Development of Methods for Detecting Viruses in Solid Waste Landfill Leachates

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Methods were developed for detecting and concentrating enteric viruses in municipal solid waste landfill leachates. Poliovirus added to a leachate was not readily detectable, possibly because the virus was adsorbed to the leachate particulates. The masking effects associated with suspended solids in the leachate were overcome by adding a final 0.1 M sodium (tetra)ethylenediaminetetraacetate concentration to the leachate. A sodium (tetra)ethylenediaminetetraacetate-treated leachate could be clarified by filtration at pH 8.0 without a loss of virus. The clarified and sodium (tetra)ethylenediaminetetraacetate-treated leachate contained interfering materials of an anionic nature which prevented virus adsorption to epoxy-fiber glass filters. This interfering effect was overcome by treating the leachate with an anion-exchange resin. Viruses in the resin-treated leachate were concentrated by adjusting the leachate to pH 3.5, adding AlCl₃ to a final 0.005 M concentration, adsorbing the viruses to an epoxy-fiber glass virus adsorbent, and eluting the adsorbed viruses in a small volume. When this method was used to concentrate poliovirus 100-fold in a variety of leachates, the average virus recovery efficiency was 37%. With the methods described in this study, it should be possible to efficiently monitor solid waste disposal site leachates for enteric viruses.

The presence of untreated human and animal fecal material and/or raw or digested sewage sludges in solid waste disposal sites raises the possibility of human enteric pathogens, including viruses, finding their way into dump or landfill leachates, thereby creating a potential health hazard (6; M. L. L. Peterson, Ph.D. thesis, Univ. of Michigan, Ann Arbor, 1972). Because leachates differ substantially from other polluted waters in their chemical composition and properties (6, 8), and because the ability to detect viruses in water often depends upon water quality (4), this study was conducted to develop efficient quantitative methods for virus detection in disposal site leachates.

MATERIALS AND METHODS

BK cells. Baboon kidneys (BK) were trypsinized and grown as previously described (7). These cultures were used for the growth and assay of the virus.

Virus and virus assays. Type 1 poliovirus, strain LSc, was selected as representative of the enteric viruses potentially present in human solid waste, and was used exclusively in this study. Virus assays were performed in monolayer cultures of BK cells by the plaque-forming unit (PFU) method (7).

Leachates. Leachate samples obtained from test cell no. 1 of an experimental lysimeter, operated by the Environmental Protection Agency in Boone County, Ky., were used in most of the experiments. All leachate samples were shipped to Houston via air and stored at 4°C upon receipt in our laboratory.

Virus adsorbents. Epoxy-fiber glass filters (series AA, Cox Instruments, Detroit, Mich.), 5 and 0.45 μm porosity in series, were used to concentrate viruses from leachates. The basic methods used to concentrate viruses on adsorbents have been described in detail (11, 12). Adsorbed viruses were eluted from Cox filters with 0.05 M glycine buffered to pH 11.5 with 1 N NaOH (11, 12). Prior to assaying for viruses, eluates were neutralized with 0.05 M glycine buffered to pH 1.0 with 1 N HCl and rendered isotonic with NaCl.

RESULTS

Effects of leachates on virus assay procedures. Because the ability to detect waterborne viruses often depends upon water quality, a series of experiments was conducted to evaluate the effect of leachates on the virus assay system. The Environmental Protection Agency leachates used in this study contained a variety of dissolved and suspended constituents (Table 1).

To determine if leachates interfered with virus adsorption to cells, BK-cell monolayers were treated for 1 h at 37°C with twofold dilutions of a leachate in pH 7.5 diluent. The
diluent consisted of 0.05 M glycine made isotonic with NaCl. The cultures were then rinsed with isotonic diluent and challenged with approximately 100 PFU of poliovirus in 0.1 ml of diluent. After a 30-min adsorption period, the cultures were overlaid with a nutrient agar and incubated at 37 C for 2 days (7). It was found that the leachate dilutions of 1:4 or less were toxic to the BK cells. At higher leachate dilutions the virus titers were essentially the same as those in the control cultures treated with diluent only. There was no evidence from this experiment to indicate that the leachate interfered with virus adsorption to cells. However, it was evident that, due to leachate cytotoxicity, viruses would have to be removed from the leachate to be detected, or the leachate would have to be diluted.

The ability to assay poliovirus in the leachate by direct plating was studied. Equal quantities of type 1 poliovirus were added to unfiltered and to 0.2 μm porosity, Cox-filtered leachates, and to a virus diluent that had been adjusted to the same pH as the leachate. After mixing, the samples were immediately diluted 10- and 100-fold in pH 7.5 diluent for plating. The results for three experiments are shown in Table 2. The assays for virus in the unfiltered leachate resulted in nonproportional plaque counts between the 10- and 100-fold dilutions, whereas the plaque counts for the 10- and 100-fold dilutions of filtered leachates and virus diluents showed a proportional relationship. One possible explanation for these results is that the viruses in the undiluted, unfiltered leachate were adsorbed to leachate particulates. When the unfiltered leachate was diluted 100-fold, it is possible either that the virus was released from the particulates or that the particulates were dissolved. The undiluted leachate had a pH of 5.5, which is conducive for virus adsorption to particulates. The 10-fold dilution of leachate had a pH of about 6.5 and was turbid, indicating that particulates to which the virus could possibly be adsorbed were still present. A pH of 6.5 is not capable of causing virus release from particulates. In leachate-free salt solution, the efficiency of virus plating was the same for both pH 6.5 and 7.5 solutions. When a similar experiment was conducted with increased virus levels so that leachate dilutions of 100- and 1,000-fold were required for plating, proportional virus assays were obtained with the unfiltered leachate, again suggesting that leachate particulates do not interfere with virus assay when the dilution factor is sufficiently large.

**Effects of chelation on virus detectability**

| Parameter | Value |
|-----------|-------|
| pH        | 5.5   |
| Conductivity (mg/liter of NaCl) | 8,000 |
| Turbidity (JTU) | 1,200 |
| Total solids (mg/liter) | 32,100 |
| Suspended solids (mg/liter) | 550 |
| Total organic carbon (mg/liter) | 14,000 |
| Hardness (mg/liter) | 3,600 |

* EPA, Environmental Protection Agency.

* pH was measured with a Corning pH meter (model 110); conductivity was measured with a Myron L Co. conductivity meter (model 532T1); turbidity was measured with a Hach Chemical Co. turbidimeter (model 2100A); and total solids, suspended solids, total organic carbon, and hardness were determined according to Standard Methods (1).

* JTU, Jackson turbidity units.

**Table 2. Effect of leachate on the plating efficiency of poliovirus**

| Sample | Average no. of PFU/0.1 ml detected in leachate dilutions | Expected plaque counts at the 10-fold dilution* |
|--------|----------------------------------------------------------|-----------------------------------------------|
|        | 10-fold | 100-fold |                                      |
| Unfiltered leachate: | | | |
| Exp 1 | 27 | 17 | 170 |
| Exp 2 | 7 | 7 | 70 |
| Exp 3 | 5 | 2 | 20 |
| Filtered leachate: | | | |
| Exp 1 | 132 | 15 | 150 |
| Exp 2 | 75 | 8 | 80 |
| Exp 3 | 28 | 3 | 30 |
| Virus diluent: | | | |
| Exp 1 | 143 | 16 | 160 |
| Exp 2 | 77 | 8 | 80 |
| Exp 3 | 31 | 3 | 30 |

* Based on the PFU detected at the 100-fold dilution.

**Table 1. General characteristics of EPA* leachate**

**and leachate clarification.** Concentration of viruses from water by adsorption to filter surfaces usually requires the removal of suspended solids from the water to prevent clogging of virus adsorbents. In our previous studies, clarification was effected by filtration through a series of coarse filters which do not adsorb viruses (5, 12). Attempts to clarify poliovirus-containing leachates resulted in the clogging of clarifying filters and a loss of virus. The leachate had a pH of about 5.5, and because viruses may adsorb to filter surfaces under acidic conditions an attempt was made to raise the leachate pH to the alkaline range before clarification. This treatment resulted in mas-
sive precipitation of dissolved leachate components. The leachate contained high levels of polyvalent metal cations as indicated by its high hardness concentration (Table 1). Because the precipitate, which formed when we attempted to adjust the leachate to an alkaline pH, may have been due to polyvalent metal cation salts, sodium (tetra)ethylenediaminetetraacetate (EDTA) was added to the leachate in an attempt to chelate these metal cations and to prevent precipitation upon pH adjustment. A final EDTA concentration of 0.1 M in the leachate not only prevented precipitation of leachate components at pH 8.0, 9.0, and 10.0, but also resulted in a dramatic clearing of the leachate, as indicated by the turbidity data in Fig. 1. Apparently, both soluble and precipitated metal cations were extensively chelated by the EDTA.

The effect of EDTA treatment on viruses in the leachate was investigated. Type 1 poliovirus was added to the leachate and to a pH 5.5 diluent to contain about 10^4 PFU/0.1 ml. The leachate was immediately divided into two portions; one was treated with EDTA to give a final 0.1 M concentration. All samples were assayed for viruses at zero time and after 3 h of contact at room temperature. Poliovirus added to the leachate and immediately plated gave nonproportional plaque assays, and after 3 h of contact the degree of non-proportionality was increased further (Table 3). However, when EDTA was present in the leachate, proportionality between dilutions was obtained, there was no virus loss after 3 h of contact, and the virus titers corresponded to those in a control sample of buffered salt solution. The results of this experiment suggested that 0.1 M EDTA in the leachate prevented the masking of viruses caused by the interactions of the virus with leachate particulates.

An experiment was performed to determine the effect of EDTA on leachate clarification. Type 1 poliovirus was added to the leachate to give a concentration of about 3.5 x 10^3 PFU/0.1 ml. The leachate-virus mixture was divided into four portions of 100 ml each. One sample was filtered, without any additional treatment, through a set of clarifiers consisting of Tween 80-treated (12), 5 and 1 μm porosity Cox filters in series. The remaining three portions were treated with EDTA to give a final 0.1 M concentration, and the pH levels were adjusted with 1 N NaOH to the values indicated in Table 4. Each sample was then filtered through a set of the clarifiers described above. The untreated leachate clogged the clarifying filters before the total volume could be processed, whereas the leachate samples with EDTA were easily clarified (Table 4). The best flow rate for the leachate containing EDTA was obtained at pH 8.0. The filtrate of the untreated leachate showed a substantial loss of virus, whereas the filtrates for leachates containing EDTA showed no appreciable loss of virus. Based upon the results of these experiments, leachate samples were treated with a final 0.1 M concentration of EDTA and adjusted to pH 8.0 prior to clarification in all subsequent experiments.

**Concentration of viruses on Cox filters.** In previous studies (5, 10, 11), viruses in water and wastewater were efficiently concentrated by adjusting the water or wastewater to pH 3.5,
adding AlCl$_3$ to a final 0.0005 M concentration to enhance virus adsorption, adsorbing the viruses to a filter surface, and recovering the adsorbed virus by elution with a small volume. The effect of AlCl$_3$ concentration in a leachate containing 0.1 M EDTA on virus adsorption to Cox filters was examined. Clarified leachate (1,400 ml, pH 8.0), containing 0.1 M EDTA and about $5 \times 10^5$ PFU/0.1 ml of type 1 poliovirus, was treated with MgCl$_2$ to a final 0.02 M concentration to complex any residual free EDTA. The mixture was adjusted to pH 3.5 with 1 N HCl and divided into seven portions of 200 ml each. The portions were then filtered through a 47-mm diameter filter at a flow rate of approximately 50 ml/min, and the filtrate was assayed for viruses. Appreciable amounts of virus did not adsorb to the Cox filters, even when the AlCl$_3$ concentration was as high as 0.15 M (Table 5). Apparently, the leachate contained components that interfered with the reaction between the virus and filter surface, because the presence of excess aluminum ions did not greatly enhance virus adsorption.

The effect of leachate volume processed on the extent of leachate interference with virus adsorption to Cox filters was studied. A sample of clarified, pH 8.0 leachate, containing 0.1 M EDTA and $5 \times 10^5$ PFU of poliovirus per ml, was adjusted to pH 3.5 with 1 N HCl, treated with MgCl$_2$, and finally adjusted to a final 0.02 M concentration, treated with AlCl$_3$ to a final concentration of 0.0005 M, and divided into five portions having volumes of 10, 30, 100, 300, and 1,000 ml. Each portion was filtered through a 47-mm diameter virus adsorber and the filtrate was assayed for viruses. The percentage of initial virus present in the Cox filtrates of leachate volumes of 10, 30, 100, 300, and 1,000 ml was <1, 10, 44, 44, and 64%, respectively. Thus, the degree of interference with virus adsorption by leachate components was, to some extent, volume dependent.

To determine if the materials in a leachate that interfered with virus adsorption were membrane-coating components (13), the following experiment was conducted. A sample of a clarified, pH 8.0 leachate, containing 0.1 M EDTA, 0.02 M MgCl$_2$, and no added poliovirus, was adjusted to pH 3.5 with 1 N HCl and divided into five portions having volumes of 10, 30, 100, 300, and 1,000 ml. Each portion was filtered through a 47-mm diameter virus adsorber. Each virus adsorber was washed with 10 ml of pH 3.5 physiological saline to remove excess Al ions and then challenged with 10 ml of pH 3.5 glycine buffer (0.05 M) containing $5 \times 10^{-4}$ M AlCl$_3$ and $10^5$ PFU of poliovirus per ml. As a control, a virus adsorber which had received no leachate was also challenged with 10 ml of the same virus suspension in glycine-AlCl$_3$. The glycine-AlCl$_3$ filtrate from each adsorber was assayed for viruses to determine the extent of virus breakthrough. In each case no virus was detectable in the virus adsorber filtrates of the challenge virus, indicating that all of the viruses had adsorbed to the Cox filters. Apparently, the components in a leachate that interfere with virus adsorption were not membrane-coating components and did not accumulate on the filters.

The chemical nature of the virus-interfering components in a leachate was investigated by treating leachate samples with a variety of ion-exchange resins. The use of ion-exchange resins to remove interfering materials from wastewater (13) and to chemically characterize interfering materials in tapwater (9) has been reported previously. Fifty-milliliter volumes of a clarified, pH 8.0 leachate containing 0.1 M EDTA were treated with 100-g amounts of the resins indicated in Table 6. The resin-treated leachates and an untreated control were adjusted to pH 3.5, and AlCl$_3$ was added to a final concentration of 0.0005 M. Poliovirus was TABLE 4. Clarification of leachate containing EDTA

| pH of leachate | Filtration time (s) | Total virus in filtrate (%) |
|---------------|---------------------|----------------------------|
| untreated leachate, pH 5.6 | 9 | 0.14 |
| Leachate + EDTA: | | |
| pH 8 | 14 | 97 |
| pH 9 | 28 | 100 |
| pH 10 | 87 | 104 |

* Time required to filter the total 100-mI portions through the clarifiers.

* Filters were clogged at 42 ml.
added to each sample to give a concentration of about 5 × 10^4 PFU/ml. Each sample was filtered through a 47-mm diameter virus adsorber at a flow rate of about 50 ml/min, and the filtrate was assayed for unadsorbed viruses. The interfering components in leachates appear to be anionic compounds having a relatively weak affinity for the resins used, because they were efficiently removed only when the strong base anion resin Duolite ARA-366 was in the hydroxide form (Table 6). The chloride form of this resin removed lesser amounts of the interfering material, and the affinity of chloride for the resin relative to hydroxide is 22. However, the hydroxide form of the resin could not be used to remove interfering components from virus-containing leachates because the release of hydroxide ions in the exchange process inactivated the viruses. The mixed bed resin Duolite ARM-381 also gave good removals of interfering components in leachates. This can be explained by the fact that the anion-exchange resin in Duolite ARM-381 is identical to Duolite ARA-366.

Based upon the above observation that certain anion-exchange resins were capable of removing interfering material from leachates, a number of commercially available anion-exchange resins were tested in an attempt to find a resin that would efficiently remove interfering components from leachates without removing or inactivating the viruses. Two hundred-milliliter volumes of a clarified, pH 8.0 leachate, containing 0.1 M EDTA and 5 × 10^4 PFU of poliovirus per ml, were treated with 200-g quantities of the resins listed in Table 7. Each resin-treated leachate was reassayed for viruses, treated with MgCl₂ to a final 0.02 M concentration, adjusted to pH 3.5 with 1 N HCl, treated with AlCl₃ to a final concentration of 0.0005 M, and filtered through a 47-mm diameter virus adsorber. Each filtrate was assayed for viruses to determine the percentage that had become adsorbed to the virus adsorber. A number of the resins tested were capable of removing interfering material from leachates without appreciably reducing the amount of virus present, thereby making it possible for the virus to be more efficiently adsorbed to Cox filters (Table 7). Based upon the results of this experiment, Ionac A540 was selected for further investigation on the removal of interfering materials from leachates.

The optimal resin-leachate ratio required for effective removal of interfering materials was determined. A 500-ml sample of a clarified, pH 8.0 leachate, containing 0.1 M EDTA and 5 × 10^4 PFU of poliovirus per ml, was divided into five volumes of 100 ml each. The volumes were treated with 100-, 50-, 25-, and 12.5-g amounts of Ionac A540 resin in columns. The resin-treated leachates were given a final 0.02 M concentration of MgCl₂, adjusted to pH 3.5, given a final 0.0005 M concentration of AlCl₃, and filtered through a 47-mm diameter virus adsorber.

### Table 6. Effect of ion-exchange resins on the virus-interfering components in leachate

| Resin                  | Type          | Form          | Virus in resin-treated leachate adsorbed to Cox filters (%) |
|------------------------|---------------|---------------|------------------------------------------------------------|
| Control, no resin      |               |              |                                                            |
| Duolite® ARC-351       | Cation        | H⁺            | 51                                                         |
| Ionac® C-249           | Cation        | Na⁺           | 30                                                         |
| Duolite S-37           | Organic adsorbent | H⁺ + OH⁻     | 70                                                         |
| Duolite ARM-381        | Mixed bed     | Cl⁻           | >99                                                        |
| Dowex® 1-X8            | Anion         | OH⁻           | 62                                                         |
| Duolite ARA-366        | Anion         | Cl⁻           | >99                                                        |
| Duolite ARA-366        | Anion         |               | 84                                                         |

* Ratio of resin weight to leachate volume was 2:1 (wt/vol).
* Diamond Shamrock Co., Redwood City, Calif.
* Permuit Co., Birmingham, N.J.
* Bio-Rad Laboratories, Richmond, Calif.

### Table 7. Removal of interfering components from leachate by anion-exchange resins

| Resin                                | Remaining after resin treatment | Adsorbed to Cox filters |
|--------------------------------------|---------------------------------|-------------------------|
| Amberlite® IRA-400                   | 95                              | 70                      |
| Amberlite® IRA-401S                  | 85                              | 55                      |
| Dowex 1-X8                           | 86                              | 36                      |
| Duolite A104                         | 98                              | 65                      |
| Ionac A540                           | 95                              | 76                      |
| Control, no resin                    |                                 | 45                      |

* Ratio of resin weight to leachate volume was 1:1 (wt/vol).
* Rohm and Haas Co., Philadelphia, Pa.
adsorber. The resulting filtrates were assayed for viruses. A 100-ml leachate sample, which had not been treated with resin but otherwise was treated in the same manner, served as a control. Virus adsorption to Cox filters was improved over that of the control at a resin-leachate ratio as low as 1:8 (Table 8). However, optimal results in terms of the percent of viruses remaining after resin treatment and the percent of viruses adsorbed to Cox filters were achieved at a ratio of 1:2; this ratio was used in further experiments.

Concentration of viruses from leachates. Based upon the results of the above experiments, the following virus concentration scheme for leachates was established and tested: (i) addition of EDTA to leachates to a final 0.1 M concentration and adjustment to pH 8.0; (ii) clarification of leachates through a Tween 80-treated, 5 and 1 μm porosity Cox filter series for small leachate volumes, or a 10 and 1 μm porosity, orlon cartridge depth filter series (12) for volumes greater than 1 gallon (3.785 liters); (iii) treatment of clarified leachates with Ionac A540 anion-exchange resin at a resin-to-leachate ratio of 1:2 on a weight-to-volume basis; (iv) addition of MgCl₂ to a final 0.02 M concentration to react with residual EDTA; (v) adjustment of leachates to pH 3.5 with 1 N HCl and addition of AlCl₃ to a final 0.0005 M concentration; (vi) adsorption of viruses to a 5 and 0.45 μm porosity Cox filter series; (vii) elution of viruses in a small volume of pH 11.5 glycine sodium-hydroxide buffer; and (viii) adjustment of the eluate to pH 7.5 and isotonicity before virus assay.

This procedure was used to concentrate viruses 100-fold from 1-liter volumes of 15 different leachates having different physical and chemical properties, and which were experimentally contaminated with type 1 poliovirus at a concentration of about 5 × 10⁴ PFU/ml. Virus recoveries ranging from 2 to 62%, with an average recovery of 37%, were obtained (Table 9). The Cox filters used for virus concentration were visually examined after virus elution for the presence of residual solids. It was observed that the filters used to process the leachate from test cell no. 4 had a brown-black, oily residue on their surface. This material was not seen on the filters used to process the other leachate samples, and it may have been responsible for the poor virus recovery efficiency obtained with this sample.

Other enteroviruses, including echovirus type 7 and coxsackievirus types A9 and B3, have also been concentrated 100-fold from experimentally contaminated leachates by the procedures described above, and virus recovery efficiencies have averaged 40 to 50% (Sobsey, Wallis, and Melnick, unpublished data).

**DISCUSSION**

Initial experiments on the development of methods for detecting enteric viruses in solid waste landfill leachates were complicated by the fact that poliovirus added to leachates could not be easily recovered. In addition, the leachate was toxic for the BK cells used in assaying poliovirus infectivity unless it was diluted 1:8 or more.

Poliovirus in the untreated leachate could not be quantitatively detected unless the leachate sample was extensively diluted, whereas poliovirus in the 0.2 μm-filtered leachate could be quantitatively detected without extensive dilution. These results suggested that poliovirus in the untreated leachate may have been adsorbed to or otherwise associated with particulate ma-

| Table 9. Concentration of poliovirus from leachates |
|-----------------------------------------------|
| Leachate | Source            | Initial virus recovered (%) |
|-----------|------------------|-----------------------------|
| Test cell 1 | Boone Co., Ky. | 62 |
| 2D | Boone Co., Ky. | 54 |
| 1 | Crawford Co., O. | 46 |
| 2 | Crawford Co., O. | 29 |
| 3 | Crawford Co., O. | 35 |
| 6 | Crawford Co., O. | 42 |
| Landfill sample 1 | Lexington, Ky. | 12 |
| 2 | Lexington, Ky. | 54 |
| Test cell 1 | Madison, Wisc. | 40 |
| 2 | Madison, Wisc. | 41 |
| 4 | Madison, Wisc. | 2 |
| 5 | Madison, Wisc. | 19 |
| 6 | Madison, Wisc. | 23 |
| 7 | Madison, Wisc. | 61 |
| 8 | Madison, Wisc. | 33 |

*a* Ionac A540.
arterial. The acidic pH of the leachate (pH 5.5 to 5.7) is conducive for virus adsorption to particulates (11). Extensive dilution of an untreated leachate may have resulted in desorption of viruses or dissolution of particulates. The adsorption of viruses to a variety of salt and mineral precipitates has been previously reported (2, 3, 14) and has been used to concentrate waterborne viruses (4).

Clarification of an untreated leachate containing poliovirus resulted in the clogging of the clarifying filters and a loss of the virus. The acid pH and the particulate content of the leachate may have been responsible for these effects. EDTA was added to the leachate in an attempt to prevent precipitation of leachate components at the basic pH levels necessary for leachate clarification by filtration. It was found that a final 0.1 M concentration of EDTA (i) resulted in extensive clearing of the leachate, (ii) eliminated the apparent loss of virus added to the leachate, (iii) made it possible to adjust the leachate to basic pH levels without precipitation of leachate components, and (iv) allowed the leachate to be clarified by filtration without loss of virus. These results suggested that the added EDTA chelated soluble and particulate metal cations in the leachate.

In previous studies, viruses in water and wastewater were concentrated by adjusting the samples to pH 3.5, adding AlCl₃ to a final 0.0005 M concentration, filtering through a virus adsorbent, and eluting the adsorbed viruses in a small volume (5, 11). Poliovirus in a clarified, pH 3.5 leachate could not be concentrated on epoxy-fiber glass (Cox) filters, even when the AlCl₃ concentration was as high as 0.15 M. This observation suggested that certain leachate components interfered with virus adsorption to filter surfaces. It was found that the interfering components were not membrane-coating components and were weakly anionic in nature. These interfering components in leachates could be removed without extensive loss of virus by treating the leachate with a number of different anion-exchange resins prior to filtering through a virus adsorbent. Of the resins tested, the best results were obtained with Ionac A540, and the optimal ratio of resin-to-leachate on a weight-to-volume basis was found to be 1:2.

A method was developed for concentrating poliovirus from an experimentally contaminated leachate which consisted of treating the clarified, EDTA-treated leachate with anion-exchange resin before filtering through a virus adsorbent at pH 3.5 and in the presence of 0.0005 M AlCl₃. The adsorbed viruses were then eluted in a small volume.

These methods are presently being used in our laboratory to screen leachate samples from operating dumps and landfills for enteric viruses, and to study the fate of enteric viruses in laboratory models of municipal solid waste landfills (Sobsey, Wallis, and Melnick, manuscript in preparation).

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