Elimination of enteroviruses, other enteric viruses, F-specific coliphages, somatic coliphages and *E. coli* in four sewage treatment plants of southern Germany

Jens Fleischer, Karin Schlafmann, Rowland Otchwemah and Konrad Botzenhart

ABSTRACT

The reduction processes at four advanced sewage treatment plants in Baden-Württemberg were evaluated with regard to virus elimination and the elimination of indicator organisms from wastewater. The results of virus elimination were compared with the reduction of somatic and male specific bacteriophages and of *E. coli*. In total, 222 water samples were examined. The results obtained for the different treatment plants show reduction rates from 80.0% to 99.9% for enteroviruses, enumerated as PFU l^{-1} on BGM cell line, and reduction rates from 59.4% to 99.9% for other enteric viruses, enumerated as MPN l^{-1} on MA-104 cell line. Identification of the isolated enteroviruses yielded 88.3% for Coxsackie virus B (1-5), 18.3% were positive for Polio (1-3) and 8.3% for Echo virus (1+11). The reduction rates of somatic bacteriophages ranged from 76.4% to 99.90%, for male specific bacteriophages from 87.5% to 99.9% and for *E. coli*. from 75.0% to 99.9% respectively. Two of the plants use standard chemical precipitation and the other two employ combinations of chemical and biological elimination techniques to reduce the concentrations of phosphorus and nitrogen. A correlation between the amount of precipitators and the elimination rates of the tested microorganisms could not be demonstrated, perhaps due to the fact that the treatment conditions could not be modified by the investigators. It is concluded that the tested treatment plants using combinations of chemical and biological techniques for P and N removal show equal or higher elimination rates than conventional treatment processes using chemical elimination techniques.

Key words | biological phosphorus elimination, coliphages, *E. coli*, sewage treatment, virus adsorption, virus elimination

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INTRODUCTION

Conventional wastewater treatment technology aims at the reduction of suspended solids, BOD (biological oxygen demand) and COD (chemical oxygen demand). Advanced treatment processes as enforced by German and European directives additionally aim at the reduction of phosphorus (P) and nitrogen (N). During the last decade more and more biological phosphorus removal processes were installed in the treatment plants, which no longer require large amounts of precipitators. PO_4-precipitators, as used in nearly 90% of treatment plants in Baden-Württemberg, are known to be highly effective in reducing the concentration of viruses and other pathogens in wastewater. However, data on the efficiency of virus elimination during the different sewage treatment steps show wide variations (see Table 1) ranging from 0% for sedimentation/activated sludge (Morris 1984) to 98% for secondary effluent (Rose *et al.* 1996) and to values between 99% and 99.9% for tertiary treatment by

Table 1 | Virus and phage removal by different wastewater treatment technologies

Type of treatment technology	Virus and phage removal
Primary treatment:	
Sedimentation	0% (Morris 1984); ca. 20% (Irving ⟨ Smith 1981); 20% (coliphages, Zaiss ⟨ Hennies 1988)
Activated sludge	0%–30% (Morris 1984); 28–93% (Irving ⟨ Smith 1981); 83% (46%–100%) (Rolland *et al.* 1983); 73% (coliphages, Zaiss ⟨ Hennies 1988); 98% (Rose *et al.* 1996); 94% (Nieuwstad *et al.* 1988)
Tertiary treatment:	
Lagoons	99% (Morris 1984)
Coagulation-sedimentation-filtration	99.6% (Dryden *et al.* 1979)
Coagulation-filtration	99.7% (Rose *et al.* 1996)
Post-precipitation or post-filtration	99.6–99.8% (Nieuwstad *et al.* 1988)

lagooning (Morris 1984), coagulation-filtration (Rose *et al.* 1996) or post-precipitation and post-filtration (Nieuwstad *et al.* 1988). In this study the efficiency of virus elimination of advanced wastewater treatment processes, including P and N reduction as presently used for municipal sewage, has been evaluated. It was, however, not possible to modify the treatment conditions according to the wishes of the investigators. For concentration and recovery of viruses the glass-wool filtration technique first described by Villaginés *et al.* (1993) was optimized. For the differentiation of isolated enteroviruses, the neutralization method with different antisera pools was applied.

MATERIAL AND METHODS

Sampling data

Wastewater samples were taken from four treatment plants sited at Tübingen (TÜ), Reutlingen (RT), Heidelberg (HG) and Wendlingen (WE) which are located along the River Neckar or a tributary river (RT). Five litre samples of primary treated sewage (which had passed through settling basins) were taken by autosamplers (Bühler) as 24 h mixed samples in proportion to the quantity of incoming wastewater. At the same time 10 l samples were taken at the end of the treatment processes (after the last settling basin) at each plant. Samples were taken from August 1995 to September 1996 each alternate week. Simultaneously, 250 ml samples were taken for bacterial assays.

Treatment plants

Tübingen (TÜ)

The treatment plant at Tübingen is directly located on the River Neckar and is charged with the sewage from the city of Tübingen and six small villages located in the Ammer valley. The maximum capacity amounts to about 200,000 population equivalents (pe). The Tübingen plant, expanded in 1979, works as a multistage plant with mechanical and biological sewage and sludge treatment. To fulfil the directives for phosphate in the treated water, discontinuous simultaneous precipitation with $Al_2(SO_4)_3$ (see Table 2) has been installed. Discontinuous simultaneous precipitation is where the dosage of precipitator is

Table 2 | Average concentrations of the precipitators and COD in the primary treated sewage during the sampling period at the four different treatment plants

Treatment plant	Average load of COD [kg d^{-1}]	Maximum capacity [pe*]	Precipitator	Average molar concentration of precipitator [mol l^{-1}]
Reutlingen	5388	300,000	$FeCl_3$	0.119
Tübingen	7423	200,000	$Al_2(SO_4)_3$	0.007
Heidelberg	21,939	380,000	$FeCl_3$	0.110
Wendlingen	6723	170,000	$FeCl_3$	0.025

*Population and population equivalents.

proportional to the amount of incoming wastewater or the end concentration of PO_4-P in the sewage effluent (discontinuous), and the precipitator is dosed at the start of, or during, the biological treatment step (simultaneous). At the time of sampling the plant was working to capacity and lacked nitrification or denitrification techniques, but directives demanded that the facilities be extended in subsequent years.

Reutlingen-West (RT)

The treatment plant at Reutlingen, last extended in 1984 and located on the River Echaz, releases treated wastewater indirectly into the River Neckar. Reutlingen works as a multistage plant with mechanical and biological sewage and sludge treatment. It also has continuous simultaneous phosphate precipitation, where the precipitator is added in excess of, and not proportional to, the amount of incoming wastewater or the end concentration of PO_4-P in the sewage effluent. This means a significantly high dose of precipitation agents in comparison with the other plants (see Table 2). The result is a very efficient plant in general. The maximum capacity is about 300,000 pe.

Wendlingen (WE)

The extension was completed in 1994. The plant is charged with the sewage water from the cities of Wendlingen, Kirchheim-u.T. (under Castle Teck) and Weilheim, and their neighbouring communities: Dettingen-u.T., Holzmaden, Köngen, Oberbohingen, Ohmden and Unterensingen. The Wendlingen treatment plant is a multistage plant with mechanical and biological sewage and sludge treatment and simultaneous phosphate precipitation. The biological P-removal is highly efficient, but a chemical precipitator is used in addition. To fulfil the directives, a discontinuous simultaneous precipitation has been installed. The dosage of the precipitator is in proportion to the online registered actual amount of ortho-phosphate PO_4-P in the treated water. The maximum capacity amounts to about 170,000 pe. The Wendlingen plant is apparently the best equipped of the four plants investigated in this study.

Heidelberg (HG)

The extension of the plant, located on both sides (plant south and north) of the River Neckar, dates from 1994. The plant is charged with the sewage water from the City of Heidelberg. The Heidelberg treatment plant is a modern multistage plant with mechanical and biological sewage and sludge treatment. The required phosphate concentrations in the effluent are obtained by simultaneous phosphate precipitation at the end of the biological treatment step. The dosage of the precipitator is related to the redox potential in the anoxic zone of the tertiary treatment stage. The maximum capacity is about 380,000 pe.

Concentration of viruses

Viruses in the wastewater were concentrated by a combined filtration and elution method (Fleischer & Botzenhart 1996). The 5 l or 10 l water samples were initially adjusted to pH 3.5 with 1 M HCl after the addition of $AlCl_3$ to a final concentration of 0.5 mM. Samples were then filtered under positive pressure through glass wool (Orgel; Rantigny France, R725) packed columns (Ø 40 mm). The adsorbed viruses were then eluted with 300–500 ml of 0.1% (w/v) skim milk powder (Oxoid) in 0.05 M glycine-buffer, pH 9.5. To reduce the volume of skim milk in the primary eluates to a volume of 15–40 ml, the eluates were reconcentrated by organic flocculation (Safferman *et al.* 1988) (pH 4.5) 1–1.5 h after elution, and centrifugation (4500 × g for 30 min). The resulting pellets were dissolved in 15–40 ml 0.15 M Na_2HPO_4 (pH 7.4) and kept overnight at 4°C. After resolubilization each sample was filtered (Sartorius Minisart filters; 0.45 μm; previously saturated with 1.5% (w/v) beef extract solution adjusted at pH 7.4) to remove bacterial contamination. The samples were stored at −70°C until further processing.

Plaque assay

For the overlay technique buffalo green monkey kidney cells (BGM-Flow) were cultivated. First, 10 × 1 ml of each thawed concentrate was added to a 3 day old BGM monolayer in 90 mm Ø petri dishes (Falcon). After incubation for 1–1.5 h at 37°C in a 5% CO_2 atmosphere, excess eluate was removed and the cells were overlaid with medium and agar. After 4–6 days incubation at 37°C and 5% CO_2, the cultures were stained with crystal violet and plaques were counted. The virus concentration was estimated as the number of plaque-forming-units/litre (PFU l^{-1}).

MPN technique

For the quantification of virus concentration as most probable number (MPN), foetal Rhesus monkey kidney cells (MA-104) were cultivated in 24-well-plates as a monolayer. After incubation for 1–1.5 h at 37°C in a 5% CO_2 atmosphere, excess eluate was removed and the cells overlaid with medium. Cells were incubated for at least 14 days and permanently controlled for cytopathogenic effects. Virus concentration was estimated as the most probable number/litre (MPN l^{-1}).

Identification of enteroviruses

In total, 137 plaques from 60 different samples were taken from the petri dishes and incubated again for 3 days in BGM cells. The supernatants of the infected monolayers in the 24-well-plates were stored at −70°C for further processing. Neutralisation tests were carried out after the protocol from RIVM Holland with Melnick antisera pools against Polio 1–3, Coxsackie A9 and B1–B6, Echo 1–7, 9, 11–14, 20–22, 25, 27, 29, 30 and 33.

Bacterial and phage assays

For the quantification of *E. coli* in wastewater, 0.1 ml aliquots of dilutions (10-fold) were directly plated on antibiotic (Cefsulodin®) treated Chromocult®-Agar (Merck; Darmstadt). Somatic coliphages were detected by a single agar layer method (host strain: *E. coli* ATCC 13706) as described by Grabow & Coubrough (1986), and F-specific RNA-coliphages were detected by a double agar layer method according to ISO-10705-1 using the host strain *Salmonella typhimurium* WG49 described by Havelaar *et al.* (1993). Samples were filtered (Sartorius Minisart filters; 0.45 μm; previously saturated with 1.5% (w/v) beef-extract adjusted to pH 7.4) to remove bacterial contamination. All agar plates were incubated inverted at 37°C for at least 24 h. Concentrations were estimated as CFU ml^{-1}.

RESULTS

The results obtained for the concentrations of the microorganisms in primary treated sewage and sewage effluent,

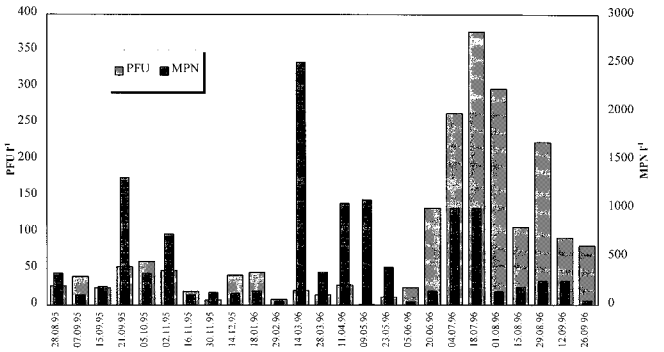

Figure 1a | Concentrations of viruses in primary treated sewage (l^{-1}), results for the plant at TÜ. The results given as PFU show concentrations of enteroviruses enumerated as plaque-forming-units, results given as MPN show concentrations of other enteric viruses enumerated as most probable number.

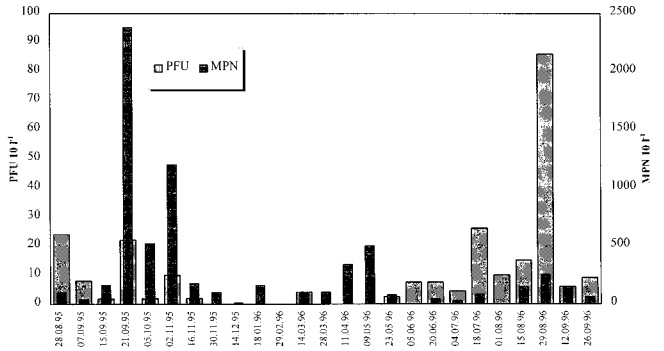

Figure 1b | Concentrations of viruses in sewage effluent (10 l^{-1}), results for the plant at TÜ. The results given as PFU show concentrations of enteroviruses enumerated as plaque-forming-units, results given as MPN show concentrations of other enteric viruses enumerated as most probable number.

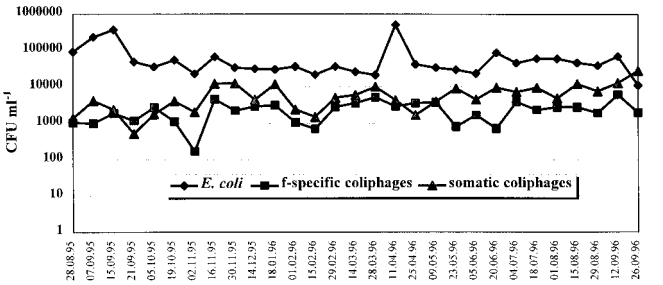

Figure 2a | Concentrations of *E. coli*, somatic and f-specific coliphages (log cfu ml^{-1}) in primary treated sewage, results for the plant at TÜ.

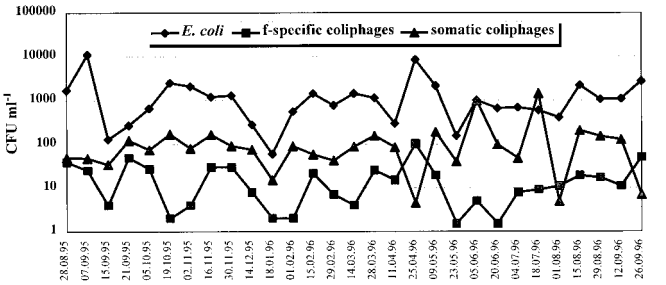

Figure 2b | Concentrations of *E. coli*, somatic and f-specific coliphages (log cfu ml^{-1}) in sewage effluent, results for the plant at TÜ.

and their seasonal distribution (viruses only), for the different plants are shown in Figures 1a–8b.

As expected, the concentrations of viruses showed large variations between sampling dates, sometimes differing by a factor of 100. The enteroviruses, determined as PFU with the plaque assay, showed seasonal fluctuations with significantly higher virus concentrations in late summer and autumn at all four treatment plants. The concentrations of enteric viruses (enteric viruses cultivatable on the MA-104 cell line which means enteroviruses and rotaviruses showing certain CPEs (cytopathogen effects), determined as MPN, reached additional peaks during the year. The additional peaks of the enteric viruses occurred at different times at the different plants.

The concentrations of the viruses as determined by the plaque assay ranged from zero to ca 500 PFU l^{-1} in primary treated sewage, and from zero to ca 8 PFU l^{-1} in the effluent. When calculated as MPN from cytopathogenic effects in cell culture, the concentrations ranged from zero to ca 3,500 MPN l^{-1} after the first settling step and from zero to ca 240 MPN l^{-1} in the effluent. Higher concentrations in primary treated wastewater were usually associated with above-average findings in the secondary effluent.

In every sample *E. coli* was present, with concentrations ranging most frequently between 10^4–10^5 CFU ml^{-1} in primary treated wastewater (see Table 3) and 10^2–10^3 CFU ml^{-1} in effluent. Successive samples showed big differences in concentrations of *E. coli,* which was also observed with viruses. The numbers of f-specific and somatic coliphages were always below those of *E. coli*.

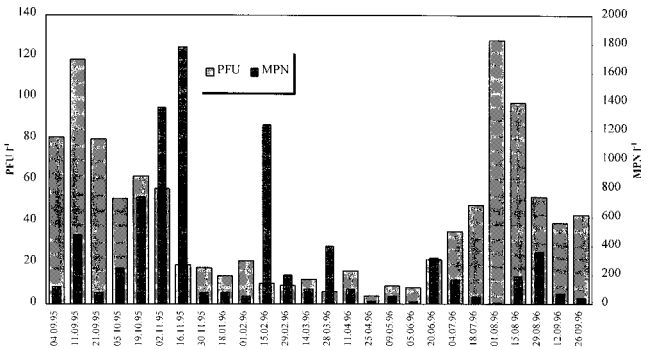

Figure 3a | Concentrations of viruses in primary treated sewage (l^{-1}), results for the plant at RT. The results given as PFU show concentrations of enteroviruses enumerated as plaque-forming-units, results given as MPN show concentrations of other enteric viruses enumerated as most probable number.

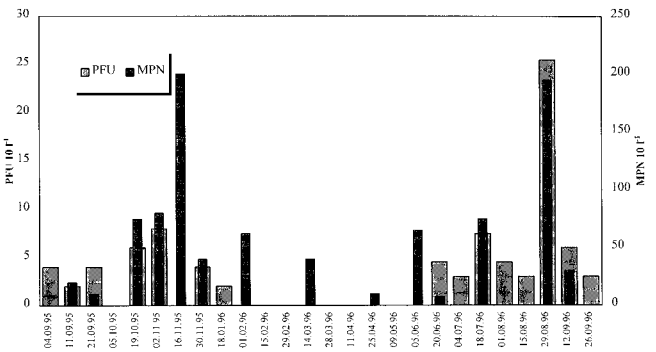

Figure 3b | Concentrations of viruses in sewage effluent (10 l^{-1}), results for the plant at RT. The results given as PFU show concentrations of enteroviruses enumerated as plaque-forming-units, results given as MPN show concentrations of other enteric viruses enumerated as most probable number.

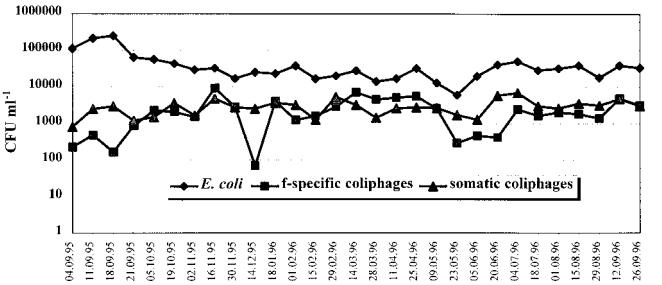

Figure 4a | Concentrations of *E. coli*, somatic and f-specific coliphages (log cfu ml^{-1}) in primary treated sewage, results for the plant at RT.

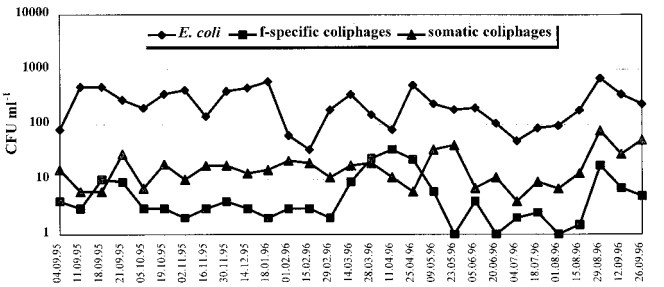

Figure 4b | Concentrations of *E. coli*, somatic and f-specific coliphages (cfu ml^{-1}) in sewage effluent, results for the plant at RT.

The f-specific coliphages seem to get reduced more effectively during the wastewater treatment than the somatic phages. Neither the bacteria nor the phages showed any seasonal fluctuations. Figure 9 shows the reduction capacities for the different microorganisms at the four plants. Enteroviruses and f-specific coliphages are retained more efficiently and with smaller variations compared to *E. coli*, somatic coliphages and the enteric viruses. Enteroviruses were reduced by 80.0% to 99.9%, while the enteric viruses showed the lowest reduction rates (59.4% to 99.9%) with the greatest deviations of the parameters tested. The treatment plant RT attained the highest reduction in four of the five parameters and the lowest deviations, followed by WE, HG and TÜ.

The six combinations of isolated and identified enteroviruses are shown in Table 4. Figure 10 shows the percentage of the different enterovirus genera in positive sewage samples including the samples positive for more than one genus. Of the tested samples, 45 ($n = 60$) contained only Cox B virus, four of the samples contained only Polio virus and two of the samples contained only Echo virus. Nine samples contained two or more different enterovirus genera. For confirmation of the results obtained in our laboratory, and for further differentiation of Polio and Echo viruses, 23 isolates were sent to the National Reference Centre for Enteroviruses at the Robert-Koch-Institut (RKI) in Berlin, Germany. RKI confirmed our results pertaining to the enterovirus classification and found Polio virus Type 1 in 10 isolates, Type 2 in six isolates and Type 3 in one isolate. Only one sample contained Type 1 + 2. All isolates were identified as Sabin-like-Polio. Echo

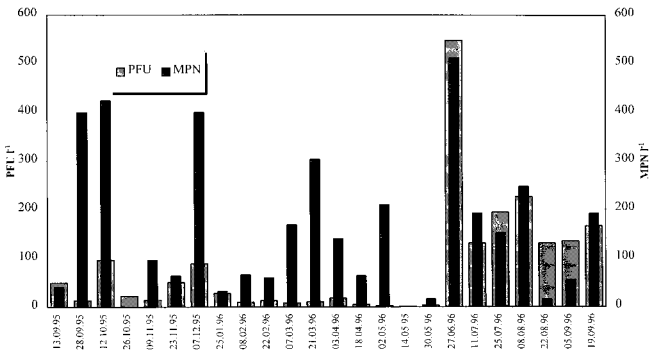

Figure 5a | Concentrations of viruses in primary treated sewage (l^{-1}), results for the plant at WE. The results given as PFU show concentrations of enteroviruses enumerated as plaque-forming-units, results given as MPN show concentrations of other enteric viruses enumerated as most probable number.

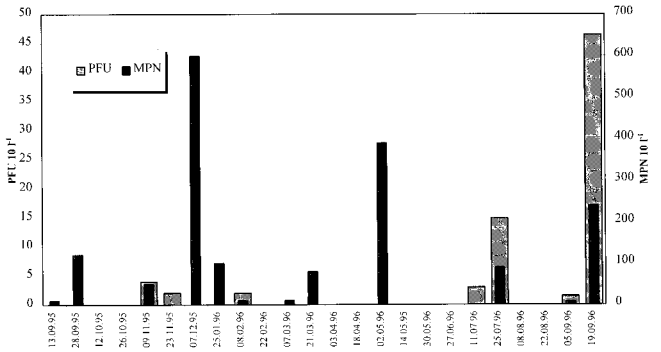

Figure 5b | Concentrations of viruses in sewage effluent (10 l^{-1}), results for the plant at WE. The results given as PFU show concentrations of enteroviruses enumerated as plaque-forming-units, results given as MPN show concentrations of other enteric viruses enumerated as most probable number.

11 was found in all echopositive isolates, three of them contained Echo 1 + 11.

DISCUSSION

Dependent on the amounts of precipitators used (Table 2), the four treatment plants present differences in their reduction capacities for the examined microorganisms. All four plants use simultaneous precipitation. The TÜ treatment plant, which employs chemical phosphorus elimination, required only 2.35 kg (0.007 mol) of $Al_2(SO_4)_3$ per 1,000 m^3 preconditioned wastewater, whilst the RT plant, using $FeCl_3$ for chemical phosphorus elimination, needed 34.24 kg (0.119 mol) of the precipitating agent. The WE and HG plants, in contrast, use a combination of chemical and biological phosphorus elimination and required 6.88 kg (0.025 mol) and 29.74 kg (0.110 mol), respectively, of precipitators to attain either 1 mg PO_4-P l^{-1} or 0.5 mg PO_4-P l^{-1} in the sewage effluent.

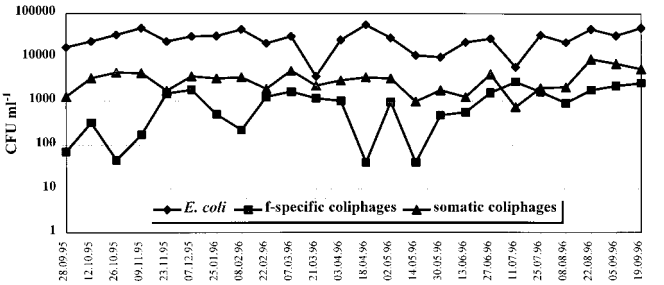

Figure 6a | Concentrations of *E. coli*, somatic and f-specific coliphages (log cfu ml^{-1}) in primary treated sewage, results for the plant at WE.

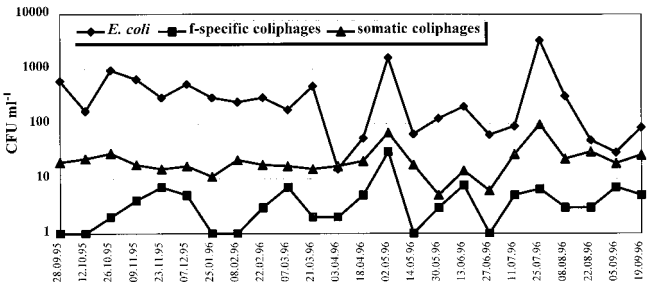

Figure 6b | Concentrations of *E. coli*, somatic and f-specific coliphages (log cfu ml^{-1}) in sewage effluent, results for the plant at WE.

Biological treatment alone, as used today, is not sufficient to fulfil the directives for wastewater treatment regarding the elimination of N and P at these four plants. Therefore the dosage of precipitators is still inevitable, even at plants which perform the newer, biological P-removal technologies.

With respect to the good reduction potential of $FeCl_3$ for viruses, F-specific and somatic coliphages, as well as *E. coli*, the results of this work are supported by the findings of Morris (1984), Lewis & Metcalf (1988) and Steinmann &

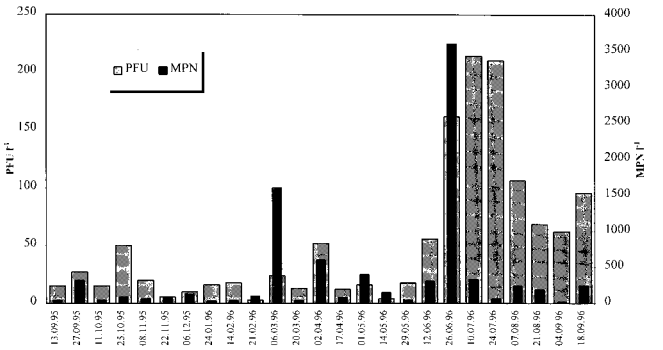

Figure 7a | Concentrations of viruses in primary treated sewage (l^{-1}), results for the plant at HG. The results given as PFU show concentrations of enteroviruses enumerated as plaque-forming-units, results given as MPN show concentrations of other enteric viruses enumerated as most probable number.

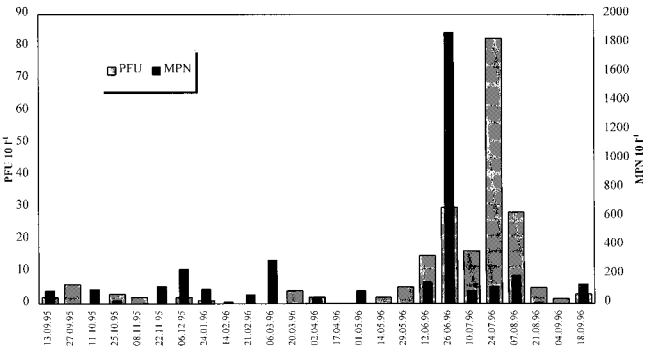

Figure 7b | Concentrations of viruses in sewage effluent (10 l^{-1}), results for the plant at HG. The results given as PFU show concentrations of enteroviruses enumerated as plaque-forming-units, results given as MPN show concentrations of other enteric viruses enumerated as most probable number.

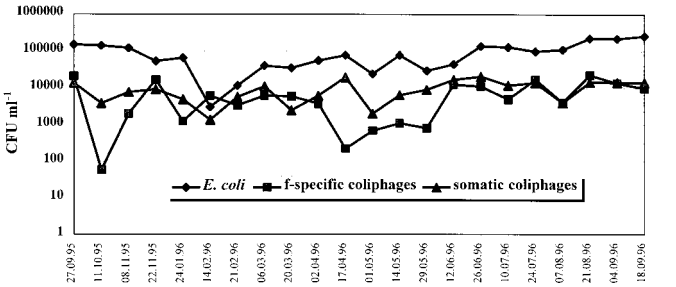

Figure 8a | Concentrations of *E. coli*, somatic and f-specific coliphages (log cfu ml^{-1}) in primary treated sewage, results for the plant at HG.

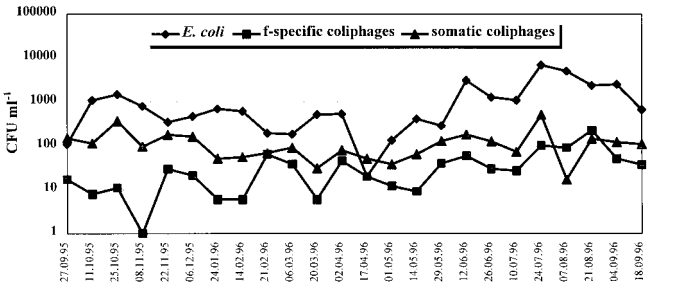

Figure 8b | Concentrations of *E. coli*, somatic and f-specific coliphages (log cfu ml^{-1}) in sewage effluent, results for the plant at HG.

Havemeister (1982). Additional statistical analysis produced no relationships between the concentration of precipitators and the reduction of pathogens in the treated sewage water. The seasonal distribution and the amount of viruses found in this study are similar to the findings of Buras (1976), Schaub *et al.* (1980), Irving & Smith (1981) and Krikelis *et al.* (1984). Some authors describe always finding lower concentrations of enteroviruses than adenoviruses (Pina *et al.* 1996) or rotaviruses (Gerba *et al.* 1996) in sewage water. Another point is the different survival rates in wastewater (Enriquez *et al.* 1995). The peaks for enteroviruses, detected in late summer and in the autumn, may equally result from different epidemiological factors and distributions among the population or other hosts (Hovi *et al.* 1996), and from the purification capacity of the treatment plants. This assumption is supported by the findings of Hurst & Goyke (1986) and Payment *et al.* (1983, 1986), who worked on the stability of enteroviruses in sewage and sewage sludge treatment.

The presence of somatic coliphages and *E. coli* was nearly 100% in the tested samples, therefore both organisms seem to be a good indicator for the presence of infectious enteroviruses or enteric viruses in wastewater (Gantzer *et al.* 1998). In the sewage effluent they show similiar peaks, whereas the concentration of f-specific coliphages dropped to zero.

Considering these items, it is evident that concentration effects of the precipitators alone cannot be responsible for the elimination of the pathogens in wastewater. Hence other parameters, such as the maximum working load of the plant, the buffering capacity and the wastewater quality, may either increase or decrease the reduction potential of precipitating agents on pathogen clearance

Table 3 | Maximum and minimum concentrations of the tested organisms in primary treated wastewater

Treatment plant	Enteroviruses (pfu l^{-1})	Enteric viruses (mpn l^{-1})	*E. coli* (cfu ml^{-1})	Somatic coliphages (cfu ml^{-1})	F-specific coliphages (cfu ml^{-1})
Reutlingen	Max: 128	Max: 1780	Max: 2.5×10^5	Max: 6.9×10^3	Max: 9.2×10^3
	Min: 1	Min: 8	Min: 6.0×10^3	Min: 8.0×10^2	Min: 7.0×10^1
Tübingen	Max: 376	Max: 2504	Max: 5.1×10^5	Max: 2.8×10^4	Max: 6.1×10^3
	Min: 1	Min: 32	Min: 11.0×10^3	Min: 5.2×10^2	Min: 1.7×10^2
Heidelberg	Max: 214	Max: 3600	Max: 2.6×10^5	Max: 2.1×10^4	Max: 2.3×10^4
	Min: 3	Min: 1	Min: 3.0×10^3	Min: 1.4×10^3	Min: 5.8×10^1
Wendlingen	Max: 548	Max: 512	Max: 5.6×10^4	Max: 9.2×10^3	Max: 2.8×10^3
	Min: 1	Min: 8	Min: 3.7×10^3	Min: 7.4×10^2	Min: 4.0×10^1

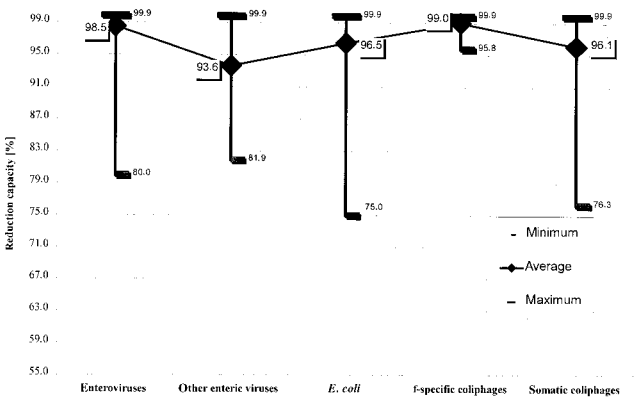

Figure 9a | Reduction capacities (%) for the different microorganisms at the TÜ plant with average, minimum and maximum reduction capacity.

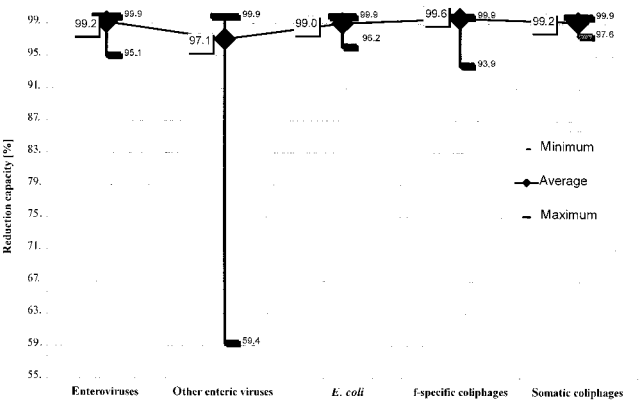

Figure 9b | Reduction capacities (%) for the different microorganisms at the RT plant with average, minimum and maximum reduction capacity.

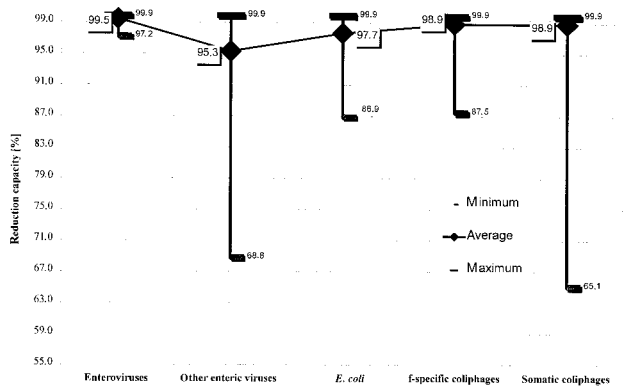

Figure 9c | Reduction capacities (%) for the different microorganisms at the WE plant with average, minimum and maximum reduction capacity.

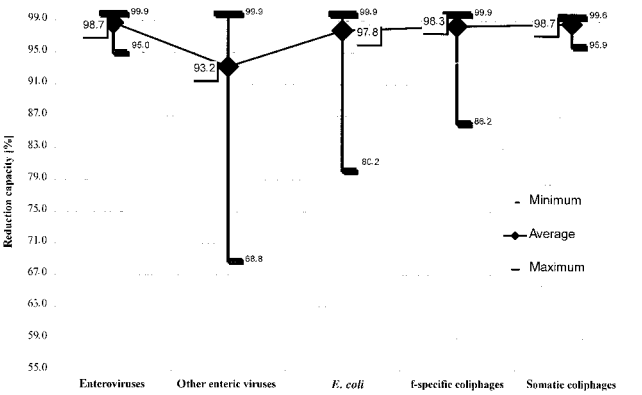

Figure 9d | Reduction capacities (%) for the different microorganisms at the HG plant with average, minimum and maximum reduction capacity.

Table 4 | Combinations of different enterovirus genera in sewage water

Different combinations of enterovirus genera	Number of samples containing virus groups
Cox B Virus (Type 1–6)	45
Polio Virus (Type 1–3)	4
Echo-Virus (Type 11)	2
Cox B-Virus + Polio-Virus	6
Cox B-Virus + Echo-Virus (Type 1 + 11)	2
Polio-Virus + Echo-Virus (Type 1 + 11)	1

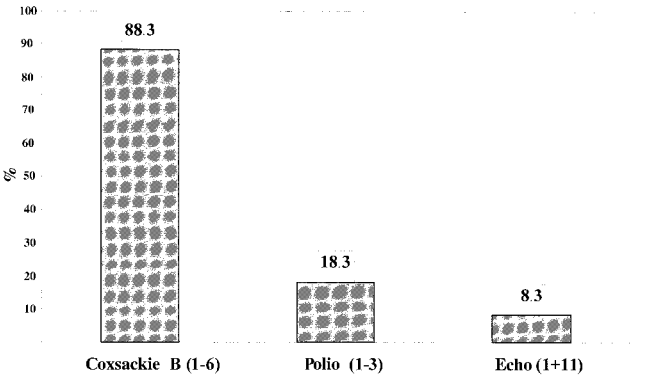

Figure 10 | Percentage (%) of the different enterovirus genera in positive sewage samples including the multiple positive samples.

and should be taken into consideration. Looking at the results for the plants at TÜ and HG, which show larger reduction oscillations especially for the enteric viruses, the influence of wastewater quality and the working capacity of the treatment plant are clearly shown. The TÜ plant working at its maximum capacity and the HG plant with its three-fold higher COD load compared with the RT plant (see Table 2), cannot obtain continuously the same reduction of microorganisms as RT and WE (Fleischer 1998).

A general problem in wastewater management is the absence of regulations and directives for the microbiological quality of the sewage effluent, at least in Germany. The disinfection of treatment plant effluents has never been seriously considered, except in the vicinity of bathing beaches and some exceptional situations. Compared with the efforts for removing BOD, COD, N and P from wastewater, the release of pathogenic microorganisms into surface waters is neglected. Notwithstanding the fact that pathogens were reduced on average by >95% in all of the tested treatment plants, the high numbers of virus-positive sewage effluents are not acceptable. Virus concentrations of up to 200 infectious particles per litre are tantamount to a high risk of infection for humans coming into contact with that water. The receiving water bodies may be used for the production of drinking water, for the watering of crops or gardens and for recreational activities. The effluents or the receiving waters should comply with the regulations of the European Community for surface water used for the production of drinking water (75/440/EWG) and for bathing water (76/160/EWG). Different methods for wastewater disinfection have been described and practised (ATV 1998). A 4 log (10) reduction of pathogenic bacteria and a 3 log (10) reduction of enteropathogenic viruses are necessary to meet the quality requirements of the European Community for recreational waters and to minimize the risk of infection for users (Fleisher *et al.* 1993). None of these four examined treatment plants is able to fulfil these demands today.

CONCLUSIONS

In the examined wastewater treatment plants, biological P and N elimination processes did not make the use of chemical precipitating agents superfluous. The efficiency of virus elimination varied considerably, possibly depending on numerous parameters not within the control of the investigators such as BOD and COD concentrations, the amount of precipitators used, the reduction of suspended solids or the maximum working load. The extent of virus elimination in general was insufficient to provide water complying with the EG-directives for bathing water or for surface water used for the production of drinking water.

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REFERENCES

ATV (Abwassertechnische Vereinigung) 1998 Disinfection of biological treated wastewater. *Regelwerk Abwasser Abfall Hinweisblatt* M205.

Buras, N. 1976 Concentration of enteric viruses in wastewater and effluent: A two year survey. *Wat. Res.* **10**, 295–298.

Dryden, F. D., Chen, C. & Selwa, M. W. 1979 Virus removal in advanced wastewater treatment systems. *J. Wat. Pollut. Control. Fed.* **51**(8), 2098–2109.

EG-Richtlinie 75/440/EWG 1975 Richtlinie des Rates über die Qualitätsanforderungen an Oberflächenwasser für die Trinkwassergewinnung vom 16.06.1975. *Amtsblatt der EG* L 194.

EG-Richtlinie 76/160/EWG 1976 Richtlinie des Rates über die Qualität der Badegewässer vom 8.12.1975. *Amtsblatt der EG L 31*.

Enriquez, C. E., Hurst, C. J. & Gerba, C. P. 1995 Survival of the enteric adenoviruses 40 and 41 in tap, sea and wastewater. *Wat. Res.* **29**, 2548–2553.

Fleischer, J. & Botzenhart, K. 1996 Enterale Viren. In: *Hygienisch-mikrobiologische Wasseruntersuchungen* (E. Schulze, Ed.). Gustav Fischer Verlag, Jena.

Fleischer, J. 1998 Elimination von enteropathogenen Viren und Fäkalindikatoren bei der erweiterten biologischen Abwasserreinigung: Vergleich zwischen 4 Anlagen des Neckarraumes. *Dissertation der Fakultät für Biologie der Eberhard-Karls-Universität, Tübingen.*

Fleisher, J. M., Kay, D., Stanwell-Smith, R., Wyer, M. & Morano, R. 1993 Water and non-water related risk factors for gastroenteritis among bathers exposed to sewage contaminated marine waters. *Int. J. Epidemiol.* **22**, 698–708.

Gantzer, C., Maul, A., Audic, J. M. & Schwartzbrod L. 1998 Detection of infectious enterovirus, enterovirus genomes, somatic coliphages, and *Bacteroides fragilis* in treated wastewater. *Appl. Environ. Microbiol.* **64**(11), 4307–4312.

Gerba, C. P., Rose, J. B., Haas, C. N. & Crabtree, K. D. 1996 Waterborne rotavirus: A risk assessment. *Wat. Res.* **30**, 2929–2940.

Grabow, W. O. K. & Coubrough, P. 1986 Practical direct plaque assay for coliphages in 100-ml samples of drinking water. *Appl. Environ. Microbiol.,* **52**, 430–433.

Havelaar, A. H., van Olphen, M. & Drost, Y. C. 1993 F-specific RNA bacteriophages are adequate model organisms for enteric viruses in fresh water. *Appl. Environ. Microbiol.* **59**, 2956–2962.

Hovi, T., Stenvik, M. & Rosenlew, M. 1996 Relative abundance of enterovirus serotypes in sewage differs from that in patients: clinical and epidemiological implications. *Epidemiol. Infect.* **116**, 91–97.

Hurst, C. J. & Goyke, T. 1986 Stability of viruses in wastewater sludge eluates. *Can. J. Microbiol.* **32**, 649–653.

Irving, L. G. & Smith, F. A. 1981 One-year survey of enteroviruses, adenoviruses, and reoviruses isolated from effluent at an activated-sludge purification plant. *Appl. Environ. Microbiol.* **41**, 51–59.

Krikelis, V., Spyrou, N., Markoulatos, P. & Serie, C. 1984 Seasonal distribution of enteroviruses and adenoviruses in domestic sewages. *Can. J. Microbiol.* **31**, 24–25.

Lewis, D. & Metcalf, T. G. 1988 Removal of viruses in sewage treatment: assessment of feasibility. *Microbiol. Sci.* **5**, 260–264.

Morris, R. 1984 Reduction of naturally occurring enteroviruses by wastewater treatment processes. *J. Hyg. Camb.* **92**, 97–103.

Nieuwstad, T. J., Mulder, E. P., Havelaar, A. H. & van Olphen, M. 1988 Elimination of microorganisms from wastewater by tertiary precipitation and simultaneous precipitation followed by filtration. *Wat. Res.* **22**, 1389–1397.

Payment, P., Ayache, R. & Trudel, M. 1983 A survey of enteric viruses in domestic sewage. *Can. J. Microbiol.* **29**, 111–119.

Payment, P., Fortin, S. & Trudel, M. 1986 Elimination of human enteric viruses during conventional wastewater treatment by activated sludge. *Can. J. Microbiol.* **32**, 992–925.

Pina, S., Puig, M., Lucena, F., Jofre, J. & Girones, R. 1996 Human adenoviruses are more frequently detected in environmental samples than enteroviruses, hepatitis A or hepatitis E viruses, presented at IAWQ Congress on Health Related Water Microbiology, Oct 1996 Mallorca (unpublished).

Rolland, D., Jore, J. C., Vieeval, F., Block, J. C. & Hartemann, P. 1983 Sampling strategy for detecting viruses in a sewage treatment plant. *Appl. Environ. Microbiol.* **45**(6), 1767–1774.

Rose, J. B., Dickson, L. J., Farrah, S. R. & Carnahan R. P. 1996 Removal of pathogenic and indicator microorganisms by a full-scale water reclamation facility. *Wat. Res.* **30**, 2785–2787.

Safferman, R. S., Rohr, M.-E. & Goyke, T. 1988 Assessment of recovery efficiency of beef extract reagents for concentrating viruses from municipal wastewater sludge soils by the organic flocculation procedure. *Appl. Environ. Microbiol.* **54**, 309–316.

Schaub, S. A., Kenyon, K. F., Blediak, B. & Thames, R. E. 1980 Evaluation of the overland run-off mode of land wastewater treatment and virus removal. *Appl. Environ. Microbiol.* **39**, 127–134.

Steinmann, J. & Havemeister, G. 1982 Eliminierung von Bakterien und Viren durch Flockung im vorgereinigten Abwasser. *Zbl. Bakt. Hyg., I. Abt. Orig.* **B176**, 546–552.

Vilaginès, P., Sarrette, B., Husson, G. & Vilaginès, R. 1993 Glass-wool for virus concentration at ambient water pH level. *Wat. Sci. Tech*. **27**(3–4), 299–306.

Zaiss, U. & Hennies, H. H. 1988 Quantitative studies of the elimination of coliphages and other fecal indicators during wastewater treatment. *Zentrbl. Bakteriol. Mikrobiol. Hyg.* **B186**(5–6), 512–525.