

AN EXPERIMENT WITH *ESCHERICHIA COLI* T
BACTERIOPHAGE AS TRACER IN RIVER FLOW STUDIES

BY

S. NIEMELÄ and K. KINNUNEN

Department of Microbiology, University of Helsinki

A b s t r a c t

Bacteriophages (bacterial virus particles) were used in the Kymi River for measurement of flow time between three points. The data presented demonstrate even the possibility of utilizing bacteriophages for more sophisticated hydrological purposes.

In search of new tracer substances which could be used for river flow studies an experiment was made with bacteriophages (bacterial viruses) which, to the best of our knowledge, have not been previously used for the said purpose. Bacterial virus particles have some useful properties in this respect. They are harmless to man and other higher animals, infecting only very specific types of bacteria. Due to their specificity it is almost certain that a viral tracer with minimal background concentration could be found for any environment. Bacteriophages are relatively stable — their concentration stays unaltered for several months in laboratory conditions. Certain bacteriophage types can be generated in concentrations up to 10^{12} per ml without excessive trouble, and on the other hand, analytical methods allow detection of concentrations down to one particle per ml. Although bacteriophages are very small as biological units (200—1000 Å diameter) they are larger than molecules by several orders of magnitude, and their adsorptive properties are certain to differ from those of molecular size particles. The density of phage cell substance is approximately 1.5 g/cm^3 [4].

For further information on bacteriophages the reader is referred to ADAMS' excellent monograph [1].

Detection and quantitative determination of phage particles is based on their ability to infect and kill their specific host bacterium. In practice the phage count is made by adding approximately 10^9 cells of the sensitive host bacterium to the water sample. After mixing, the suspension is spread on a nutrient surface for development. In places where bacterial growth is prevented by bacteriophages clear areas, so called plaques, remain in an otherwise homogeneous bacterial «lawn» (cf. Fig. 1). The plaques can be counted after 6–10 hours' development.

Distribution of phage particles among parallel random samples follows the Poisson distribution, and has accordingly a standard deviation of $m^{1/2}$, where m = mean number of particles per sample. The relative random error can thus be diminished by increasing the total number of observed particles. Mathematical methods for correcting the coincidence or overlap error in these biological counts are sufficiently developed [3, 6], although somewhat cumbersome. With present methods sample size is limited to 1 ml or less, but means of concentrating larger samples into convenient size are being investigated by us and others [2, 5].

A test with an *Escherichia coli* bacteriophage of the T series as tracer was performed on October 18th 1968 in the Kymi River (Q: 262 m³/s). The main object was to test the phage method in general and to obtain a rough estimate of flow times in the river between some points of biological interest. Therefore, little effort was spent on obtaining sufficient statistical precision of each separate count, which would be necessary if bacteriophages were used for discharge measurements, for example. For the same reason no attempt was made to detect the entire tracer wave in the river. As biological considerations determined the sampling

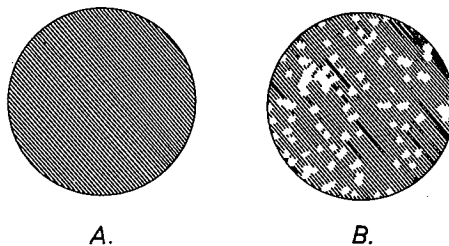


Fig. 1. A. Uniform bacterial growth in the absence of bacteriophages.
B. Plaques formed in the bacterial growth by the action of bacteriophages.

places it was not possible to follow the usual hydrological practice of sampling immediately below rapids. Both sampling stations happened to lie in relatively slow sections of the river.

$1.15 \cdot 10^{14}$ coliphage particles were introduced into the Kymi River at 00 hours by breaking a bottle containing 4.60 liters of bacteriophage suspension (concentration $2.50 \cdot 10^{10}$ particles per ml) approximately 3 meters above the water. This was effected by breaking the bottle with a hammer while standing on the power dam at Keltti hydroelectric plant.

First samples of river water were taken at 06.30 hours at a site 8.2 km downstream. The water was collected in the middle of the river with a sinking 100 ml bottle. Sampling was continued for 5.5 hours at 30 minute intervals (with minor variations). Later, the sampling site was moved further downstream to a point 17.2 km below Keltti. There samples were taken from 16.30 until 22.00 o'clock — again at 30 minute intervals. Results are presented in Fig. 2 as phage counts per ml of river water against passage time. Ten parallel 1 ml samples were counted from each sampling bottle. Lowering and possibly widening of the maximum in the concentration curve from station 1 to station 2 seems evident in the result.

Three days before the experiment the background phage concentration was determined at Keltti and at sampling station No. 2 with the

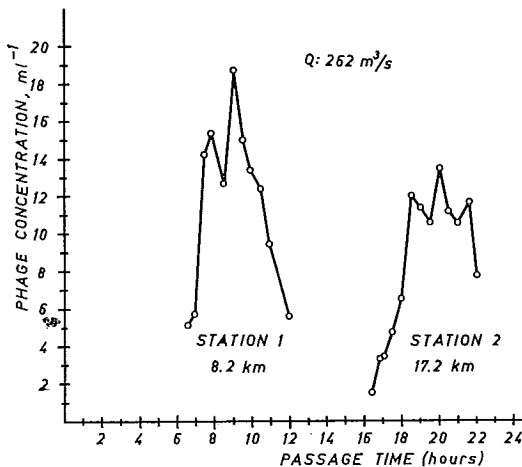


Fig. 2. Recovery of bacteriophages at two sampling stations in the Kymi River.

conveniently small results of 2 and 3 particles per 10 ml, respectively.

It seems safe to conclude from this first experience that phage particles show considerable promise as tracer substances. The recovery of phages from the river was perhaps not fully efficient, but it seemed to us that this was largely due to analytical procedures rather than actual losses in the river.

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