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Effects of Humic Materials on Virus Recovery from Water

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Humic and fulvic acids were tested for their ability to interfere with virus recovery by microporous filters. Two electropositively charged types of filter (Seitz S and Zeta Plus 60S) were used to concentrate poliovirus in the presence of humic materials. Humic acid inhibited virus adsorption, but even at the highest humic acid concentrations tested (200 mg/liter), 30 to 40% of the virus was recovered by the filters. Fulvic acid, tested with Zeta Plus filters, did not affect virus recovery. For comparison, two electronegatively charged filter types were tested (Cox and Balston). These two types of filter were more sensitive to interference at lower concentrations of humic acid than the more positively charged filters. With Balston filters, at humic acid concentrations above 10 mg/liter, most of the virus was recovered in the filtrate. Fulvic acid, tested with Balston filters, did not interfere with virus recovery. With the electropositively charged filters, the humic materials adsorbed efficiently, even at high input concentrations. Interference with virus adsorption occurred at humic acid concentrations which were below the level of saturation of the filters. In addition, in high-volume experiments, humic acid led to premature blockage of the filters. The efficiency of virus recovery by a second concentration step, organic flocculation of the filter eluate, was tested. For all the filter types tested, this procedure was not affected by the presence of humic or fulvic acid in the input water.

The standardization of techniques for assaying viruses in water, and knowledge of their limitations, are necessary to accurately assess the degree of viral contamination of natural waters. Currently used methods of virus concentration by adsorption to microporous filters are often influenced by the presence of interfering factors in the water samples (12, 20). The degree of interference depends on the method used (18), as well as the type of water tested (16). Humic substances, which comprise the majority of organic material in natural waters (2), are candidate interfering factors in the recovery of viruses by microporous filters (3, 5).

Humic materials are widely occurring organic contaminants of natural waters (2, 19) and effluents (11, 14), which are formed as degradation products of organic matter in soil (9). They are highly colored complex polymeric nonvolatile acidic materials which can chelate metals. The alkali-soluble humic materials may be classified as humic or fulvic acid on the basis of their solubility in acid. The fulvic acids are soluble in acid, whereas the humic acids are acid insoluble. The fulvic acids have a lower molecular weight and higher total acidity and carboxyl values than the humic acids (21).

Recently, electropositively charged microporous filters have come into use for recovering waterborne viruses, and these filters are reportedly less sensitive to water quality changes than more negatively charged filters (15). In this paper, we report the results of experiments testing the sensitivity of two electropositively charged types of filter to interference by humic substances in comparison with results obtained with more electronegatively charged filters.

MATERIALS AND METHODS

Virus and virus assay. Poliovirus type I (Brunhilde strain) was grown and titrated by plaque assay on BGM (African green monkey kidney) cells as described (5).

Humic materials. Commercial humic acid (Aldrich Chemical Co., Milwaukee, Wis.) and fulvic acid extracted from Hula Valley peat (the kind gift of R. Ikan and P. Ioselis) were prepared as previously described (5, 6). Relative concentrations of the materials were determined by measuring the A_{280} in a Varian Techtron Spectrophotometer at pH 7.0.

Virus concentration methods. Virus was seeded into deionized water (1-liter volumes unless otherwise indicated), with or without humic materials, as indicated in the text. For Zeta Plus 60S (0.45-μm nominal pore size; cellulose-diatomaceous earth-charge-modified resin filter; AMF, Cuno Division, Meriden, Mass.) or Seitz S (0.5-μm nominal pore size; asbestos-cellulose filter; Republic Seitz Filter Corp., Milldale, Conn.) 142-mm-diameter disk filters, the pH of the input water was adjusted to 6.0 before filtration, and the virus was eluted with 3% beef extract (Lab-Lemco; Oxoid, Ltd., Long, England). For Cox AA (0.45-μm nominal pore size; Type M-780 fiber glass-asbestos-epoxy 142-mm-diameter disk filter; Cox Instruments, Detroit, Mich., with a Sartorius SM 13430 fiber glass prefilter) or Balston grade C (8-μm nominal pore size; 17.8-cm-long fiber glass-epoxy filter tube; Balston, Inc., Lexington, Mass.) filters, the pH of the water was adjusted to 3.5 before filtration, and the virus was eluted with 1% beef extract.

Reconcentration was by organic flocculation of the filter eluate, as described (7). The percentage of the input virus which was in each fraction (filtrate, eluate, organic floc, and supernatant of the organic floc) was determined by plaque assay.

RESULTS

Humic acid and Seitz filters. Seitz filters have not been widely tested for their ability to recover viruses from water, in spite of their reportedly efficient adsorption of poliovirus over a wide pH range (3.5 to 9.0) and successful virus elution with beef extract (17). To verify this finding, a preliminary experiment was performed in which poliovirus was concentrated from 2 liters of tap water (pH 7.5) by passage through a Seitz filter and elution with 1% beef extract (pH 9.0). The amount of virus recovered in the eluate was 67% of the input, in agreement with published results (17).

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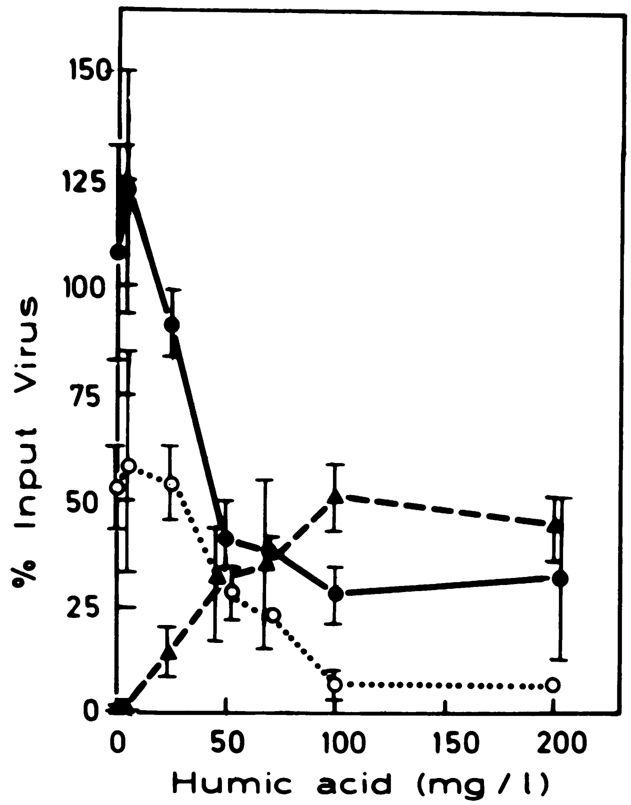

FIG. 1. Effect of humic acid on virus recovery by Seitz filters. Poliovirus (2.0×10^5 to 1.2×10^8 PFU) was added to 1 liter of water containing humic acid at the indicated concentrations, and the virus was concentrated by filtration through Seitz filters and organic flocculation as described in the text. Virus recovery in the eluate (●), filtrate (▲), and organic floc (○) is shown as a percentage of the input virus. Each point is the average of two to five experiments.

To test the effect of humic acid on virus recovery by these filters, a series of experiments was performed in which the fate of poliovirus in humic acid-containing water was followed during adsorption to and elution from Seitz filters and reconcentration by organic flocculation. In the filtration step, virus recovery in the eluate, filtrate, and organic floc was monitored, at humic acid concentrations ranging from 5 to 200 mg/liter in the input water (Fig. 1). At concentrations of 50 mg/liter and above there was a decrease in virus recovery in the eluate. By 50 mg/liter, virus recovery in the eluate was reduced to 40% of the input and remained at the 30 to 40% level up to 200 mg of humic acid per liter. In parallel, virus recovery in the filtrate increased and remained at 30 to 50% of the input for concentrations of humic acid above 50 mg/liter. Thus, the adsorption of the virus to the filters was apparently inhibited by the humic acid. In addition, at humic acid concentrations of 50 mg/liter and above, the elution efficiency (calculated from the amount of adsorbed virus which was eluted) was decreased to 60%, indicating the possibility of some interference with elution as well. However, at all concentrations of humic acid, 73% or more of the virus was accounted for in the eluate and filtrate, so that the loss due to a decreased elution efficiency was less than 14% of the input.

After elution, the virus in the eluate was reconcentrated by organic flocculation. The efficiency of organic flocculation was 50 to 66% at low humic acid concentrations, and was slightly lower at humic acid concentrations of 100 mg/liter and above. This incomplete recovery was not due to virus remaining in the supernatant of the organic floc. The supernatant was assayed, and 0 to 0.8% of the input virus was found in that fraction (data not shown). Thus, in general, the organic flocculation step was less efficient for these filters than for more electronegative filters (7), but it was not greatly affected by the presence of humic acid in the input water.

Humic acid and Zeta Plus filters. The effect of humic acid on the recovery of poliovirus was explored for a second type of electropositive filter, Zeta Plus, which has been used successfully for virus concentration from tap water (4, 17) and wastewater (1). Experiments testing the effect of humic acid on poliovirus recovery by these filters were performed with input water containing concentrations of humic acid ranging from 0.5 to 200 mg/liter (Table 1). At 70 mg of humic acid per liter and above, there was a reduction in virus recovery to between 36 and 49% of the input virus. As was seen with the Seitz filters, at low humic acid concentrations there was little effect, but at a certain level interference was detected, which remained fairly constant at higher concentrations. The amount of virus in the filtrate generally accounted for the reduction in virus in the eluate, indicating that the interference was primarily at the level of adsorption. Efficiency of elution of the adsorbed virus was generally above 70%, with the exception of a humic acid concentration of 200 mg/liter, where the elution efficiency was 55%. In all cases, more than 70% of the virus was in the eluate plus filtrate.

Organic flocculation recovered approximately half of the virus contained in the eluate, and its efficiency was not strongly affected by the presence of humic acid in the input water; the supernatant of the organic floc contained little or no virus (data not shown). The loss of virus during organic flocculation of the filter eluate has been noted with tap water (5a) and is similar to what was found with the Seitz filters.

Fulvic acid and Zeta Plus filters. Fulvic acid, the acid-sol-

TABLE 1. Effect of humic and fulvic acids on virus recovery by Zeta Plus filters

Humic material and concn (mg/liter)	No. of expts	% Virus recovered in[a]: Eluate	Filtrate	Organic floc
Humic acid				
0	11	82 ± 16	4 ± 2.7	49 ± 12
0.5	4	69 ± 17	4 ± 3.4	39
5	2	89 ± 23	3.5 ± 2	ND
10	3	87 ± 32	6 ± 2	42 ± 20
50	6	79 ± 14	25 ± 21	45 ± 13
60	3	40 ± 12	50 ± 23	22 ± 27
70	5	43 ± 10	61 ± 12	17
100	3	49 ± 21	60 ± 33	27 ± 21
150	2	36 ± 8	56 ± 37	29 ± 15
200	3	36 ± 14	35 ± 14	25 ± 8
Fulvic acid				
0	5	74 ± 24	1 ± 0.7	35 ± 7
0.2	2	62 ± 11	0.5 ± 0.7	ND
2	2	111 ± 54	3.5 ± 2	ND
20	4	74 ± 29	2.1 ± 2	24 ± 6
100	3	89 ± 17	0.7 ± 1	39 ± 3
200	2	104 ± 4	0.25 ± 0.01	56 ± 1

[a] Percentage of input virus in each fraction ± standard deviation. ND, Not done. Organic flocculation was not performed in all experiments.

uble fraction of soluble humic materials, was tested for its effect on virus recovery from water by Zeta Plus filters in a series of experiments similar to those described above for humic acid. At concentrations of 0.2 to 200 mg of fulvic acid per liter, there was no effect on virus recovery in the eluate, and little or no virus was found in the filtrate (Table 1). In addition, virus recovery in the organic floc remained at 50% of the eluate. Thus, the fulvic acid apparently did not interfere with virus recovery by these filters.

Humic materials and electronegative filters. For comparison, the results of a similar series of experiments assessing the effect of humic materials on virus recovery by two electronegatively charged filter types are presented. Cox filters were tested with humic acid added to the input water, and interference was detectable at 25 mg of humic acid per liter (Fig. 2). Virus recovery in the eluate was less than 10% at 100 and 200 mg of humic acid per liter, with the majority of the virus appearing in the filtrate. At all concentrations of humic acid, 70% or more of the virus was accounted for in the eluate and filtrate fraction, and organic flocculation remained efficient.

Balston filters were tested with both humic and fulvic acids. With humic acids, virus recovery was even more sensitive to interference than was found with the Cox filters. Recovery in the eluate was low at input humic acid concentrations of 10 mg/liter and above, and most of the input virus was in the filtrate (Table 2). Organic flocculation was not affected by the humic acid in the input water. With both Cox

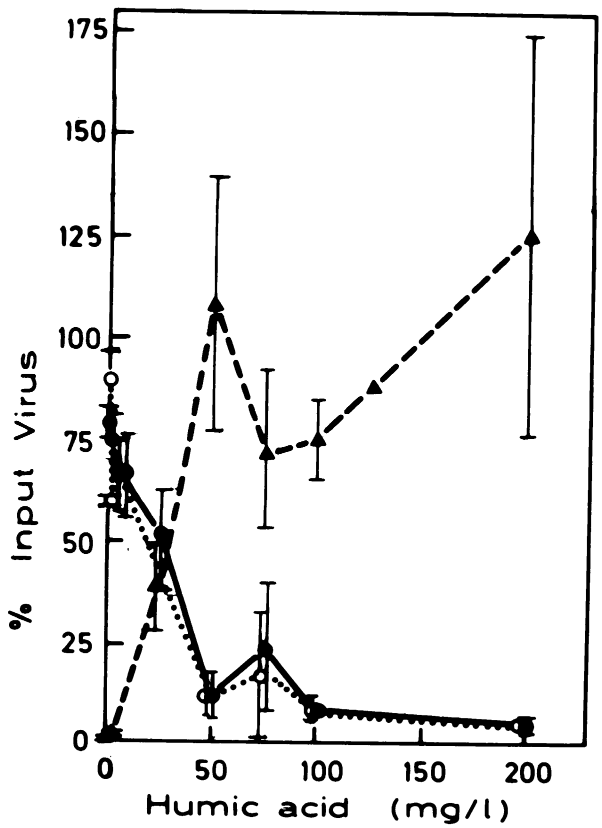

FIG. 2. Effect of humic acid on virus recovery by Cox filters. The experimental procedure and symbols are given in the legend to Fig. 1, except that Cox filters are used. Each point is the average of one to eight experiments.

TABLE 2. Effect of humic and fulvic acids on virus recovery by Balston filters

Humic material and concn (mg/liter)	No. of expts	% Virus recovered in[a]: Eluate	Filtrate	Organic floc
Humic acid				
0	6	73 ± 13	2 ± 2	70 ± 25
5	3	85 ± 8	0.6 ± 0.3	68 ± 14
10	4	12 ± 9	82 ± 46	16 ± 0
25	3	17 ± 3	65 ± 3	8 ± 1
50	3	28 ± 18	79 ± 50	19 ± 21
100	3	10 ± 1.2	80 ± 38	7 ± 5
200	2	8 ± 5	62 ± 3	6
Fulvic acid				
0	3	87 ± 17	4 ± 3	ND
2	1	71	2	ND
10	1	61	10	ND
20	3	79 ± 1.6	3 ± 2	ND
100	1	137	0	ND
200	1	103	0	ND

[a] Percentage of input virus in each fraction ± standard deviation. ND, Not done. Organic flocculation was not performed in all experiments.

and Balston filters, interference was primarily at the level of virus adsorption to the filters.

Balston filters were assayed with fulvic acid, and as was found with the Zeta Plus filters, there was no effect on virus adsorption or recovery by the filters (Table 2).

In summary, the four types of filter differed in their sensitivities, both in terms of the humic acid concentration at which interference was detected and the degree of interference attained. The more electronegatively charged filters were more sensitive than the more electropositively charged filters in both respects.

Adsorption of humic materials. Since adsorption of poliovirus to Zeta Plus filters was interfered with by humic acid but not fulvic acid, the adsorption of these materials to the filters was monitored at the same range of concentrations as in the above experiments. In addition, the adsorption of humic acid was assessed for Seitz filters. Humic acid adsorbed to both types of filters (Fig. 3). The Seitz filters were exposed to up to 100 mg of humic acid per liter and adsorbed essentially all of the input; 86 to 100% of the humic acid was adsorbed. The Zeta Plus filters were tested at a wider range of concentrations, and at the highest level of input humic acid, 75% (158 mg) was adsorbed. Overall, adsorption ranged from 42 to 97% of the input humic acid. Thus, at pH 6.0, the negatively charged humic acid adsorbed efficiently to the relatively positively charged filters. It is therefore possible that the humic acid interfered with virus adsorption by direct competition for sites of attachment on the filters.

In addition, fulvic acid was tested for adsorption to Zeta Plus filters (Fig. 3). This was of interest since, in contrast to humic acid, the fulvic acid did not interfere with virus adsorption. It was found that the fulvic acid adsorbed well to the filters, and at a maximum input concentration of 211 mg/liter, 60% (131 mg) was adsorbed. Fulvic acid adsorption ranged between 61 to 86% of the input.

Virus recovery from large volumes of water. The effect of humic acid on the recovery of poliovirus from water volumes larger than the 1 liter used in the initial experiments was investigated by using two concentrations of humic acid (Table 3). At an input concentration of 5 mg/liter, it was possible to filter 9 liters of water, in contrast to the 65 liters

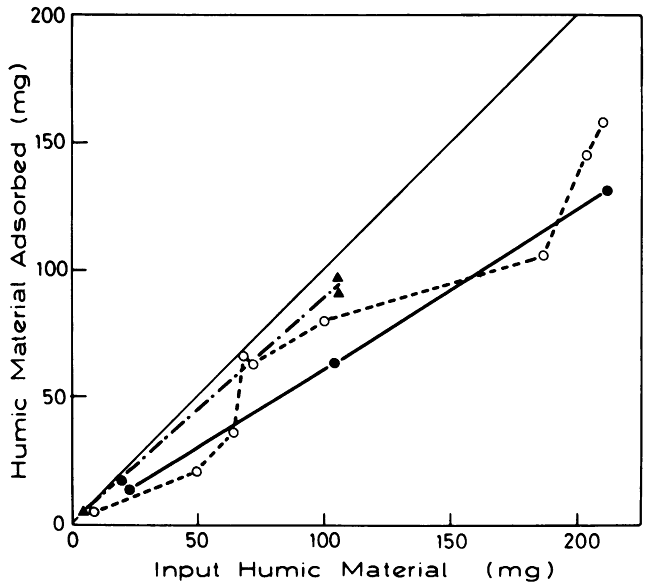

FIG. 3. Adsorption of humic materials to positively charged filters. Humic materials were added to 1-liter volumes of water and filtered through Seitz or Zeta Plus filters. The amount adsorbed was calculated from the input minus the filtrate. Symbols: ○, humic acid and Zeta Plus filter; ▲, humic acid and Seitz filter; ●, fulvic acid and Zeta Plus filter; ——, theoretical line of complete adsorption.

of tap water which can normally be processed (4). This amount of humic acid did not interfere with virus recovery in the smaller volume experiments (1 liter of input water). In spite of the blockage of the filter, 60% of the input virus in the eluate was recovered, both in the absence and presence of humic acid. Thus, although the larger volume decreased elution efficiency, humic acid interference with virus adsorption was not observed.

At the higher humic acid concentration of 70 mg/liter, only 4 liters could be filtered before blockage occurred. In this case, virus was detected in the filtrate, although at a lower percentage than that found for the smaller volume experiments. The recovery in the filtrate and eluate again totalled 60% of the input virus.

DISCUSSION

The effects of humic materials on virus recovery by four types of microporous filter were investigated in this paper. Humic acid interfered with virus recovery by all four filter types, whereas fulvic acid had no effect on recovery by the two types of filter (Balston and Zeta Plus) which were tested.

The negatively charged filters were more sensitive to interference by humic acid than were the positively charged filters at all concentrations of humic acid tested. Recovery by Balston filters was inhibited at concentrations as low as 10 mg of humic acid per liter, whereas the Zeta Plus filters recovered essentially all of the virus at humic acid concentrations of up to 50 mg/liter. At higher humic acid concentrations, less than 10% of the virus was recovered by the Balston filters, whereas the positively charged filters continued to recover 30% or more of the virus. In both cases, the primary level of interference was prevention of virus adsorption, since the virus not found in the eluate could generally be accounted for by the amount of virus in the filtrate.

The results obtained with the electronegatively charged filters suggested a relatively simple mechanism of interference, in which the humic acid, which bound to the Balston filters (5), prevented virus adsorption by occupying virus attachment sites. The fulvic acid, which was not adsorbed by the filters (5), did not interfere with virus attachment. In these experiments, carried out at pH 3.5, the virus is primarily positively charged (8) and would be attracted by the negatively charged filter surface. The soluble fulvic acid would be negatively charged and not adsorbed, whereas the less soluble and less acidic humic acid might be in the form of flocs or have some local positive charge which would allow binding to the filter. In this model, the virus and humic acid sites of attachment to the filter would be the same, and interference would be due to direct competition for available sites.

The effects of the humic materials on virus recovery by the electropositively charged filters were less easily explained by a model based solely on electrostatic interactions. In the absence of humic materials, the virus particles adsorbed efficiently to the filters at pH 6.0. At pH 6.0, the charge on the Zeta Plus filters has been reported to be negative (15) or close to neutral (17). At this pH, poliovirus particles are a mixture of two forms with different isoelectric points (10), although the positively charged form is likely to predominate. Thus, the primary adsorption of the virus may be simply electrostatic.

In the presence of humic materials, however, the results were more complex. Maximal interference with virus adsorption occurred before saturation of the filters with humic acid, although at the highest humic acid concentrations tested, virus was still partially adsorbed by the filters. In contrast, fulvic acid adsorbed to the filters but did not interfere with virus adsorption. It would seem that the humic acid has components which can compete with the virus for adsorption sites on the filter, which are absent from the fulvic acid. In addition, a fraction of the virus may be able to adsorb to additional filter sites or to the humic acid on the filter, since the degree of interference reached a plateau at high humic acid concentrations. Nonelectrostatic bonds (13) may be responsible for partial adsorption of some of the components, including polar and nonpolar interactions. More research is needed to assess the relative importance of the different possible modes of virus adsorption to filters.

In terms of the practical aspects of virus concentration from water, it is clear from these results that humic acid may indeed play a role in decreasing concentration efficiency (3), both for positively charged and negatively charged filters. In addition, these results support the findings of Sobsey and Glass (15) that the positively charged filters are less sensitive to water quality changes than the negatively charged filters. In this respect, of the four filters tested here, the Zeta Plus was the most resistant to interference by humic acid. It is of

TABLE 3. Effect of humic acid on virus recovery from large volumes of water[a]

Humic acid (mg/liter)	No. of expts	Input vol (liters)	% Virus recovered in: Eluate	Filtrate
0	2	9	60 ± 4	0.4
5	2	9	60 ± 11	1 ± 0
70	3	4	37 ± 6	12 ± 5

[a] Water containing the indicated concentration of humic acid was filtered through a Zeta Plus filter until the filter clogged. The largest volume containing humic acid which could be processed was used in the control experiments. Virus recovery expressed as a percentage of the input virus ± standard deviation.

interest to note the lack of interference by fulvic acid, which is frequently found in higher concentrations than humic acid in water (2, 19), indicating that both the type and quantity of organic contaminants are important in predicting virus concentration efficiency from a given type of water. The concentrations of humic substances tested were within the range (up to 300 mg/liter) reported for natural waters (2).

The second concentration step used in these experiments to concentrate the virus was organic flocculation. Organic flocculation from the filter eluates was not influenced by the amount of humic or fulvic acid in the input water. In general, however, this method was not as efficient in recovering virus from the eluate of the positively charged filters as from the negatively charged filters.

The universal applicability of a virus concentration technique should take into account unexpected changes in water quality. Recovery is a complex phenomenon, and further work needs to be done to elucidate the mechanisms of virus adsorption to filters, and the mechanisms of interference with adsorption, to enable the development of methods invariant under a variety of conditions.

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