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BACTERIA AND PHAGES IN A RIVER AND IN A SEWAGE EFFLUENT

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The occurrence of bacteria and phages was followed for a period of one year in a sewage effluent from an activated sludge treatment plant and in the River Vantaanjoki. The plant and the river discharge to the same area in the Gulf of Finland but they are not otherwise connected. The bacteria determined were faecal coliform bacteria, total bacteria and gram negative bacteria. The phages determined were coliphages, salmonellaphages and phages of *Pseudomonas aeruginosa*. In sewage effluent the correlations calculated between phages, bacteria, temperature and discharge indicated mainly the input of bacteria and phages into sewage. In the river water the correlations indicated, besides input of bacteria and phages into river, the effects of survival characteristics of bacteria and phages.

Index words: Bacteria, faecal coliforms, bacteriophages, wastewater treatment plant, sewage.

1. INTRODUCTION

The effect of season on occurrence of bacterial indicators of water hygiene has been pointed out in several investigations, e.g. Carney et al. (1975), Davenport et al. (1976), Harms et al. (1975) and Stephenson (1978). The occurrence of coliphages in contaminated waters has been investigated in a number of studies e.g. Dhillon and Dhillon (1974), Dhillon et al. (1970), Durham and Wolf (1973), and Niemi and Niemi (1979). However, no information is available concerning the effect of season on occurrence of phages.

The aim of this work was firstly to determine the concentrations of phages of some *Escherichia coli* and *Salmonella* strains and of phages of *Pseudomonas aeruginosa* simultaneously with the concentrations of faecal coliform bacteria, total bacteria and gram negative bacteria for a period of one year both in river water and in the effluent from an activated sludge treatment plant, secondly to investigate if correlations between temperature, discharge and the concentrations of the determined micro-organisms can be found, thirdly to find out whether seasonal patterns in the occurrence of phages and bacteria can be observed and finally to investigate the survival characteristics of these micro-organisms, especially in the river water.

2. MATERIALS AND METHODS

Samples: Duplicate water samples from the River Vantaanjoki and from sewage effluent at the Viikki activated sludge treatment plant in Helsinki were taken at two week intervals. Both the river and the sewage effluent discharge into the Gulf of Finland, but they are not otherwise connected. Samples were taken into sterile glass bottles at c. 8.30 a.m., at the beginning of the experiment on Tuesdays and subsequently on Thursdays. The samples were plated within two hours of sampling. Water temperature was measured at sampling. The discharge values were provided for the River Vantaanjoki by Helsinki City Waterworks and for the sewage effluent by the Viikki treatment plant.

Niemi and Niemi (1979) determined the flow time of sewage through the Viikki activated sludge treatment plant. On the basis of this investigation the daily fluctuations in the concentrations of micro-organisms in the effluent could be determined. It was observed that a plateau in the loading curve occurs between 7 and 9 a.m. and therefore water samples were decided to be taken ca 8.30 a.m. in this work. It was assumed that the daily plateau in the loading remains constant troughout the year.

Bacterial analyses: The methods for determining faecal coliform bacteria and viable count of total bacteria have been described by Niemi and Niemi (1979). The viable count for gram negative bacteria was carried out as described for total bacteria but using medium containing 2 iu ml^{-1} of penicillin (Penicillin G Hoechst).

Phage assay: Phages were determined using the agar layer method of Adams (1966), the medium described by Niemi and Niemi (1979) and incubation at 28 ^{o}C overnight. The bacteria used for determination of phages were *Escherichia coli* B M219 (rough strain, Zechoslovak National Collection of Type Cultures), *E. coli* C (rough strain, Bertani (1968), provided by Mr Dennis Baumford), *Salmonella typhimurium* SH 4247 (smooth LT2 strain, provided by prof. P.H. Mäkelä), and *S. typhimurium* SL 1102 (deep rough strain, Witkinson et al. (1972), provided by prof. P.H. Mäkelä), and *Pseudomonas aeruginosa* 10145 (The American Type Culture Collection). The stability of surface structures of *Salmonella* strains was investigated at the Central Public Health Laboratory in Helsinki several times using several phages which have different receptor sites.

3. RESULTS

3.1 Sewage treatment plant

The discharge of the sewage treatment plant was rather even except in spring during the snowmelt (Fig. 1). The effluent temperature varied from 6 to 19 ^{o}C. The concentrations of bacteria (Fig. 2) and phages (Fig. 3) varied as follows: faecal coliform bacteria from 100 to 45500 ml^{-1}, total bacteria from $8 \cdot 10^5$ to $155 \cdot 10^5$ ml^{-1}, gram negative bacteria from $3 \cdot 10^5$ to $55 \cdot 10^5$ ml^{-1}, phages of *E. coli* B M219 from 6 to 545 PFU ml^{-1}, phages of *E. coli* C from 44 to 48000 PFU ml^{-1}, phages of *S. typhimurium* SH4247 from 0 to 3100 PFU ml^{-1}, and phages of *P. aeruginosa* from 0 to 63 PFU ml^{-1}.

The concentrations of bacteria and phages were low when the discharge was large (Table 1, the negative correlations). This phenomenon can be explained by the diluting effect of snowmelt and rain waters.

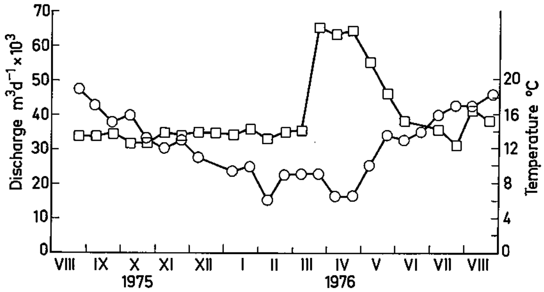

Fig. 1. Discharge (□—□) and effluent temperature (○—○) at the Viikki activated sludge treatment plant.

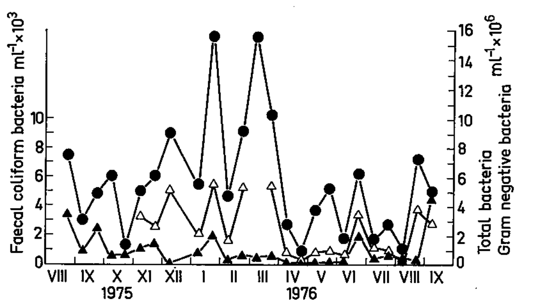

Fig 2. Concentrations of bacteria in the effluent from the Viikki activated sludge treatment plant. Each point is the average of two duplicate samples. ▲—▲ = faecal coliform bacteria, ●—● = total bacteria, and △—△ = gram negative bacteria.

The positive correlations between faecal coliform bacteria and phages of *E. coli* B M219 and *E. coli* C were strong (Table 1), indicating their simultaneous occurrence in effluent water. There was a weak correlation between concentration of faecal coliform bacteria and salmonellaphages (Table 1). The concentrations of coliphages and salmonellaphages correlated weakly with concentrations of total bacteria and gram negative bacteria. The correlation between concentrations of phages of *E. coli* B M219 and *E. coli* C was strong and positive. Positive correlation was observed between the concentrations of coliphages and salmonellaphages.

The effluent temperature did not in general correlate with microbiological parameters. However, there was a weak positive correlation be-

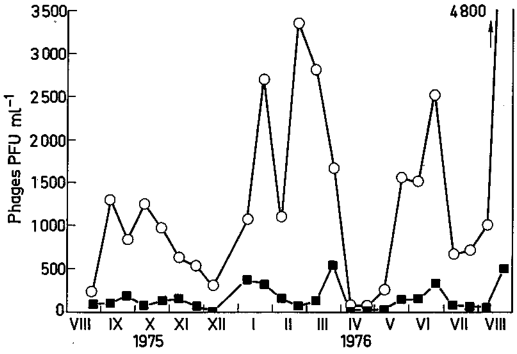

Fig. 3. Concentrations of phages of *E. coli* B M219 (■—■) and *E. coli* C (○—○) in the effluent from the Viikki activated sludge treatment plant. Each point is the average of two duplicate samples.

Table 1. Correlation coefficients (r) between phages, bacteria, temperature and discharge in the River Vantaanjoki and in sewage effluent from the Viikki activated sludge treatment plant. The logarithmic transformation was calculated for all values of bacteria and phages. When the discharge of the river was less than 0.1 m^3s^{-1}, it was approximated to 0.1 m^3s^{-1}. (n = number of observations, ★ = significant with 5 % risk, ★★ = significant with 1 % risk, ★★★ = significant with 0.1 % risk).

Variables		River water		Sewage effluent	
x	y	n	r	n	r
conc. of phages of *E. coli* B M219	temperature			24	0.28
conc. of phages of *E. coli* C	temperature	24	(−0.78★★)	24	0.28
conc. of phages of *S. typhimurium* SH 4247	temperature			24	0.19
conc. of phages of *E. coli* B M219	discharge			24	−0.65★★★
conc. of phages of *E. coli* C	discharge	24	0.24	24	−0.63★★★
conc. of phages of *S. typhimurium* SH 4247	discharge			24	−0.47★
conc. of phages of *E. coli* B M219	conc. of faecal coliforms			24	0.72★★★
conc. of phages of *E. coli* C	conc. of faecal coliforms	24	0.25	24	0.60★★
conc. of phages of *S. typhimurium* SH 4247	conc. of faecal coliforms			24	0.44★
conc. of phages of *E. coli* B	conc. of total bacteria			24	0.41★
conc. of phages of *E. coli* C	conc. of total bacteria	24	0.14	24	0.46★
conc. of phages of *S. typhimurium* SH 4247	conc. of total bacteria			24	0.41★
conc. of phages of *E. coli* B M219	conc. of gram negative bacteria			18	0.54★
conc. of phages of *E. coli* C	conc. of gram negative bacteria	19	0.77★★	18	0.56★
conc. of phages of *S. typhimurium* SH 4247	conc. of gram negative bacteria			18	0.49★
conc. of phages of *E. coli* B M219	conc. of phages of *E. coli* C			24	0.80★★★
conc. of phages of *E. coli* B M219	conc. of phages of *S. typhimurium* SH 4247			24	0.52★★
conc. of phages of *E. coli* C	conc. of phages of *S. typhimurium* SH 4247			24	0.63★★★
conc. of faecal coliforms	temperature	24	(−0.23)	24	0.50★
conc. of total bacteria	temperature	24	(−0.17)	24	−0.09
conc. of gram negative bacteria	temperature	24	(−0.73★★)	24	−0.02
conc. of faecal coliforms	discharge	24	0.28	24	−0.84★★★
conc. of total bacteria	discharge	24	0.26	24	−0.41★
conc. of gram negative bacteria	discharge	19	0.43	18	−0.64★★
conc. of faecal coliforms	conc. of total bacteria	24	0.40	24	0.42★
conc. of faecal coliforms	conc. of gram negative bacteria	19	0.16	18	0.58★★
conc. of total bacteria	conc. of gram negative bacteria	19	0.28	18	0.93★★★

tween the occurrence of faecal coliform bacteria and temperature (Table 1). There was a correlation between the concentrations of different bacterial groups. Gram negative bacteria appeared to be a constant proportion of total bacteria.

3.2 The River Vantaanjoki

The discharge of the river varied from less than 0.1 to 11.5 m^3s^{-1} and water temperature from 0.0 to 18.5 °C during the study (Fig. 4). The concentrations of bacteria (Fig. 5) and phages (Fig. 6) varied as follows: faecal coliform bacteria from 0 to 520 ml^{-1}, total bacteria from $0.9 \cdot 10^5$ to $10 \cdot 10^5$ ml^{-1}, gram negative bacteria from $0.1 \cdot 10^5$ to $6.7 \cdot 10^5$ ml^{-1}, phages of *E. coli* B M219 from 0 to 61 PFU in 5 ml, phages of *E. coli* C from 1 to 235 PFU in 5 ml, phages of *S. typhimurium* SH 4247 from 0 to 8 PFU in 5 ml, phages of *S. typhimurium* SL 1102 from 0 to 16 PFU in 5 ml, and phages of *P. aeruginosa* from 0 to 13 PFU in 5 ml.

The values for temperature were not normally distributed and therefore the data were not suitable for statistical treatment. It was evident, however that the concentration of coliphages were high during the cold season (Figs. 4 and 6). The river was icebound when the water temperature was low, and it was therefore not possible to distinguish between the effects of sunlight and temperature on the persistence of phages.

No correlation was observed between discharge and microbiological parameters.

No correlation was observed between the concentration of phages of *E. coli* C and the concentrations of faecal coliform bacteria and total bacteria. However, a strong positive correlation was observed between the concentration of phages of *E. coli* C and that of gram negative bacteria (Table 1).

The effects of temperature and of discharge on concentration of total bacteria appeared to be opposite (Figs. 4 and 5). In the autumn the concentrations of total bacteria increased when the temperature decreased. However, the concentration of bacteria began to decrease again when the discharge decreased. In the spring the rise in discharge led to an increase in concentration of total bacteria. However, in spite of high discharge, with increasing temperature the concentration of bacteria in the river again decreased.

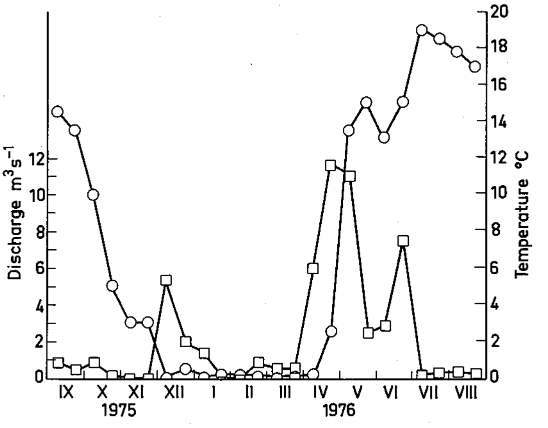

Fig. 4. Dishcharge (□—□) and water temperature (○—○) in the River Vantaanjoki.

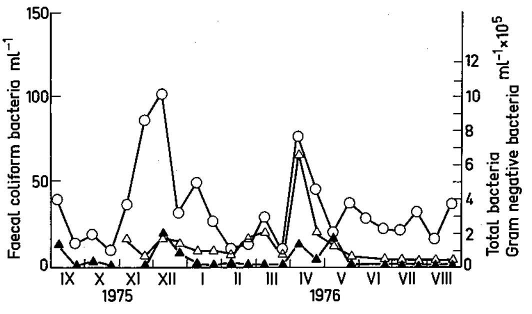

Fig. 5. Concentrations of bacteria in the River Vantaanjoki. Each point is the average of two duplicate samples. ▲—▲ = faecal coliform bacteria, ○—○ = total bacteria and △—△ = gram negative bacteria.

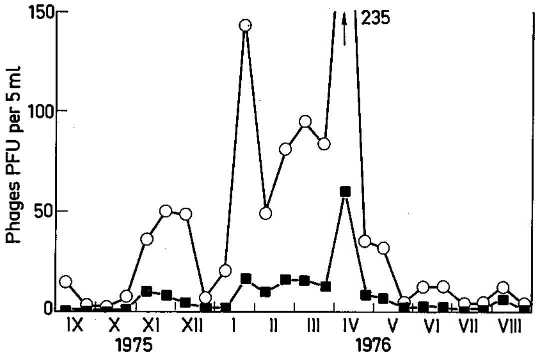

Fig. 6. Concentrations of phages of *E. coli* B M219 (■—■) and *E. coli* C (○—○) in the River Vantaanjoki. Each point is the average of two duplicate samples.

4. DISCUSSION

The bacterial strain used for determining phages from water samples must be chosen carefully, because each bacterial strain selects a specific pattern of phages. Rough *E. coli* strains are known to yield high values for phages, because their receptors are not very specific (Dhillon et al. 1970, Dhillon and Dhillon 1974).

The highest concentration of phages observed in the river water was 47 PFU ml^{-1} and in sewage effluent 4800 PFU ml^{-1}. These concentrations were obtained using *E. coli* C as the indicator bacterium. The average concentrations observed for the phages in the river water and in the effluent were 8 and 1350 PFU ml^{-1}, respectively. The average phage concentrations in sewage effluent were for *E. coli* B M219 phages 152 PFU ml^{-1} and for *S. typhimurium* SH 4247 phages 541 PFU ml^{-1}. The concentration of phages of the smooth *Salmonella* strain was surprisingly high. Dhillon et al. (1970) have reported concentrations of between 36 and 15900 PFU ml^{-1} in untreated sewage for phages of *E. coli* K–12 F^{+} in Hong Kong.

The ratio of phages to faecal coliform bacteria varied considerably. The average ratio for phages of *E. coli* C in sewage effluent was 1:14, for phages of *E. coli* B M219 1:86 and for phages of *S. typhimurium* SH 4247 1:67. The ratios of phages to faecal coliform bacteria are higher in river water than in effluent water, especially during the winter. For example the ratio of phages of *E. coli* C to faecal coliform bacteria in river water was on average 1:8. The increase in this ratio indicates that the inactivation rate of phages was lower than the die-off rate of coliform bacteria in river water.

The weak positive correlation between temperature and amount of faecal coliform bacteria was the only correlation observed between effluent temperature and microbial parameters. The effluent temperature has two kinds of impact. Firstly it indicates the proportion of domestic wastewater in sewage that is the input of faecal bacteria over short time intervals. In this study this effect was eclipsed by the effect of season on water temperature. Secondly water temperature affects microbial survival. The fact that high temperature decreases the die-off rate of bacteria might be eclipsed by the higher bacterial input entering the treatment plant with warm waste waters.

Simultaneous occurrence of low temperature and ice-cover in the river during the winter favoured the persistence of phages (Fig. 4 and 6).

The high discharges in the autumn and spring cause a considerable input of total bacteria into the river water, probably due to runoff waters (Fig. 4 and 5). The survival of bacteria in cold water under ice-cover seems to be better than during the warm season, but not as good as the persistence of phages.

The correlations observed between various microbiological parameters and between microbiological parameters and temperature as well as between microbiological parameters and discharge indicated the input of bacteria and phages in the effluent. In the river these correlations also indicated besides the input of bacteria and phages into the river, additional effects due to inactivation of phages and die-off of bacteria.

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Helsinki, May 1980

Maarit Niemi, Jorma Niemi

LOPPUTIIVISTELMÄ

Viikin jätevedenpuhdistamon sekä Vantaanjoen bakteerien ja bakteriofaagien pitoisuuksia seurattiin yhden vuoden ajan kahden viikon välein. Puhdistamo ja Vantaanjoki laskevat molemmat samaan merenlahteen, mutta ne eivät ole muuten toisiinsa yhteydessä. Määritetyt bakteeriryhmät olivat fekaaliset koliformiset bakteerit, kokonaispesäkeluku ja gram negatiiviset bakteerit. Määritetyt faagit olivat kolifaageja, salmonellafaageja sekä *Pseudomonas aeruginosan* faageja. Puhdistetussa jätevedessä bakteerien, faagien, lämpötilan ja virtaaman välillä lasketut korrelaatiot osoittivat pääasiassa bakteerien ja faagien kulkeutumista puhdistamolle. Vantaanjoessa lasketut korrelaatiot osoittivat paitsi bakteerien ja faagien kulkeutumista jokeen myös niiden säilyvyyttä jokivedessä.

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