Microbial tracers in groundwater research

Mihael Bricelj

National Institute of Biology, 1000 Ljubljana, Večna pot 111, R Slovenija;
E-mail: mihael.bricelj@nib.si

Abstract: For the research of karst environment and its water connections in Slovenia, Austria and Greece, bacteriophage P22H5 was chosen with the host bacteria Salmonella typhimurium. Average flow velocity of the phage tracer P22H5 varied from one injection to another, with regard to the traced distance and geological structure. It varied from 4.000 to 36 m/day.

Keywords: karst, underground water, tracer, bacteriophage P22H5

Introduction

Microbial tracers that are used in tracing experiments are bacteria, bacterial spores, yeasts, bacteriophages and viruses (Käss, 1998). Microbial tracers are introduced for several reasons, namely for determination of water flow velocity, dilution of polluted water and partition of water between sinkholes and springs. Nonharmful strains of microbes are often used as the model organisms for research of persistence and trickling of the pathogen counterparts through unsaturated and saturated zones to aquifer. Because of very special propagation of bacterial viruses, within the host bacteria, bacteriophages represent a valuable tracer in underground tracing experiments. In the environment they act as an inert complex macromolecule, build-up mostly of DNA and protein envelope, with special morphology and the size between 400 and 800 nm. They are relatively stable in environment and survive for a long time.

The selection of bacteriophage and its host is crucial for the tracing experiments. Very valuable for the freshwater tracing are bacteriophages of the bacteria from the sea (Rossi, 1994) or bacteriophages of bacteria that seldom occur in water (Seeley, 1982). Most common bacteriophages in the faecally polluted water are coliphages, with occasionally high background numbers that could overnumber the coliphage tracer in diluted samples. For the research of karst environment and its water connections in Slovenia, Austria and Greece, bacteriophage P22H5 was chosen with the host bacteria Salmonella typhimurium, which is rarely found in waters (Krivic, 1987; Behrens, 1992; Bricelj, 1994). In the case of salmonella phage never occurred the background of natural phages that could interfere with the injected tracer. This feature is very important in the case of maximal dilution of the tracer. The phage tracer P22H5 was injected on ten locations in 14 tracer experiments, mostly in running water and in three occasions in unsaturated karstic zone on high plateau of Nanos. The results of the tracer test in unsaturated zone are presented in the paper.

Scientific paper

RESULTS AND DISCUSSION

The bacteriophage tracer P22H5 was injected, subsequently three times at the location Zavrhovec - Otlica, which is a kilometre away from the Hubelj spring on high plateau of Nanos. The hole with the diameter of 5 cm was drilled in the floor of valley, 5,5 m deep into permeable strata in the first tracing experiment. Before the injection of tracer, the drill hole was splashed with 1.5 m^3 of water, following with 16,500 ml of phage broth that was subsequently washed with additional 3,5 m^3 of water. The injection of phage tracer was continuing for 3 minutes, and the total concentration of injected phage particles was 3.0 x 10^{15} pfu.
In the second tracing experiment tracer was injected into a crack in other valley next to the place of the first injection. A crack at the bottom of valley, was first washed with 3.5 m^3 of water, than lithium chloride in quantity of 110 l (30 kg) was poured into the fissure, followed by the washing of 1.0 m^3 of water. Then the phage tracer in the quantity of 20.500 ml was poured and then washed with 3.0 m^3 of water. The total quantity of phage tracer was 3.75 x 10^{15} pfu.
The third injection of phage tracer was was done on the same place as the second one. The quantity of 26,000 ml of phage was poured into the same crack, at the bottom of valley. Injection began with the washing of the crack with 3.5 m^3 of water, than phage tracer was poured into crack, followed by washing with additional 3.5 m^3 of water. The total quantity of phage tracer was 6.6 x 10^{15} pfu.
The samples for the determination of phages were taken at sampling point Hubelj spring.
The recovery of tracer in the first tracing experiment was 0.78 %. The gravity centre position of tracer curve t_g, as described by KINNUNEN (1978), was calculated to 94.32 hours (see Figure 1). The recovery of tracer in the second tracing experiment was 0.012 %. The gravity centre position of tracer curve t_g was calculated to 99.61 hours. The recovery of tracer in the third tracing experiment was 0.007%. The gravity centre position of tracer curve t_g was calculated as 678.2 hours (see Table 1).

Table 1. Measured and calculated data for the three tracing experiments with phage tracer P22H5, injected at the location Zavrhovc, a = deactivation factor calculated from injected and recovery values; av_p = average precipitation in the month period before tte injection of phage took place; $av.Q_{inj}$ = average day's throughflow in the time of injection of tracer; t_g = time calculated from following equation $\Sigma c_i * t_i / \Sigma c_i$; v_{tg} = velocity calculated with t_g; recovery = calculated recovery of the phage tracer from injected and recovered quantity of phage tracer

Tracer experiment	a	av_p	av. Q_{inj}	t_g	v_{tg}	recovery
		mm	$m^3 sec^{-1}$	h	m/day	%
October 1993	1.3 x 10^2	15.4	2.81	94.7	253.9	0.780
April 1994	8.3 x 10^3	11.8	6.33	99.6	241.4	0.012
August 1995	1.0 x 10^5	2.8	0.51	678.2	35.5	0.007

The empirical formula $P_{trac} = a * t_{trav} * Q$ (P_{trac} = quantity of tracer needed in tracing experiment; a = deactivation factor; t_{trav} = time of tracer travel in sec; Q=waterflow in $mlsec^{-1}$; Q was taken as average waterflow, including the data from the first appearance of tracer, to the last positive result) was used to calculate the needed quantity of phage tracer for a = 1, Q = 27.79 m^3sec^{-1} and t_{trav} = 94.7 h. The resultant quantity was 9.5 x 10^{12} pfu. The quantity injected was 3.0 x 10^{15} pfu, therefore the calculated inactivation factor is in the magnitude of 316.6. The real inactivation factor calculated from the recovery value 2.43 x 10^{13} was 128.0 that means 2.8 times lower than the calculated one. The calculated inactivation factor in the second tracing experiment was in the magnitude of 2,354.6 and in the third tracing experiment 846.4.

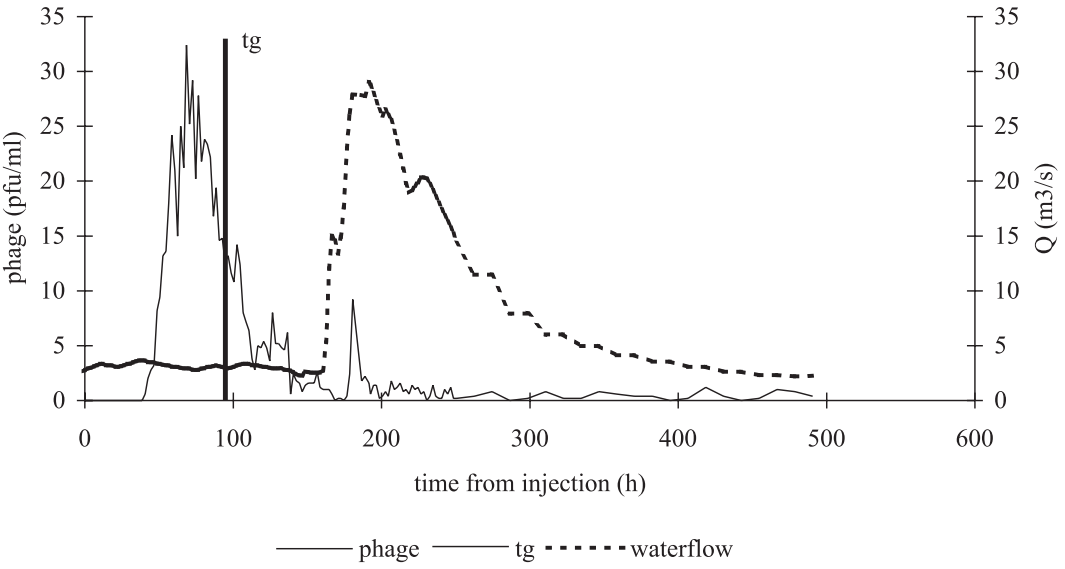

Figure 1. The breakthrough curve of phage tracer P22H5 at the Hubelj spring of the first tracing experiment, in October 1993

Conclusions

The tracing experiments with phage P22H5 and other tracers on the Nanos plateau were programmed in three different water level situations, low, high and medium. The first tracing experiment was performed in the medium level of water in the spring of Hubelj. The average precipitation in the month period before the injection was 15.4 mm. In such conditions the bacteriophage tracer was injected to the permeable strata for the first time instead of directly into the water as was commonly done in preceding experiments in several karst locations. The recovery of tracer in the first experiment was the highest comparison to the recovery values of the two subsequent tracer experiments (see Table 1), although a part of phage tracer was adsorbed to the underground surfaces. This can obviously be seen in the

breakthrough curve of phage tracer at the Hubelj spring (see Figure 1), where the second peak of phage tracer strictly follows the sudden augmentation of water throughflow at the Hubelj spring.

Second tracing experiment was performed after the melting of the snow, which gave high average day's throughflow of 6.33 $m^3 s^{-1}$ in the time of the injection. The recovery of the phage tracer was lower than in the first tracing experiments because of the high dilution of phage tracer and possible dispersion of the phage tracer in highly saturated strata, away from the main flow. The difference in the values of deactivation factor in both experiments could be contributed to the dispersion of the phage tracer rather than to the enhanced adsorption.

Completely different conditions were in the time of the third tracing experiment, when the average precipitation in the monthly period before the injection was only 2.8 mm. Recovery value of 0.007 % could be contributed partly to the deactivation of adsorption to different underground surfaces and partly to dilution of phage tracer because of the sudden augmentation of the ground water levels. Keeping in mind the time of the passage of phage tracer in first and second tracing experiment, that was 94.7 and 99.6 hours respectively and the day values of precipitation at Otlica in the time of reappearance of phage tracer; we can conclude that the main water which pushed the phages into the Hubelj spring came from other direction than from the background of Otlica.

The recovery values for phage tracer P22H5 in both three experiments at Nanos plateau are for several magnitudes lower than in the preceding tracing experiments in different karst regions, where bacteriophage was injected directly into flowing waters. Nevertheless, the results of all the three tracing experiments with the phage P22H5 on high plateau of Nanos confirm, that the phage tracer could also be injected into permeable strata with additional washing, where the water flow doesn't occur. The best recovery values for phage tracer can be expected, when permeable strata are sufficiently saturated due to longer rainy periods.

REFERENCES

BRICELJ, M. (1994): *Underground Water Tracing with the Phages of Salmonella taphimurium*. Ph.D. Thesis, Ljubljana, University of Ljubljana; 113 p.

BEHRENS, H., BENISCHE, R., BRICELJ, M., HARUM, T., KÄSS, W., KOSI, G., LEDITZKY, H. P., LEIBUNDGUT, C., MALOSZEWSKI, P., MAURIN, V., RAJNER, V., RANK, D., REICHERT, B., STADLER, H., STICHLER, W., TRIMBORN, P., ZOJER, H., ZUPAN, M. (1992): Investigations with natural and Artificial Tracers in the karst Aquifier of Lurbach System (Peggau-Tanneben-Semriach, Austia). *Steir. Beitr. z. Hydrologie 43*, pp. 9-158, Graz.

KÄSS, W. (1998): Tracing Technique in Geohydrology. A.A. Balkema, Rotterdam, Brookfield.

KINNUNEN, K. (1978): Tracing water movement by means of Escherichia coli bacteriophages. Publication of The Water Research Institute, Vesihallitus - National Board of Waters, Finland, Helsinki, No 25.

KRIVIC, P., BRICELJ, M., TRIŠIČ, N., ZUPAN, M. (1987): Water Tracing in the Rižana Spring Ground Water basin. *Acta Carsologica XVI(4)*, pp. 83-104.

ROSSI, P. (1994): *Advances in biological tracer techniques for hydrology and hydrogeology using bacteriophages*. Ph.D Thesis, Neuchatel, University of Neuchatel; 200 p.

SEELEY, N. D., PRIMROSE, S. B. (1982): The isolation of the Bacteriophage from the Environment. *J. Appl. Microb. 53*, pp. 1-17.