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SMALL SCALE WASTE MANAGEMENT PROJECT

Removal of Virus From Septic Tank Effluent By Sand Columns

by

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REMOVAL OF VIRUS FROM SEPTIC TANK EFFLUENT BY SAND COLUMNS

K.M. Green D. O. Cliver

Sand columns have been used to model virus removal from septic tank effluent by sandy disposal beds. Sand has been shown to be strongly retentive of poliovirus, but its retentiveness is reduced by use, by low temperatures, and by high flow rates. Retained virus is inactivated at a rate which is dependent upon temperature.

Mounds constructed of 60 cm of sandy fill between a soil-covered seepage bed and the original topsoil have been designed to provide on-site treatment and disposal of septic tank effluent (STE;1). As a means of evaluating the efficiency of these mounds in removal of human intestinal viruses from STE, a variety of sand-filled columns have been employed as models of the field system. Previous studies have shown varying degrees of retention of viruses by sand filters, depending upon conditions (4,6-8); but little attention has been paid to the effect that long-term application of wastewater may have on the system.

METHODS

The columns have been of two types: 60 cm of medium sand (1) in 14.6 or 7.7 cm ID PVC tubing, corresponding to the path length of the field system; and 2-4 cm (10-20 g) of sand in 1.9 cm ID tubing. The 60 cm columns have fritted glass bulbs inserted at intervals for fluid sampling, and removable rubber stoppers for access to fill material. The columns were dosed with poliovirus type 1 (strain CHAT) grown in tissue culture (either primary monkey kidney or HeLa cells) and suspended in STE. The STE is delivered weekly to the laboratory and stored at 8 C until used. In some instances the virus was tagged with ^{32}P before use (5); test samples were dried on aluminum planchets and assayed with a gas-flow GM counter. Viral infectivity assays were performed by passing samples through 0.2 μm Gelman cellulose triacetate membrane filters to remove bacterial contamination (2), making serial dilutions, and testing in tissue cultures by the plaque technique (3). Virus was eluted from fill material by adding 1 ml of fetal or agamma calf serum per gram of fill and stirring for 1 minute. Particulate matter in suspension was removed by filtration through a Millipore AP 04 prefilter.

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RESULTS

Virus removal from STE has been considered in relation to four variables: conditioning, dose rate, temperature, and inactivation.

(1) Conditioning. The nutrients in the STE support microbial activity in the columns, which alters the characteristics of the sandy fill and reduces its retentiveness for virus. This was shown by adding, to 2 cm of moist sand at room temperature, 1 ml of ^{32}P labeled virus in phosphate buffer followed by 9 ml of buffer without virus. Clean sand retained 96% of the labeled virus, whereas conditioned sand retained only 50%.

Two 60 cm columns at 8 C, one filled with clean sand, the other conditioned by 3 weeks of daily dosing with STE at room temperature, were given a single 5 cm (250 ml) dose of STE containing 6.0×10^8 plaque forming units (PFU) of virus, followed by daily 5 cm doses of STE without virus. After 3 days virus had penetrated to 45 cm depth in the conditioned column, but was only detected as far as 20 cm depth in the clean sand. (Fig. 1).

(2) Dose rate. Normal operation of 60 cm columns was considered to be a once-daily 5 cm dose of STE, all of which entered the sand bed within 30 minutes (unsaturated flow). This was contrasted with a situation where sufficient fluid was applied to maintain a layer of fluid above the surface of the sand for a long period (saturated flow).

A 3 cm conditioned sand column at room temperature was dosed with 10 ml of ^{32}P labeled virus in phosphate buffer. The sand was ponded for 2 minutes and retained 61% of the labeled virus. When the same dose was applied dropwise over 2.5 hours (no standing fluid) to a similar column, 96% of the labeled virus was retained.

A 60 cm column at room temperature, which had been receiving 5 cm (850 ml) of STE containing $>10^5$ PFU of virus daily for more than a year with only a single PFU detected in the effluent, was given a 50 cm (8.5 l) dose of STE containing 4.6×10^5 PFU/ml. A sample taken the next morning at 60 cm depth contained 4.5×10^2 PFU/ml (Fig. 2).

(3) Temperature. Two sets of 4 cm columns were constructed with conditioned sand. Two ml of virus in STE was added to each column; then one set was placed at 8 C, and the other set was kept at room temperature (20-22 C). Each column was dosed daily thereafter with 2 ml of STE (no virus). At weekly intervals two columns from each set were examined for virus by removing the sand and eluting the virus in calf serum. The effluents from those columns were also assayed for virus. After 4 weeks the room temperature fill contained 2.5%, and the effluent 0.08% of the virus, a 97% net inactivation. The columns in the cold retained 57% of

the virus activity in the fill and 5.9% in the effluent after 4 weeks, or 37% inactivation (Fig. 3).

(4) Inactivation vs removal. Distinguishing between virus inactivation (loss of ability to replicate in tissue culture), detention (reduced rate of passage through the fill) and retention (immobilization of the virus on fill material) is difficult, as all three occur concurrently. Retention is suggested by the observation that only ~ 70% of ^{32}P labeled virus adsorbed to fill material can be eluted with calf serum.

Detention was shown by adding 1.5×10^{11} PFU in 5 cm (850 ml) of STE to a conditioned 60 cm column and 24 hours later removing ~ 5 g samples of fill material from various depths along the column, eluting with calf serum, and assaying for virus infectivity. This was followed by daily 5 cm doses of STE without virus. The fill was again sampled and assayed. Two days later, virus titers had decreased at 7 cm depth and increased at 14, 22 and 28 cm. However, no virus was ever detected at 45 cm or below, although the initial volume of fluid in which the virus was suspended passed through the column by day 3 (Fig. 4).

Inactivation was shown in the experiment described previously, in which only 2.5% of the virus was recovered after 4 weeks' time in 4 cm conditioned sand columns at room temperature (Fig. 3). Inactivation at room temperature was also demonstrated in the 60 cm conditioned column which received a single inoculation of 1.5×10^{11} PFU. Virus recoveries at all depths decreased with time after day 3, with no indication that the virus had been relocated downward (Fig. 4).

DISCUSSION

Sand has been shown to be effective in removing poliovirus, a presumably typical human intestinal virus, from septic tank effluent. A properly operating sand filtration system should produce effluents that present no hazards from human enteric viruses. However, there are factors which must be taken into account in the design of such systems. The longer the path through the sand, the better, although we have not always found a direct relationship between column length and virus removal, and there may be a point of diminishing returns. The temperature of operation is important: at low temperatures the sand is less retentive and the virus is inactivated more slowly, if at all. Conditioning must also be taken into consideration. The retentiveness of the fill decreases markedly after a few weeks of operation. Finally, dose rate is critical; if the pores between the sand grains are continuously saturated with fluid, a significant proportion of the virus will not adsorb to the sand.

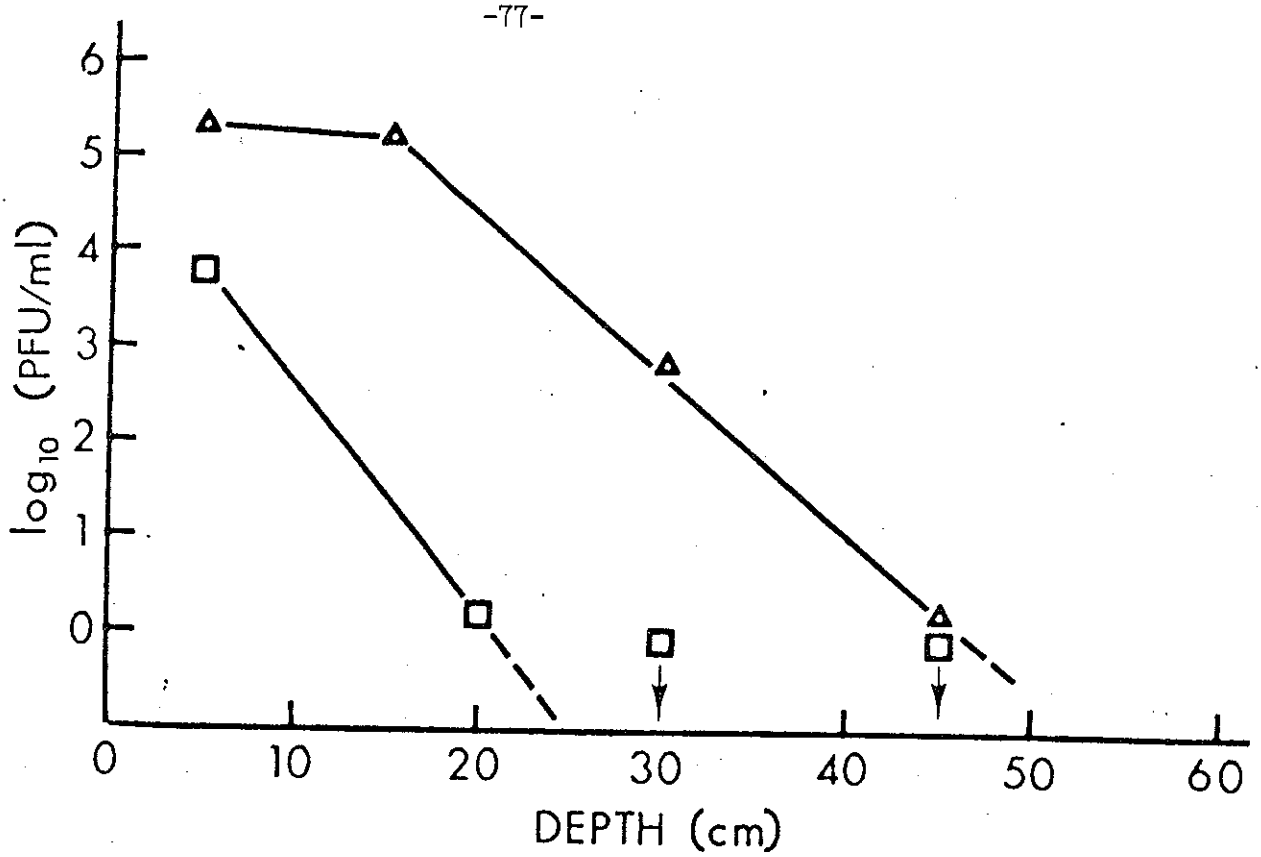

Fig. 1. Penetration of poliovirus into 60 cm sand columns at 8°C three days after inoculation with 5 cm (250 ml) of STE containing 6.0×10^8 PFU/ml. Fluid samples (△ conditioned sand, □ clean sand, ↓ < indicated value).

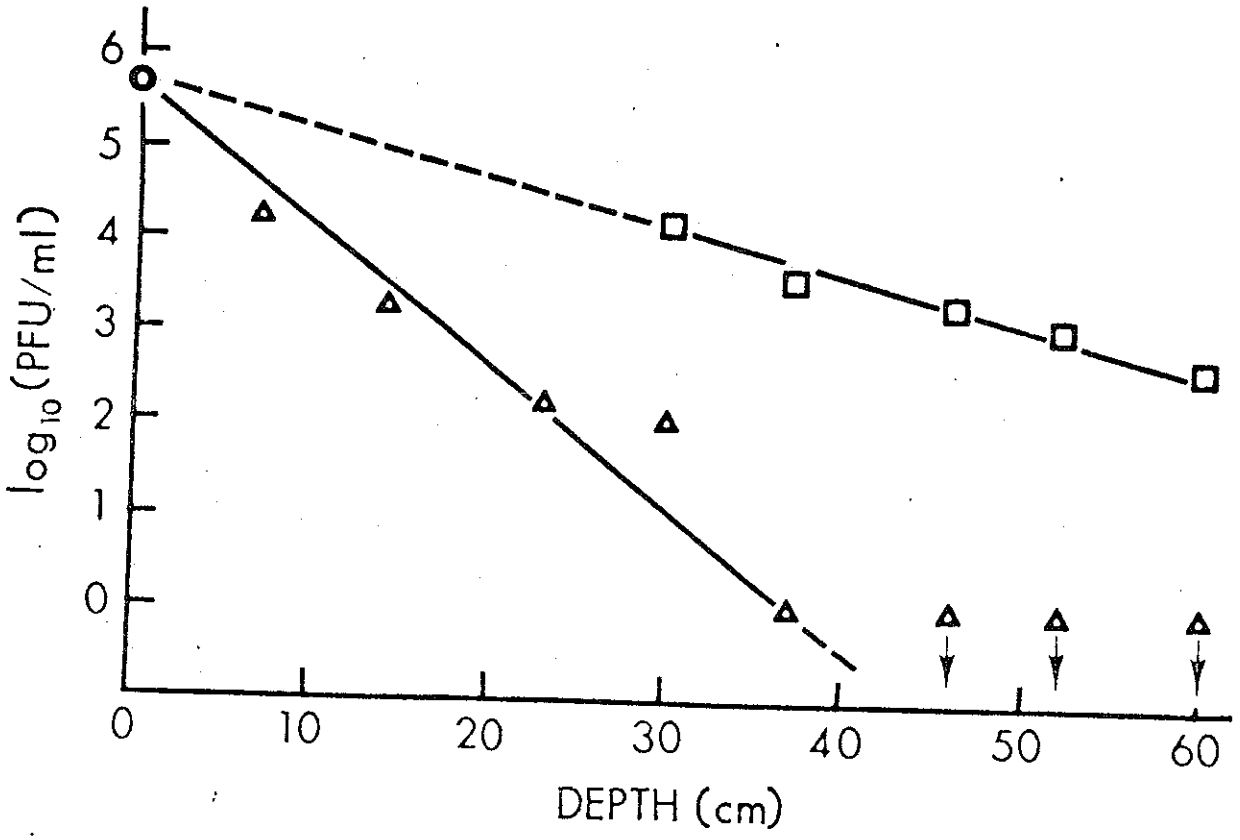

Fig. 2. Penetration of poliovirus into a 60 cm conditioned sand column at room temperature. Fluid samples (△ 5 cm (850 ml) dose, □ 50 cm (8.5 l) dose, ● input titer, ↓ < indicated value).

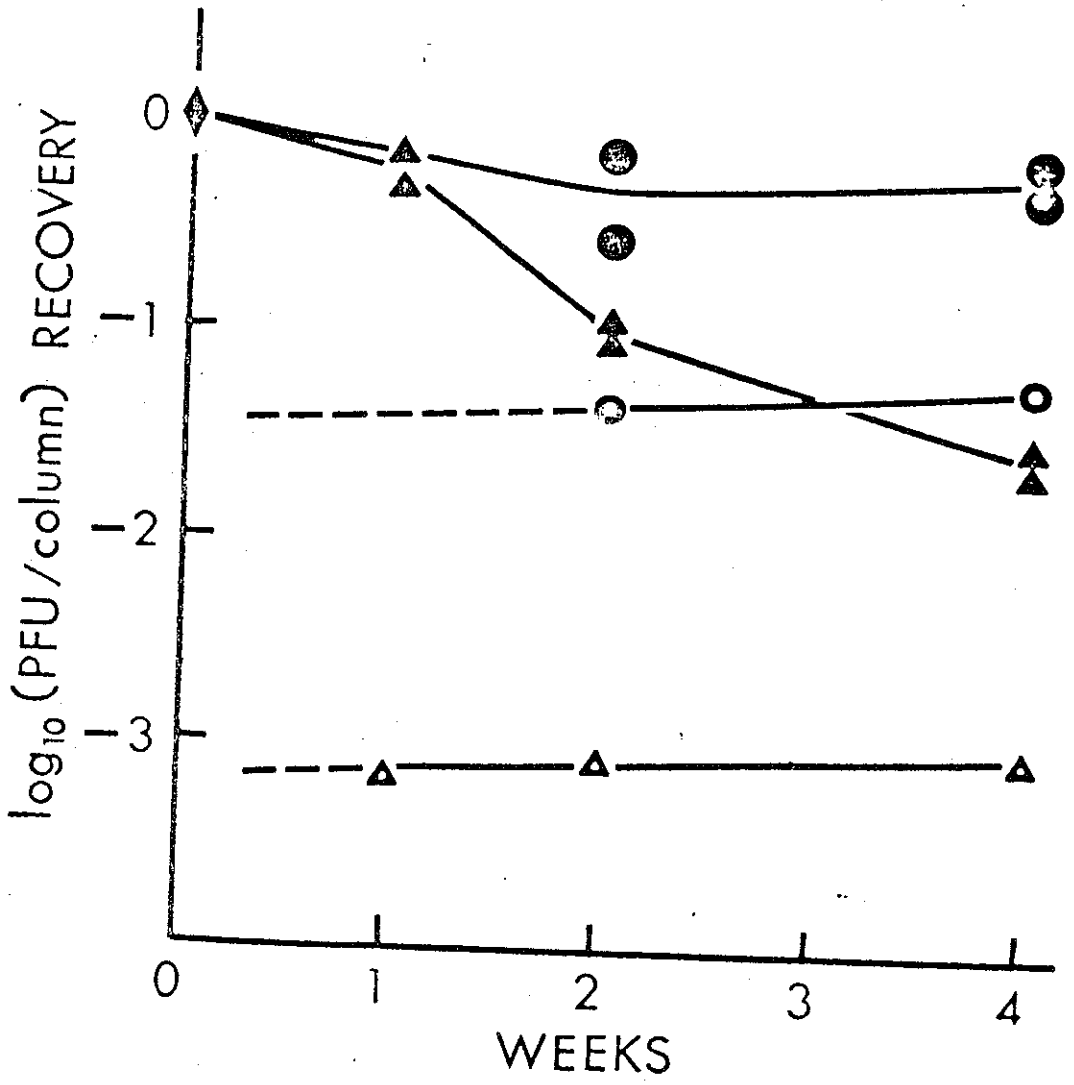

Fig. 3. Recovery of poliovirus from 4 cm conditioned sand columns (● 8°C, fill; ○ 8°C, effluent; ▲ room temperature, fill; △ room temperature, effluent).

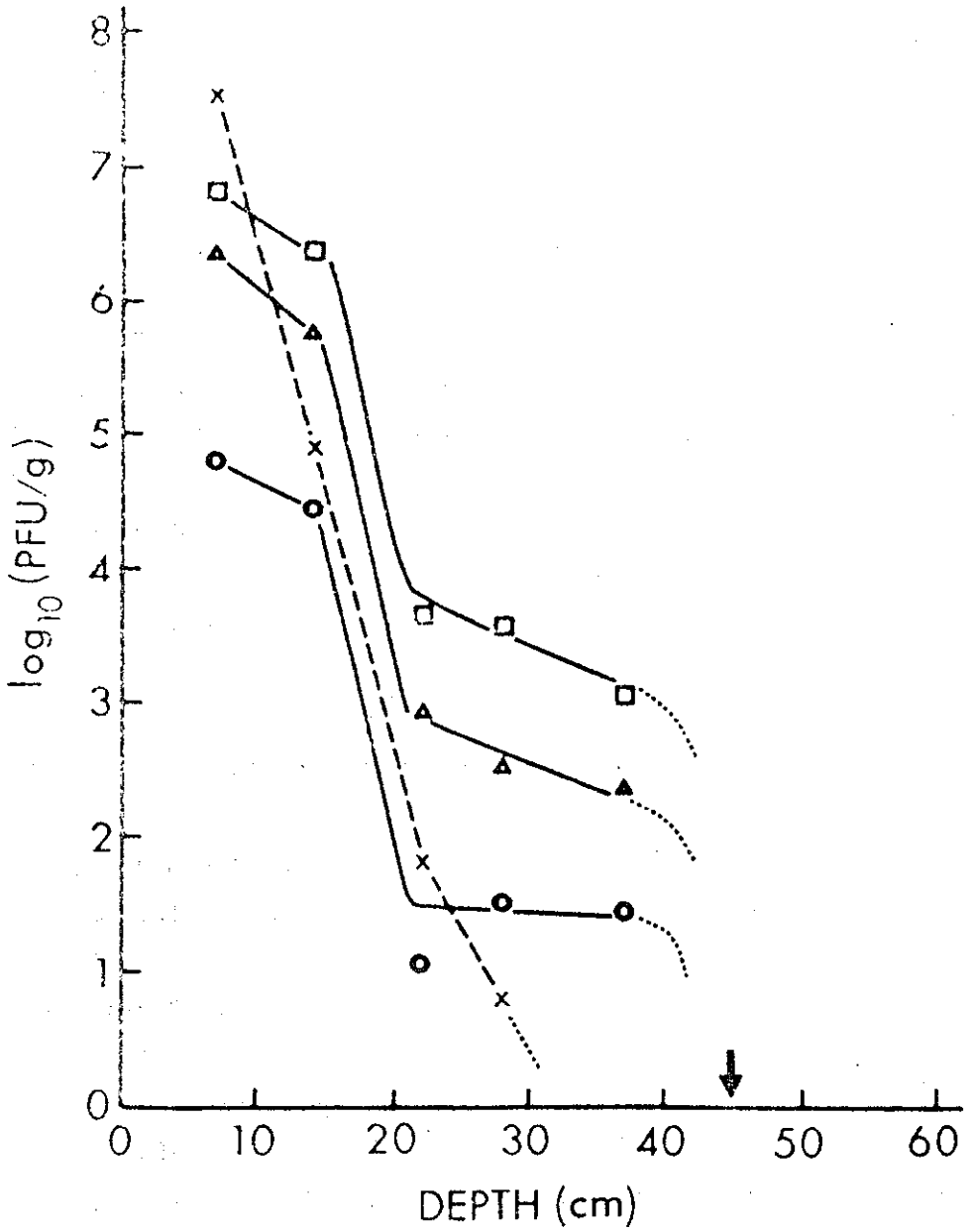

Fig. 4. Penetration of poliovirus into a 60 cm conditioned sand column at room temperature. Fill samples (X 1st day, □ 3rd day, △ 7th day, ○ 24th day after dosing, ↓ no virus detected at this depth).

ACKNOWLEDGMENTS

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