Effect of Ionic Composition of Suspending Solution on Virus Adsorption by a Soil Column

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The effect of various electrolytes on the adsorption of poliovirus was measured in 250-cm-long soil columns with ceramic samplers at different depths. Viruses suspended in deionized water moved much farther through the soil than those suspended in tap water, whereas movement in sewage water was intermediate. The salt content of the tap water and sewage water promoted virus adsorption, but evidently the organic compounds in sewage retarded adsorption. When viruses were suspended in chloride solutions of K^+, Na^+, Ca^+, and Mg^{2+}, virus adsorption increased as the cation concentration and valence increased. The depth of virus penetration was related to the ionic strength of the solutions. Virus penetration data for NO_3^-, SO_4^{2-}, and $H_2PO_4^-$ salts of K^+, Na^+, and Ca^{2+} indicated that other anions were more effective than Cl^- in promoting virus adsorption. Also, NH_4^+ was more effective than other cations in limiting the penetration depth of viruses. It seems that ions composed of radicals are more effective than ions composed of single atoms in promoting virus adsorption. Al^{3+} was the most effective ion in limiting virus penetration, probably owing to flocculation of the viruses. Adding $AlCl_3$ concentrations to secondary sewage effluent to provide an Al^{3+} concentration of 0.1 mM reduced the virus penetration depth to 40 cm. These studies show that the ionic composition of the suspending solutions must be considered in predicting virus penetration depths, and it may be practical to add low concentrations of a flocculating agent such as $AlCl_3$ to sewage water to limit virus movement through very porous soils.

Treatment of sewage water by land application is economical and produces renovated water suitable for reuse. Careful management of land treatment systems is required, however, to produce high-quality renovated water. Removal of viruses during land treatment is important because sewage water contains a number of pathogenic viruses and monitoring virus concentrations in water resources is difficult and expensive. Many laboratory and field experiments (3, 5, 8, 10, 16–19, 21) have shown that most soils have a high adsorptive capacity for viruses. Virus adsorption of soils seems to be due to Van der Waals forces (1, 6). Previous experiments with long soil columns that were demonstrated to be good models of a field groundwater recharge system have indicated that soil permeability is the most important soil characteristic affecting virus movement through most soils (11, 22). One exception is organic soil, which has an inherently low capacity for virus adsorption because Van der Waals interactions between organic colloids are weak (17). Also, soluble organic compounds in predominantly organic soils may compete with viruses for adsorption sites on the mineral components of the soils.

The composition of the suspending solution also significantly affects virus adsorption. Although dissolved organic compounds retard virus adsorption (2, 6, 17), soils readily adsorb viruses from primary and secondary sewage effluents. Carlson et al. (4) have found that adsorption of phage T2 on clay minerals increases with increasing concentrations of NaCl and $CaCl_2$ and that $CaCl_2$ is as effective as NaCl at 1/10th the concentration. Sobsey et al. (19) have also shown that virus adsorption in batch studies is enhanced more effectively by divalent than by monovalent cations (19). Taylor et al. (20) have found that virus adsorption by sand and montmorillonite increases with increasing concen-

trations of NaCl, $CaCl_2$, and Na_2SO_4; however, the multivalent ions Al^{+3} and PO_4^{-3} do not influence virus adsorption. Thus, some batch studies indicate that virus adsorption by soils might be related to the ionic strength of the electrolyte, as would be expected from the application of the Guoy-Chapman double-layer theory (1, 6), but others indicate that this relationship may not apply. Also, the applicability of data from studies to predictions of virus penetration depths in soil columns and soil profiles is questionable. Goyal and Gerba (9) have shown that adsorption of a number of enteroviruses to sand increases as the electrolyte concentration increases. However, a follow-up study has shown that batch studies with small soil samples cannot be used to assess the virus adsorption potential of a soil column (13). For example, no adsorption of echovirus type 1 to sand from secondary effluent was measured in batch studies, yet echovirus type 1 concentrations were reduced by 4 orders of magnitude in the outflow from a 250-cm-long soil column packed with the same sand. Similarly, Sobsey et al. (19) have found that sandy and organic soil materials that are poor virus adsorbers when tested in batch studies are capable of at least 95% virus removal when packed in miniature (10-cm-long) soil columns.

Previous experiments have indicated that Ca^{2+} enhances virus adsorption in the long soil columns used in the study reported here, but no other data are available on the effect of electrolyte composition and concentration on virus adsorption in long soil columns (11). Therefore, the objectives of the present study were to determine whether (i) poliovirus adsorption by long soil columns can be related to the ionic strength of the suspending solution, (ii) the effects of electrolytes on virus adsorption in soil columns can be quantitatively described, and (iii) adding low concentrations of a multivalent cation to secondary effluent will significantly improve virus removal by soil columns.

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MATERIALS AND METHODS

A soil column that had been intermittently flooded with sewage water for several years during previous wastewater renovation and virus adsorption experiments was used in these studies to compare the leaching patterns of poliovirus type 1 (LSc) suspended in various electrolytes. The column consisted of a 2.75-m length of a 10-cm (inner diameter) polyvinyl chloride pipe filled with 6 cm of pea gravel and 250 cm of loamy sand (3% clay, 8% silt, and 89% sand) from the Salt River bed. The column was uniformly hand packed to a bulk density of 1.55 g/cm^3. Secondary sewage effluent or electrolyte solutions were applied with a Mariotte siphon to maintain a 15-cm constant pressure head at the soil surface. The flow rate and cumulative flow through the column were measured by weighing the outflow daily. Details of the flow system were described previously (14, 15).

The loamy sand column was conditioned by flooding it with tap water for 3 days and drying it for 2 days before experiments on virus adsorption from salt solutions began. The column was then flooded with a series of salt solutions containing 1×10^4 to 8×10^4 PFU of poliovirus per ml. The pH of the salt solution ranged from 6.4 to 6.9. Between the application periods for each different salt, the column was flooded with tap water for 3 days. AlCl$_3$ was added to sewage water at concentrations of 0.02, 0.1, and 0.5 mM/liter to determine whether the effect of Al could be measured when organic compounds in the sewage water interfered with virus adsorption. Virus adsorption from deionized water, dechlorinated tap water, and secondary sewage effluent was also measured.

Water samples (2 to 5 ml) were extracted from ceramic samplers with hypodermic syringes at the 2-, 5-, 10-, 20-, 40-, 80-, 160-, and 240-cm depths and from the bottom of the column at 24, 48, and 52 h after the onset of flooding with each solution. Previous experiments had shown that the ceramic samplers constructed in our laboratory removed no more than 7 to 8% of the polioviruses from sewage water passed through those samplers (23). The flow rate through the column was 50 to 60 cm per day. The samples were frozen and shipped to Baylor College of Medicine, Houston, Tex., for virus assays. Virus strains were grown and assayed by the PFU method in buffalo green monkey cells as previously described (12).

RESULTS

Viruses suspended in deionized water moved farther down the column than viruses suspended in tap water (Fig. 1). Movement of viruses in sewage was intermediate between these two extremes. The increased adsorption of viruses from tap water as compared with adsorption from deionized water was probably due to its salt content and corresponding higher ionic strength. Although the salt content in the sewage water was more than twice that in tap water, viruses moved deeper in the column with the sewage water than with the tap water. Since organic compounds have been shown to inhibit virus adsorption by soils (1, 2, 17) the organic material in the sewage probably retarded virus adsorption. The apparent increase in the amount of adsorption per unit of depth from deionized water below 80 cm (Fig. 1) might have been caused by an increase in the salt contents of the soil solution to more than 100 ppm (100 µg/g), owing to the leaching of minerals from the soils by the deionized water. However, 95% of the viruses were adsorbed from deionized water in the upper part of the column before the water dissolved any appreciable amount of salt from the soil. Some

viruses did move through the column outlet when deionized water was used, whereas the maximum depth of penetration was 80 and 40 cm for the sewage water and tap water, respectively.

Virus adsorption (inferred from the removal of viruses in water moving through the soil column) increased as the concentration of chloride salts and the valence of the cation increased (Fig. 2). For example, 20 mM CaCl$_2$, the highest concentration of a divalent cation used in the study, limited virus penetration more than any other chloride salt. In every case, more than 50% of the applied virus concentration was adsorbed in the top 2 cm of soil. In earlier experiments, more than 50% of the applied virus was still adsorbed in the upper 2 cm of soil even after 27 days of continuous flooding with sewage water containing 1×10^3 to 4×10^4 PFU of poliovirus per ml (12), indicating that the virus occupied only a small proportion of the adsorbing surface of the soil. This result is in agreement with calculations from Moore et al. (17) that were based on laboratory experiments with sand and indicated that even coarse materials such as sand have a very large virus adsorption capacity and are unlikely to become saturated in natural systems. The length of time between the application of viruses at the soil surface and extraction of samples at shallow depths (2 to 5 cm) was only a few minutes. Therefore, little virus removal during that length of time could be attributed to inactivation. Also, previous experiments have shown that viruses can be eluted from the soil columns by applying deionized water to saturated soil columns that have been flooded with sewage water containing poliovirus (12, 13). Finally, laboratory studies by Goyal and Gerba (9) have shown that a number of viruses were adsorbed from various suspending solutions by the same soil used in these column experiments as well as by many other soils. Thus, we believe that adsorption is the primary retention mechanism operating in this system, and virus removal will subsequently be referred to as virus adsorption. Even though some inactivation occurs at all times, the trends shown are almost certainly due to virus adsorption.
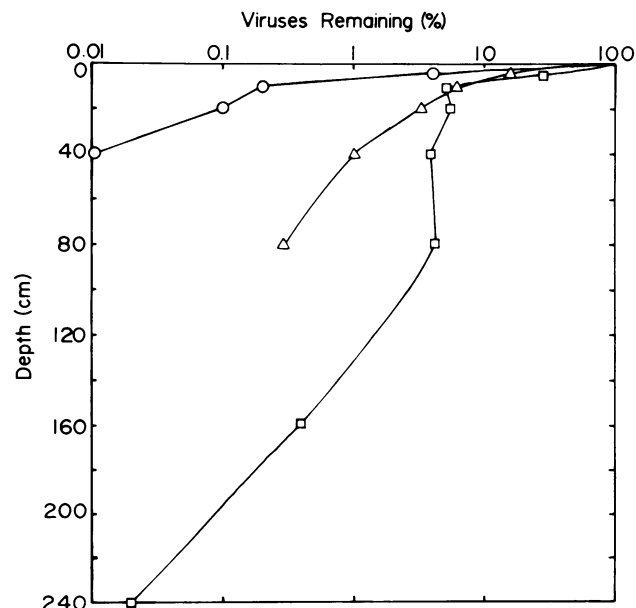

FIG. 1. Virus adsorption by soil columns from deionized water (□), tap water (○), and secondary sewage effluent (△). Data are averages for three sampling times.
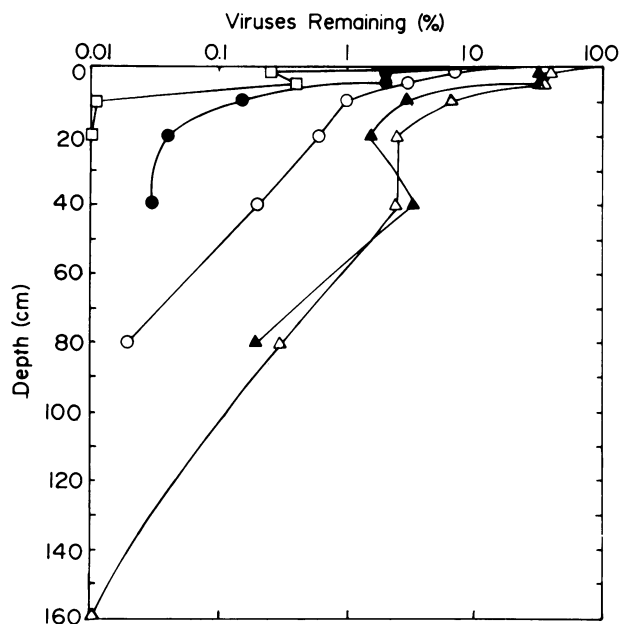
FIG. 2. Virus adsorption by soil columns from solutions of chloride salts. Data are averages for three sampling times. △, 1 mM NaCl; ▲, 10 mM NaCl; ○, 1 mM $MgCl_2$; ●, 10 mM $MgCl_2$; □, 20 mM $CaCl_2$.

The pattern of increased effectiveness with increases in salt concentration and valence suggested that virus adsorption might be related to the ionic strength of the solution. The maximum virus penetration depth for each solution was selected as the best parameter to plot versus ionic strength of the applied electrolyte to describe virus penetration in soils flooded with different electrolytes. A plot of virus penetration depth in the soil solumn versus ionic strength of several chloride salts indicated that virus adsorption increased as the ionic strength increased (Fig. 3A). The points for chloride salts were close to the line, indicating that the different cations had no specific effect apart from that on the ionic strength of the solution. Points for other anions, however, fell well off the line, indicating that they had a different effect on virus adsorption.

When the effect of several anions other than chloride were tested, however, the maximum virus penetration depth seemed to be related to the ionic strength of the solutions of various anions, with the points falling on a separate curve (Fig. 3B). Thus, nitrates, sulfates, and phosphates were more effective than chlorides in promoting virus adsorption. Tap water was more effective than chlorides but less effective than the other salts, probably owing to the fact that tap water is a mixture of all of these salts. Virus adsorption from secondary sewage effluent was similar to that from chloride salts. Apparently, the positive effect of the anions other than chloride in sewage water on virus adsorption was about equal to the negative affect of organic compounds. One chloride salt, NH_4Cl, however, was as effective as nitrate, sulfate, and phosphate in promoting virus adsorption (Fig. 4). For example, NH_4Cl was as effective as KNO_3, and both salts were much more effective than KCl, because virus penetration with both solutions was limited to 20 cm as compared with 240 cm for KCl. Apparently, ions (either cations or anions) which are radicals are more effective than ions composed of single atoms in promoting virus adsorption. Thus, complex anions were more effective than chlo-

rides, and NH_4^+ was more effective than cations consisting of single atoms.

Finally, the most effective ion in promoting virus removal by the sand column was Al^{3+}. Viruses applied in 0.2 mM $AlCl_3$ solution did not penetrate below 40 cm in the soil column (Fig. 5). When poliovirus was added to 1 or 10 mM $AlCl_3$ solutions, most of the viruses were removed or inactivated in the reservoir before application to the soil columns. Similar results were obtained with 1 and 10 mM $LaCl_3$ solutions. Thus, it appears that aluminum salts remove viruses by flocculation. When 0.1 mM $AlCl_3$ was added to secondary sewage effluent, viruses were not detected below 40 cm. Therefore, the effect of aluminum was slightly less in sewage water than in deionized water, but it still increased virus removal considerably. The reduced effect in sewage water could be due to precipitation of aluminum as aluminum phosphate or to inhibition of virus adsorption by organic matter. Increasing the aluminum concentration to 0.5 mM slightly increased virus removal. Adding 0.02 mM Al to sewage water had little effect on virus adsorption, probably because most of the aluminum was precipitated as aluminum phosphate. Flocs could be easily seen when 0.5 mM aluminum was added to sewage but were not noticeable at lower aluminum concentrations. These data indicate that it may be practical to add small concentrations of aluminum salts to sewage water to prevent virus movement through porous soils with high infiltration rates.

DISCUSSION

The adsorption of viruses by soil appears to be related to the ionic strength of the suspending solution. The effect of
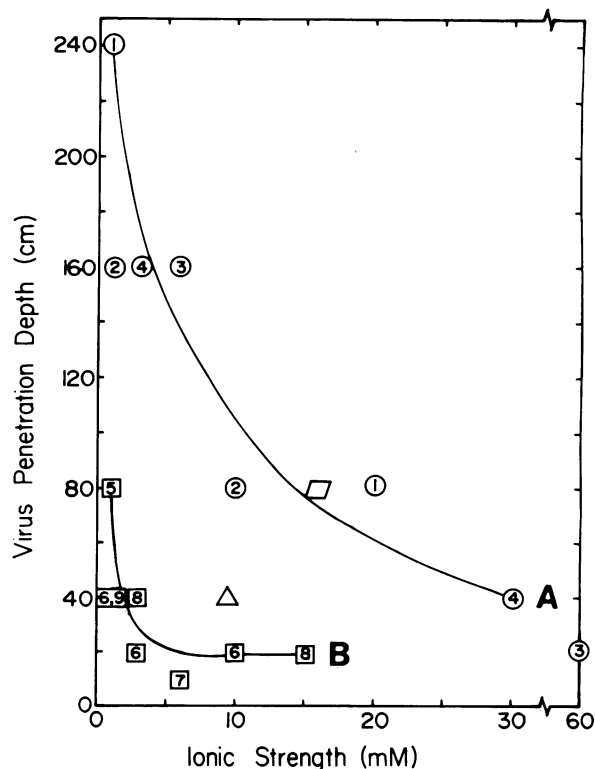

FIG. 3. Effect of ionic strength on virus penetration depth in soil columns. ○, Chlorides: 1, KCl; 2, NaCl; 3, CaCl; 4, $MgCl_2$. □, Other anions: 5, $NaNO_3$; 6, KNO_3; 7, $Ca(NO_3)_2$; 8, Na_2SO_4; 9, KH_2PO_4. △, Tap water; ▱, sewage.
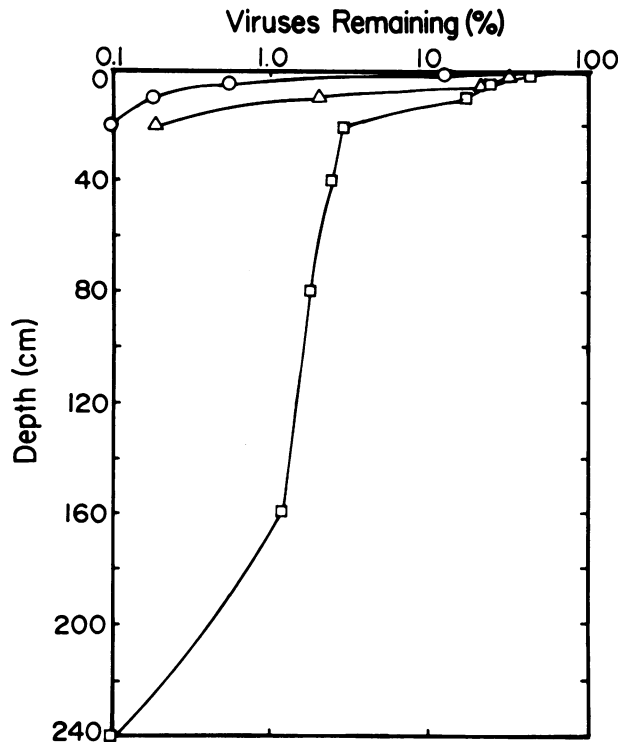
FIG. 4. Virus adsorption by soil columns from 1 mM solutions of KNO$_3$ (O), KCl (□), and NH$_4$Cl (△). Data are averages for three sampling times.

two ions with different valences is similar if the concentrations of the two salts are adjusted to provide the same ionic strength, if both cations are either single atoms or radicals, and if both anions are either single atoms or radicals. The increase in virus adsorption with increasing ionic strength is probably due to decreases in the thicknesses of the layers of charged ions around the viruses and the soil particles. This
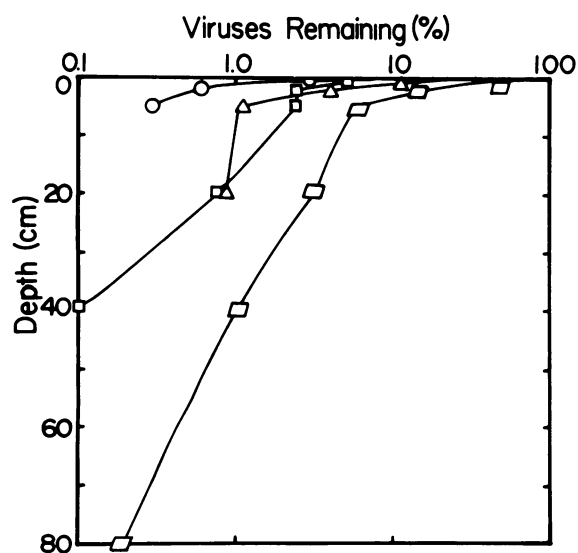

FIG. 5. Virus adsorption by soil columns for various concentrations of AlCl$_3$ in deionized water and sewage. Data are averages for three sampling times. O, 0.2 mM AlCl$_3$ in deionized water; △, 0.5 mM AlCl$_3$ in sewage; □, 0.1 mM AlCl$_3$ in sewage; ◻, sewage water.

decrease in thickness would enable the viruses and soil particles to move nearer to each other and thereby facilitate binding by Van der Waals forces. The difference in the effects of single-atom ions and radicals on virus adsorption cannot be explained by double-layer changes. It is possible that the radicals react with the viruses and change their stearic properties in some manner that improves adsorption. Since viruses are amphoteric, both cations and anions could conceivably react with the viruses. However, the effect of radicals on virus adsorption still seems to be related somehow to ionic strength, because the depth of penetration of virus in solutions of similar ions can be related to the ionic strength of the solution (Fig. 3B).

The composition of the suspending solution thus affects virus penetration in four ways: (i) virus adsorption increases as the ionic strength of the solution increases, (ii) virus adsorption increases as the proportion of radicals to single ions increases, (iii) virus removal can be increased by the addition of low concentrations of a flocculating agent such as Al^{3+}, and (iv) virus removal decreases when dissolved organic compounds are present in the suspending solution. It seems likely that the presence of suspended solids, either organic or inorganic, would also increase virus removal, but the effects of suspended solids were not tested in these experiments. The increase in virus removal in the presence of flocculating agents may occur because viruses are adsorbed to flocs and then the flocs are adsorbed or strained. The effect of dissolved organics is probably due to competition between the viruses and organic molecules for adsorption sites (2, 6). The effect of dissolved organics does not appear to be directly related to their concentration, because previous experiments have shown that virus removal from secondary and primary effluents is quite similar, even though their dissolved organic concentrations are quite different (7).

These experiments with soil columns that were shown to be good models of a ground water recharge system in previous studies show that the composition of the suspending solution must be considered in any attempt to predict the depth of virus penetration in a soil. In addition, it should be practical to enhance virus removal in land treatment systems by addition of low concentrations (0.1 mM) of a flocculation agent such as Al^{3+}.

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