700	320
Montmorillonite (Arizona)	16,000	3,000
Halloysite	1,000	<10
Glauconite	4,400	2,130
Attapulgite	58,000	<10

[a] A 500-mg amount of substrate was mixed with 2 ml of virus suspension in synthetic freshwater at 4°C.

[b] Values are based on analysis of the diluted stock suspension on the day of the experiment. Each substrate was tested in one to three experiments. The data represent PFU per count per minute of radioactive label in the supernatant after centrifugation of the suspension.

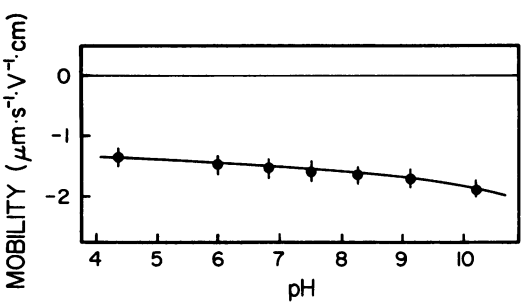

FIG. 1. Influence of pH on electrophoretic mobility of reovirus. Virus suspensions were prepared by dilution in synthetic freshwater to a concentration of 2×10^8 PFU/ml. Suspension pH was then adjusted with dilute NaOH or HCl. Mobility was measured at 25°C in the dark-field mode with a 3mW HeNe laser by determining the time required for 30 particles to travel a distance of 30 μm.

Relationship between soil properties and adsorption. Linear correlation coefficients between soil properties and virus adsorbed (inactivated) are shown in Table 4. Significant inverse correlations were observed ($P \leq 0.001$) between infectivity measurements and the content of organic matter as estimated by carbon content, apparent protein content, or weight loss on oxidation. However, when the soil with the highest organic content, muck, was omitted

TABLE 4. Linear correlation coefficients between substrate characteristics and percent adsorbed reovirus

Substrate characteristic	Correlation (r) with % virus adsorbed determined by:	
	Infectivity	Radioactivity
Surface area (m^2/g)	0.2592	0.1363
Solution pH	−0.0127	−0.2555
Weight loss by low-temperature ashing (%)	−0.6528[a]	−0.0272
Organic carbon content (%)	−0.6820[a]	−0.0572
Apparent protein content (mg/g)	−0.7168[a]	−0.1687
Capacity to adsorb PDADMA (mg/m^2)	−0.7352[a]	−0.1561
Nitric acid-extractable aluminum (μg/g)	0.2054	0.2265
Nitric acid-extractable iron (μg/g)	0.1512	0.2284
Nitric acid-extractable sodium (μg/g)	−0.0229	−0.6065[a]
Electrophoretic mobility ($\mu m\ s^{-1}\ V^{-1}\ cm$)	0.0453	0.4413[b]
Sodium content of medium (mM)	−0.0992	−0.4849[b]
Potassium content of medium (mM)	0.2436	0.2426
Magnesium content of medium (mM)	0.0289	0.1621
Calcium content of medium (mM)	0.1160	0.1682

[a] Significant at $P < 0.001$.
[b] Significant at $P < 0.01$.

from the analysis, all significant correlation with organic content was lost. Thus, this parameter was an important factor only at high values, as soils of high organic content were comparatively poor virus adsorbents. The ability of the substrates to adsorb cationic polyelectrolyte was also inversely correlated ($P \leq 0.001$) with loss of suspended infectivity. When the muck was omitted, the correlation retained significance ($r = -0.522$, significant at $P \leq 0.01$) and radiolabeled virus uptake became significantly correlated ($r = -0.615$, significant at $P \leq 0.001$) with the capacity for polyelectrolyte adsorption. Adsorption of radiolabeled virus was found to be inversely correlated with extractable Na content ($P \leq 0.001$) and Na^+ concentration ($P \leq 0.01$) in the experimental medium. However, this finding was a consequence of the fact that Wyoming montmorillonite contains large amounts of this element, which was readily released into solution. The electrophoretic mobility of each adsorbent in 10 mM NaCl near the pH of the adsorption, another measure of soil surface charge, showed correlation with radioactivity measurements for all cases ($r = 0.441$); significance was retained at the same level ($r = 0.375$, $P < 0.01$) when Wyoming montmorillonite was omitted. The scattergram (Fig. 2) shows that the materials with low electrophoretic mobility (low negative surface charge) were all effective adsorbents of viral material. Substrates of higher charge, however, were less predictable and included both weak and strong adsorbents.

A number of other properties of the adsorbents in this study had widely ranging values. The materials ranged from acidic to basic, from coarse to fine, and from low to high in extractable metal oxide content, i.e., 0.05 to 15% Fe and 0.05 to 2.6% Al. However, no other soil property, including pH, surface area, or content of any inorganic component, was significantly correlated with virus inactivation and uptake as determined by infectivity or radioactivity. The cationic content of the suspending medium, i.e., contents of K^+, Mg^{2+}, or Ca^{2+}, and the solution pH were also not significantly implicated as factors in the interaction.

DISCUSSION

For many years, electrostatic effects have been considered important in virus-soil interactions. Reovirus has a pI near 3.8 (11; unpublished data) and becomes increasingly negatively charged as pH increases from this value (26) (Fig. 1). All of the soils, minerals, and other substrates studied (Table 1) were also negatively

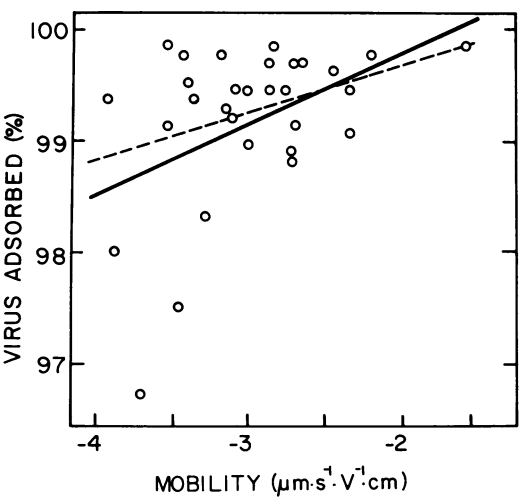

FIG. 2. Comparison of the adsorption of radiolabeled reovirus by minerals and soils from synthetic freshwater with the electrophoretic mobility of the substrates in 10 mM NaCl at a pH close to that used for the virus adsorption experiment. ——, Best fit determined by linear regression analysis of the radioactivity data for all substrates; -----, same analysis with Wyoming montmorillonite data omitted.

charged at the pH of the adsorption experiments. Hence, the interaction of reovirus with each substrate was as a charged colloid approaching a like-charged solid surface. Thus, adsorption would occur only if there were attractive interactions between the virus and the solid that exceeded the repulsive Coulombic forces from overlay of double layers at each surface.

Electrophoretic studies of negatively charged soils and clay minerals (6, 25) and viruses (26) have shown that mobility in simple electrolytes is highest in very dilute solutions of simple 1:1 salts; it is decreased by increasing concentrations of the salt and most strongly decreased by the presence of multivalent cations, including Ca^{2+} and Mg^{2+}. The enhanced adsorption found with some substrates (Table 2) as the complexity of the suspending solution was increased from deonized water and 1 mM NaCl to $CaCl_2$, $MgSO_4$, and synthetic freshwater closely parallels reductions in the electrophoretic mobility of both the virus and soil materials. These experiments suggest that Coulombic interactions may be important in inhibiting virus adsorption and that comparatively strong attractive forces between the virus and these inorganic substrates must be present, as both the virus and substrate surfaces are apparently negatively charged.

A significant finding in this study was that all of the 30 soils, minerals, and other substrates studied (Table 1) were excellent adsorbents of reovirus. Adsorption was strong not only in synthetic freshwater but also when deionized water or simple dilute electrolyte solutions (solutions in which little virus was lost from controls) were used. The concentrations of the divalent cations Ca^{2+} and Mg^{2+} which we employed in the synthetic freshwater were sufficient to substantially reduce the mobility of both the virus and the substrate and hence to favor adsorption by all substrates except the mostly highly charged and those with the lowest inherent attractive forces. In fact, the weakest adsorbent of all, montmorillonite from Wyoming, was basic and the most strongly negatively charged.

It is probable that the dominant attractive interactions when electrostatic forces are repulsive are van der Waals forces (21). The strength of van der Waals interactions between viruses and soil components will vary substantially depending upon the nature of the component. They will be comparatively weak for soil organic matter, stronger for aluminosilicates, and strongest for oxides of iron and other heavier minerals (21).

Several investigators have found variation in the ability of different soils to bind viruses (5, 7, 13, 15, 24, 28). Comparison of the adsorption of reovirus (Table 2) with that of poliovirus (12, 20, 21), which has a pI near 7.5 (27), showed that the adsorptive capacities of these substrates is similar for both viruses. When correlation coefficients were computed, the adsorption of viruses by these substrates was found to be directly correlated. Values of 0.611 ($P < 0.001$) and 0.443 ($P < 0.01$) were obtained for percent reovirus uptake as measured by infectivity and radioactivity, respectively, and percent poliovirus uptake as measured by infectivity.

Burge and Enkiri (5) reported that adsorption of a bacteriophage to four of five soils correlated with cation-exchange capacity and soil surface area. The fifth soil, which had a high organic content, was a poor virus adsorbent. Goyal and Gerba (13) found that several soil characteristics affect virus adsorption. In their study, soil pH was the most important factor and was negatively correlated with adsorption. After studying a wide range of minerals and soils, we have found that the adsorption of reovirus shows a significant correlation with the electrophoretic mobility, ability to adsorb cationic polyelectrolyte, and organic content of these substrates. Similar linear relationships between poliovirus adsorption and the ability to adsorb PDADMA and the organic matter content of these 30 soils and soil components have been reported previously (20).

Adsorption of the cationic polyelectrolyte PDADMA was studied because adsorption of this polymer is directly related to the total negative charge of each substrate (D. H. Taylor, unpublished data). When determined as polymer adsorbed per unit of weight of the adsorbent, PDADMA adsorption is closely related to the cation-exchange capacity of soils. Expressed as polymer adsorbed per unit of surface area, this parameter is an expression of the surface density of negative charge. Electrophoretic mobility is also a measure of the net particle charge. Both of these measures were inversely related to virus uptake, indicating that reovirus adsorption is stronger to surfaces with lower negative charge.

Organic matter influences virus adsorption (2, 13). Soluble organics have been shown to hinder or even reverse the adsorption of viruses (3, 4, 6, 18). Furthermore, Goyal and Gerba (13) found that adsorption of several enteroviruses is reduced when these viruses are suspended in sewage effluent. We also found that reovirus adsorption was reduced in the presence of secondary treated sewage effluent. However, we have shown here that organic matter affects reovirus adsorption only among substrates, i.e., soils, with relatively high organic content. Interestingly, reovirus infectivity, which was reduced in the presence of all of the other minerals and soils, remained at a high level in the presence of muck.

In our studies we observed a significant decrease in the specific infectivity of reovirus in the presence of a variety of minerals and soils. Preliminary studies with Ottawa sand also indicated that the loss of infectivity which occurred between 1 and 25 h at 4°C was facilitated by the substrate surface and was not due to virus aggregation. In addition, the virus had not lost its physical integrity (R. S. Moore et al., Abstr. Annu. Meet. Am. Soc. Microbiol. 1980, Q98, p. 210). Since the infectivity of the virus was stable in the presence of exopolymer-coated substrates (R. S. Moore et al., manuscript in preparation), it is likely that the observed loss of infectivity was the consequence of physical adsorption to the mineral and soil surfaces.

Our findings indicate that reovirus adsorption is a charged colloidal particle-charged surface interaction and suggest that the virus is rapidly inactivated during contact with the surface of minerals and soils. Only the presence of humic organic matter protected and prolonged virus survival. Virus uptake was closely related to the ability of the substrates to bind cationic polyelectrolyte. A measure of total negative surface charge, the polyelectrolyte is adsorbed by organic matter and strongly negative surface charges on these surfaces. Therefore, our studies have shown that the virus-adsorptive capacity of soils may be predicted to some extent by measuring the organic content and surface charge of a soil as determined by its capacity to adsorb a polyelectrolyte. This has possible implications for the land application of treated wastewater.

ACKNOWLEDGMENTS

We thank Charlotte Eastwood, Gale Schmidt, and Elizabeth Walsh for excellent technical assistance; Dale Boshart and members of the analytical chemistry department of the Environmental Health Center for chemical analysis; Donald Nuss for donating the Dearing strain of reovirus type 3; and H. Bruce Bosmann for making available the microelectrophoresis apparatus. We also express our gratitude to M. Tribley for excellent typing of this manuscript.

This research was supported by U.S. Environmental Protection Agency grant R804784 (G. Wolfgang Fuhs, project director).

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