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Reduction of naturally occurring enteroviruses by wastewater treatment processes

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SUMMARY

The levels of cytopathic enteroviruses at two wastewater-treatment works were monitored over a period of 9 months. The maximum level of virus at works 1 was 72500 p.f.u. l^{-1} and at works 2, 57500 p.f.u. l^{-1}. Examination of process efficiency showed an overall reduction of 63 % for works 1 and 26 % for works 2 when used without lagooning. When lagooning was employed at the second works, virus reduction was 97 %. Individual treatment processes showed poor reduction of virus levels. Sedimentation and rapid sand filtration had no significant effect on levels whilst both percolating filtration and activated sludge showed some reduction. Only lagooning resulted in substantial reductions of virus levels.

INTRODUCTION

The demands of modern-day society for the protection of the environment has resulted in the obligation of water undertakings to conform to often very stringent water-quality standards, whether it be effluent discharge, raw potable water or drinking water. In some parts of the world, legislation requires the disinfection of wastewater effluents before discharge, which, whilst minimizing the health risk associated with pathogens, may give rise to concern about the introduction of carcinogens and mutagens into the environment. Such disinfection of wastewater is not normally practised in the United Kingdom.

It could be argued that terminal disinfection would be unnecessary if judicious use was made of existing treatment processes. However, as wastewater treatment processes were designed specifically to meet the demands of physico-chemical standards rather than microbiological standards, any removal of pathogens must be regarded as fortuitous. The following study reports on the efficacy of a range of wastewater treatment processes in reducing the levels of naturally occurring enteroviruses in wastewater.

MATERIALS AND METHODS

Wastewater sampling. During the period from May 1982 to January 1983 samples of wastewaters from two treatment works were examined for the presence of cytopathic enteroviruses. Works 1 serves a large conurbation of about 350000

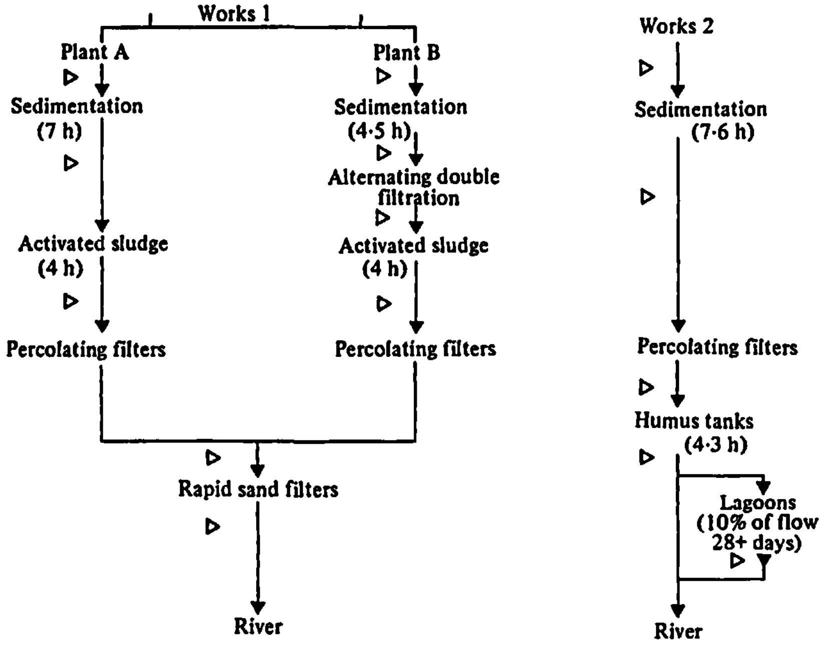

Fig. 1. Flow diagrams for the two wastewater treatment works. ▷, Sample points.

inhabitants and receives a mixture of domestic and industrial wastewater. The two incoming flows are mixed and split more or less equally to serve two independent plants within the works. After treatment the wastewater is recombined and passed through rapid sand filters prior to discharge. Sampling at this works was carried out on a rolling basis using 24 h composite samples. Such a sampling regime should minimize the differences associated with diurnal excretion patterns and weekly patterns. No allowance was made for the temporal relationship of before-and-after treatment samples. The second works serves a rural town of about 60000 inhabitants with a slightly higher proportion of the sewage being domestic when compared with works 1. The treatment process is much simpler than that at works 1 and in addition has the ability to divert up to 10% of the flow into lagoons prior to river discharge (Fig. 1). Samples at this works were taken at the same time on the same day of each week of sampling and no allowance for the temporal relationship of samples was made. In all cases, samples were of 20 cm^3 and stored prior to assay at −20 °C.

Virus assay. Plaquing was carried out using BGM cells in the agar suspended technique previously reported (Morris & Waite, 1980). Aliquots of wastewater samples were assayed by the direct inoculation method of Buras (1974) with no sample pretreatment. All data were analysed using the t (independent means) test with $N-1$ degrees of freedom. No identification of virus isolates was conducted.

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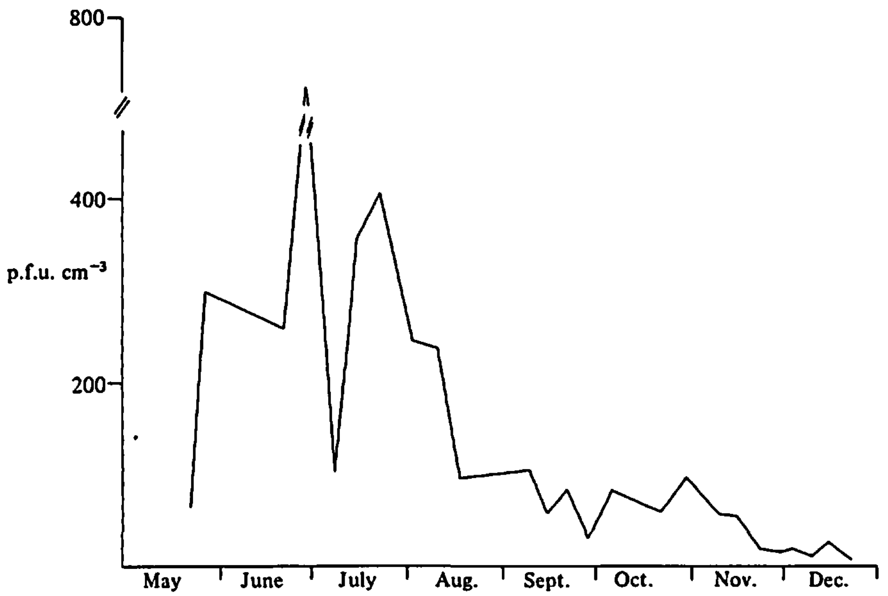

Fig. 2. Virus levels in the incoming sewage at works 1, May–December 1982.

Table 1. *Overall reduction of naturally occurring enteroviruses by two wastewater treatment works*

	% reduction (±S.D.)	Significance (*P*)
Works 1	63 (27)	0·01
Works 2		
Without lagoons	26 (28)	0·3
With lagoons	97 (7)	< 0·001

RESULTS

Virus levels in raw sewage. Levels fluctuated substantially during the period of this study. As can be seen from Fig. 2, there was some evidence of seasonal variation in the levels of virus present in the incoming sewage of works 1 but at all times it was possible to detect viruses. The highest virus levels detected in the combined sewage entering works 1 was 72500 p.f.u. l^{-1}, whilst at works 2 the maximum level detected was 57500 p.f.u. l^{-1}.

Overall performance of works. The lack of a temporal relationship between samples makes it difficult to determine accurately the performance of each works and the individual treatment processes. However, it is felt that the sampling regime at works 1 did allow analysis of treatment efficiency to be made whilst the data from works 2 reflected the trends found at works 1. Results in Table 1 show that, overall, works 1 operated at a virus reduction efficiency of 63% whereas works 2 only operated at 26% when lagooning was not included. The overall performance of works 2 was substantially improved when lagooning was included (> 90%) but it should be noted that the lagoons only treated about 10% of the effluent

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Table 2. *Effect of primary sedimentation on enterovirus levels*

Works	No of samples	Range of virus levels (p.f.u.l^{-1}) Influent	Effluent	% change (±s.d.)	Significance (P)
1A*	26	200–81600	400–71200	+93 (135)	0·2
1B*	25	1000–63400	1000–72000	+25 (60)	0·5
2*	12	1000–57500	2600–33000	+6 (59)	0·7
2†	12	3200–36500	6200–34000	+29 (52)	0·7

* Primary sedimentation. † Humus tanks.

Table 3. *Effect of percolating filtration on enterovirus levels*

Works	No. of samples	Range of virus levels (p.f.u. l^{-1}) Influent	Effluent	% change (±s.d.)	Significance (P)
1A*	28	400–75800	<200–72000	−30 (68)	0·2
1B*	25	1000–72000	400–72000	−44 (42)	0·8
1B†	28	<200–82800	200–58800	−2 (42)	0·2
2*	12	2600–3300	3200–36500	−24 (24)	0·5

* Percolating filter beds. † Alternating double filtration.

Table 4. *Effect of activated sludge on enterovirus levels*

Works	No. of samples	Range of virus levels (p.f.u. l^{-1}) Influent	Effluent	% change (±s.d.)	Significance (P)
1A	25	400–71200	400–75800	−30 (32)	0·3
1B	25	400–72000	400–75600	+32 (87)	0·8

production, thus its use in improving the quality of the final effluent was of marginal benefit.

Primary sedimentation. This process is designed for the removal of settleable suspended solids and not for improvement of microbiological quality. It would be reasonable to expect some reduction of virus levels because adsorbed and embedded particles should be removed with the solids. Our results suggest that removal was only minimal, possibly due to the elution of viruses from the solids or to the breakup of solids allowing the freeing of embedded viruses. The ineffectiveness of short-term sedimentation for the removal of viruses was confirmed by the lack of removal found in humus tanks with comparable retention times (Table 2).

Percolating filtration. This commonly used treatment process is aimed at the reduction of biochemical oxygen demand by utilizing micro-organisms to oxidize the available nutrients in the wastewater. Table 3 shows that the process is capable of reducing virus levels but not to any great extent. A variation of the percolating filter system, alternating double filtration, where humus tanks are operated between each filter bed, did not enhance virus removal.

Table 5. *Effect of tertiary treatments on enterovirus levels*

Works	No. of samples	Range of virus levels (p.f.u. l^{-1}) Influent	Effluent	% change (±S.D.)	Significance (*P*)
1*	28	< 100–30200	100–27000	+102 (284)	0·7
2†	11	4000–34000	< 50–500	−99·1 (0·8)	< 0·001

* Rapid sand filter. † Lagoons.

Activated sludge. This biological oxidation treatment process is probably the most important of the secondary treatments of wastewater. It is capable of high loadings but is susceptible to fluctuations in the quality of the incoming wastewater. Under the conditions employed at works 1, retention times were short (about 4 h) and, perhaps not unexpectedly, the removal of virus was erratic. Table 4 details the performance of two plants in works 1, and the best reduction recorded in this study was 86%. Even allowing for the lack of temporal relationship between the samples, it is obvious that short-term activated sludge treatment was ineffective for virus removal.

Tertiary treatments. Only rapid sand filtration and lagooning were considered in this study. Sand filtration of wastewater is used solely to remove any carried-over suspended solids and virus removal should be negligible unless they are solids-associated. This is confirmed by the results shown in Table 5. Lagooning, however, had a marked effect on virus removal, with > 99% reduction being achieved, although it is worth noting that viruses were still detected in about 45% of samples, albeit at low levels (Table 5).

DISCUSSION

Efficient treatment of wastewater aims to reduce the effect of effluent discharges on the receiving watercourse in terms of public nuisance and effect on animal life. In addition, the better the quality of discharged effluents, the fewer the problems that arise further downstream, where the river water may be abstracted for potable supply. The usual measure of the efficiency of wastewater treatment is based on the suspended solids – biochemical oxygen demand index but, increasingly, in many parts of the world there is a need to achieve microbiological standards with particular reference to such pathogens as viruses (White, 1982). Our results indicate that many treatments currently in use are not adequate to meet such standards.

Sedimentation has not been shown to be effective in removing viruses, probably because of the short retention times normally used. The inadequacy of the process has been noted before by many workers (Bloom *et al.* 1959; Clarke *et al.* 1961; Kelly, Sanderson & Nèidl, 1961; Mack *et al.* 1962; Malherbe & Strickland-Cholmley, 1967) but Rao, Lekhe & Waghmare (1981*a*) have advocated the system as being useful in under-developed countries where land may not be at a premium even though maximum reductions of 83% were reported. It is probable that substantial reductions can be achieved over longer periods of sedimentation-storage as reported for lagoons in this study and by Rao, Lekhe & Waghmare (1981*b*).

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Oxidation of wastewater, either by percolating filtration or activated sludge, is probably the most efficient means of improving its quality, but under field conditions reported herein reduction of virus levels was not marked. This confirms the experience of others where percolating filtration was found to be ineffective under field conditions (Malherbe & Strickland-Cholmley, 1967) although under laboratory conditions substantial reductions could be achieved (Clarke & Chang, 1975). Similarly, field experience with activated sludge has shown variable removal (Kelly & Sanderson, 1959) whilst laboratory studies have shown that 90% removal can be achieved (Lund, Hedstrom & Jantzen, 1969; Malina *et al.* 1974; Balluz, Jones & Butler, 1977).

Tertiary treatment of treated wastewater is of no consequence unless it is either long-term stabilization ponds or disinfection. Our results indicate the usefulness of lagooning, supporting the evidence of Rao *et al.* (1981*b*) and Sheladia, Ellender & Johnson (1982), although the latter workers commented on the ability to detect viruses in effluent even after 98 days retention.

Individually, none of the commonly used treatment processes affects virus levels substantially, other than disinfection and lagooning. However, the overall effect of wastewater treatment is to reduce virus levels significantly in percentage terms but not in terms of actual numbers. For example, at works 1 virus levels are reduced by 63% but this is only a reduction from $1{\cdot}4 \times 10^{12}$ p.f.u. per day to $5{\cdot}4 \times 10^{11}$ p.f.u. per day at an average daily flow of 100 Ml. Substantial reliance is thus placed upon the natural purification processes of the receiving water as well as on an adequate dilution factor. That such wastewater treatment processes and dilution in rivers gives an adequate protection to the environment and does not pose problems at downstream abstractions is supported by the failure to detect enteroviruses in drinking water derived from river water containing effluent discharges (Morris, unpublished findings). However it is recognized that disinfection of wastewater may be the only means of reducing pathogen levels in wastewater effluents where there is a need to recycle water rapidly for potable use but, bearing in mind the potential hazards of chemical compounds produced by such treatment, it would be advisable to examine closely non-disinfecting treatment processes with a view to optimizing their efficiency for virus removal.

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