

SURVIVAL OF VIRUSES IN CARSTIC FRACTURED ROCK ENVIRONMENT

Mihael Bricelj



NACIONALNI INŠTITUT ZA BIOLOGIJO

HUMAN HEALTH HAZARDOUS VIRUSES

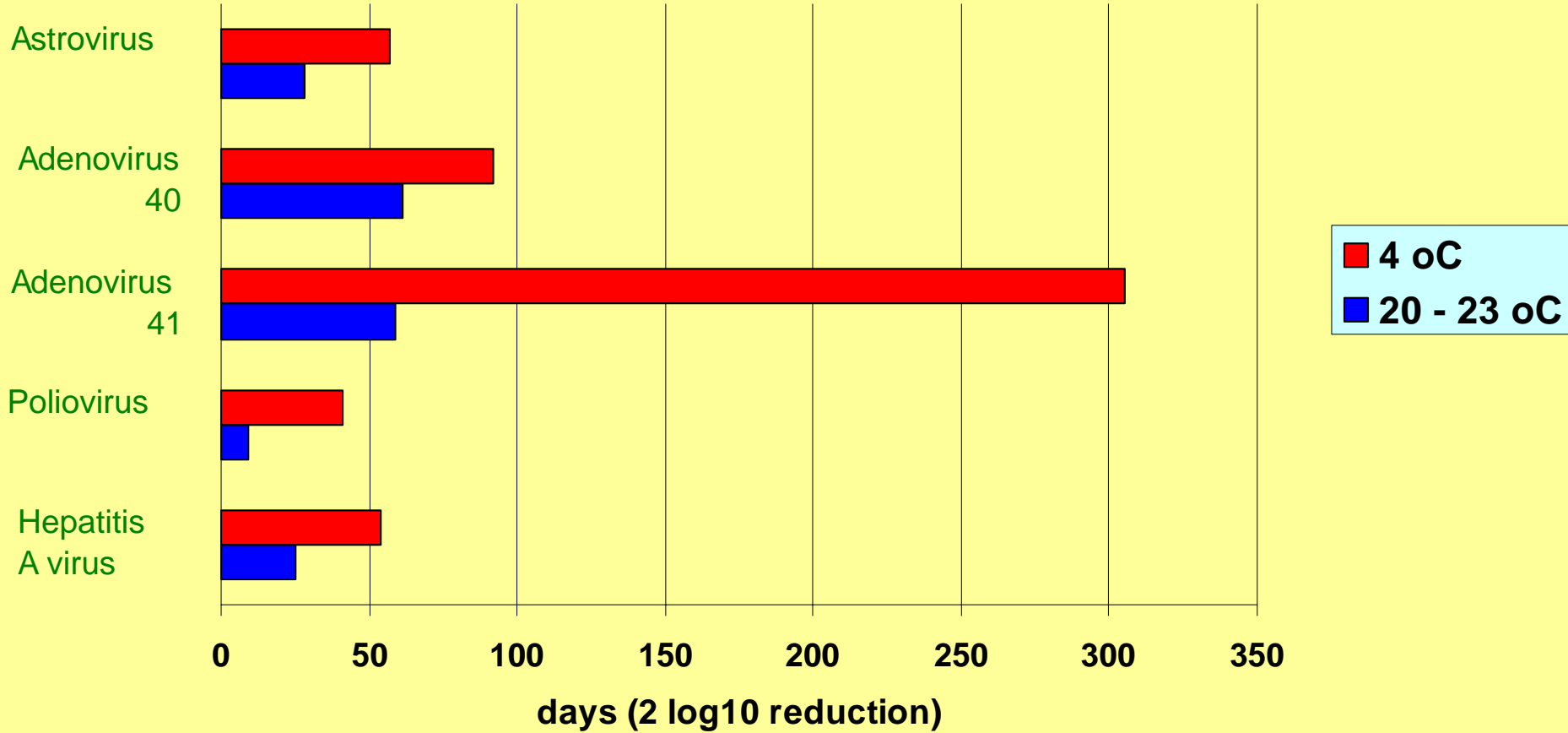
virus type	family	disease
Norwalk virus (NV)***	<i>Calciviridae</i>	acute epidemic gastroenteritis
Norwalk-like viruses***	<i>Calciviridae</i>	acute epidemic gastroenteritis
Sapporo virus (SV)	<i>Calciviridae</i>	gastrointestinal disturbances
Hepatitis A (HAV)**	<i>Picornaviride</i>	hepatitis
Hepatitis E (HEV)*	»HEV-similar viruses«	hepatitis
Rotaviruses (RV)**	<i>Reoviridae</i>	gastrointestinal disturbances
Echovirus (EV)	<i>Picornaviridae</i>	gastrointestinal disturbances
Enteroviruses (HEV-Nr.)**	<i>Picornaviridae</i>	gastrointestinal disturbances
Poliovirus (PV)	<i>Picornaviridae</i>	infantile paralysis

*in undeveloped countries 1-2% mortality in population and 30% mortality of pregnant women

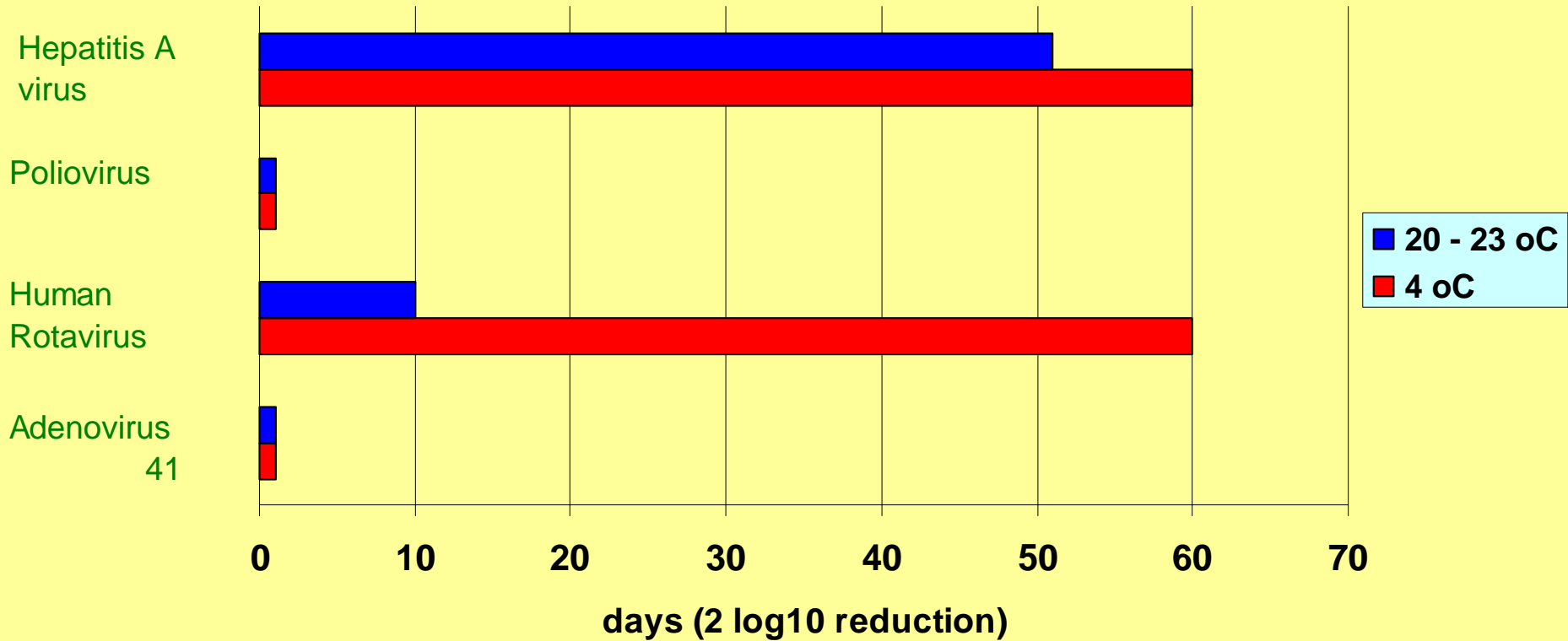
** various virus strains resistant to chlorinated water

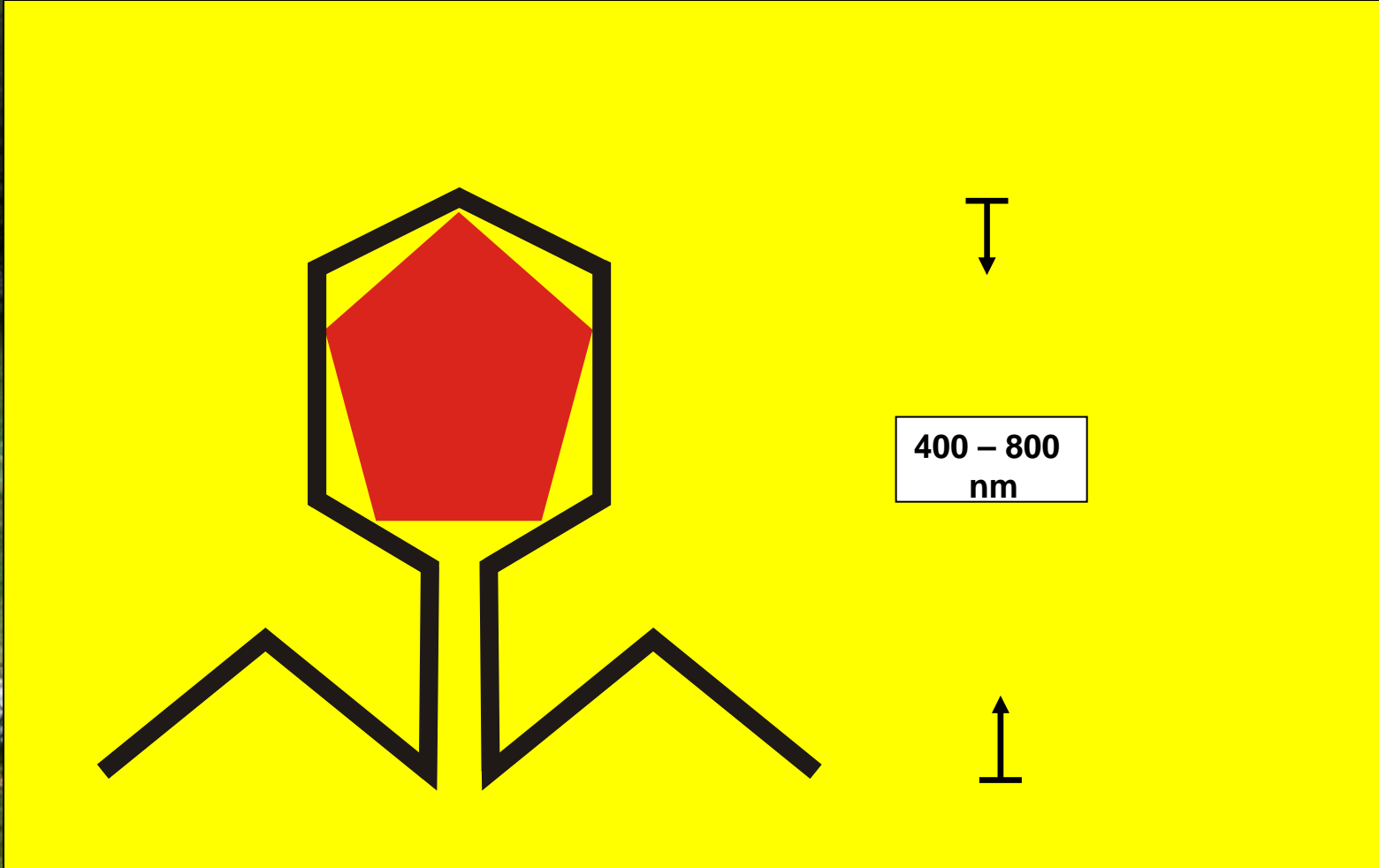
*** these viruses cause yearly only in USA disease symptoms among 23 to 40 millions inhabitants. about 50.000 hospitalizations and death of 310 patients

Survival of some enteric viruses in tap water



Survival of some enteric viruses on surfaces (aluminium)





- **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 size between 400 and 800 nm. They are relatively stable in environment and survive for a long time.**

BACTERIOPHAGE TRACER

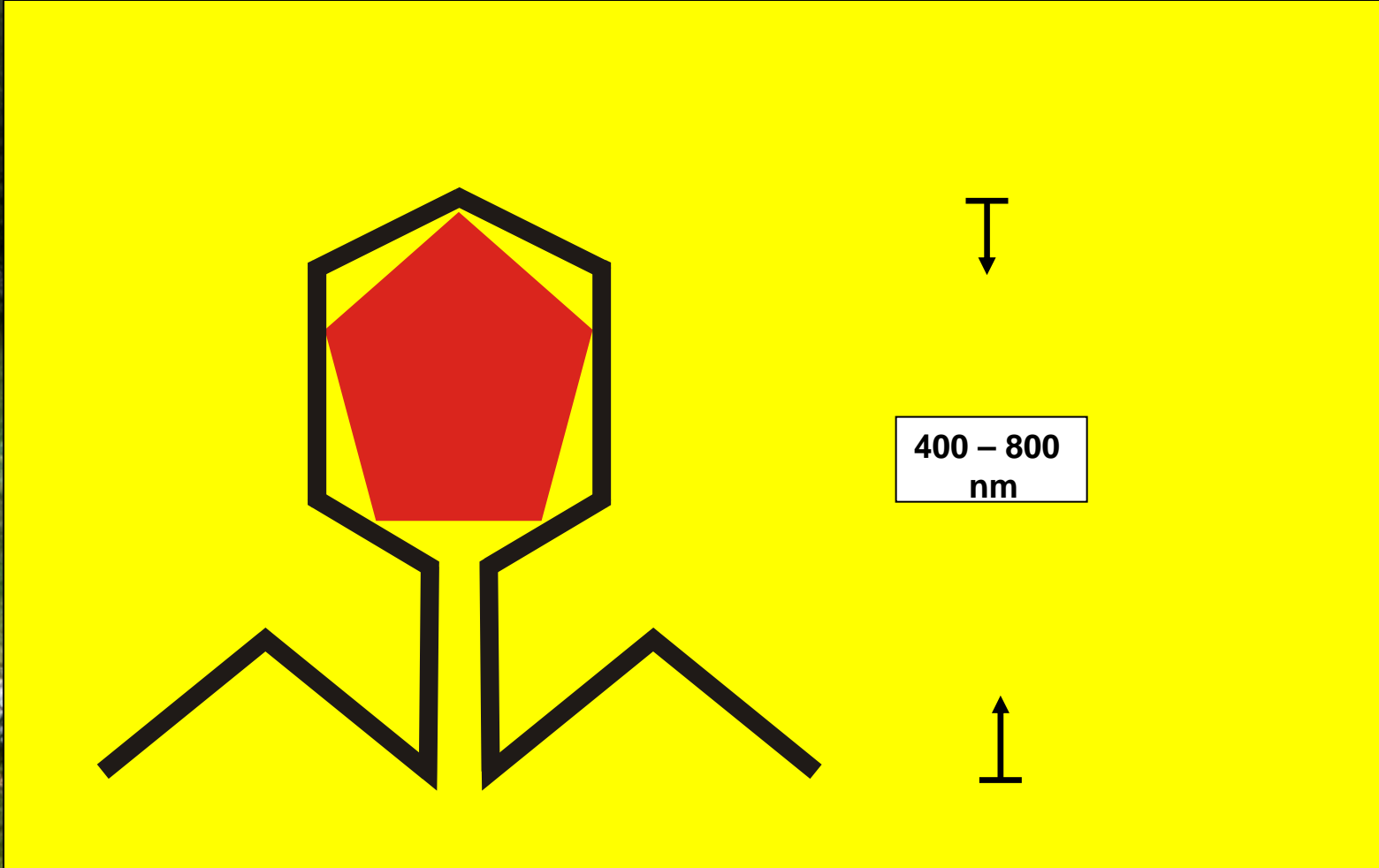
- P22H5 virulent mutant of
- *Salmonella typhimurium* strain LT2
- (NIB 22)

- ... because the phages of *Salmonella typhimurium*
- had been rarely encountered in surface waters (N.D SEELEY & S.B. PRIMROSE, 1982).

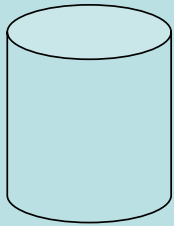
injection point	sampl. point	distance m	Tg h	vtg m/day	Recovery %
Kapsia	Kiveri	39,000	233.0	4,017	-
Smokovska vala	Rižana	3,880	348.0	268	0.006
Hotična	Rižana	12,450	482.0	620	4.5
Lurbach	Hammerbach	3,000	53.4	1,348	2.5
Kačji potok	Radeščica	19,200	511.4	901	3.0
Kačji potok	Obrh	20,000	704.6	681	-
Bajer	Krupa	6,000	99.1	1,453	-
Vrčice	Krupa	6,000	156.8	818	-
Movražka vala	Ara	800	80.8	237	-
Movražha vala	Mlini	1,000	94.6	165	3.4
Movražka vala	Sopot	1,043	145.1	173	-

Subsurface injection of bacteriophage to usaturated zone

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



Decimal diluting test tubes (FS – Na Cl 0,85%)



Sample



10^{-2}



10^{-4}



10^{-6}



10^{-8}



10^{-9}



10^{-10}

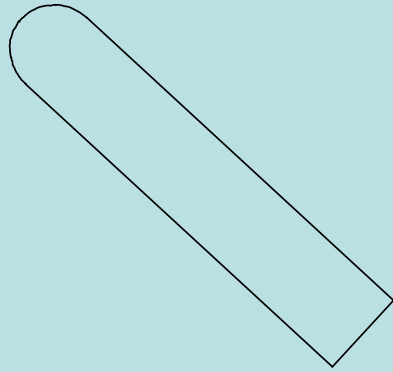
**Test tube
with soft
agar contains**



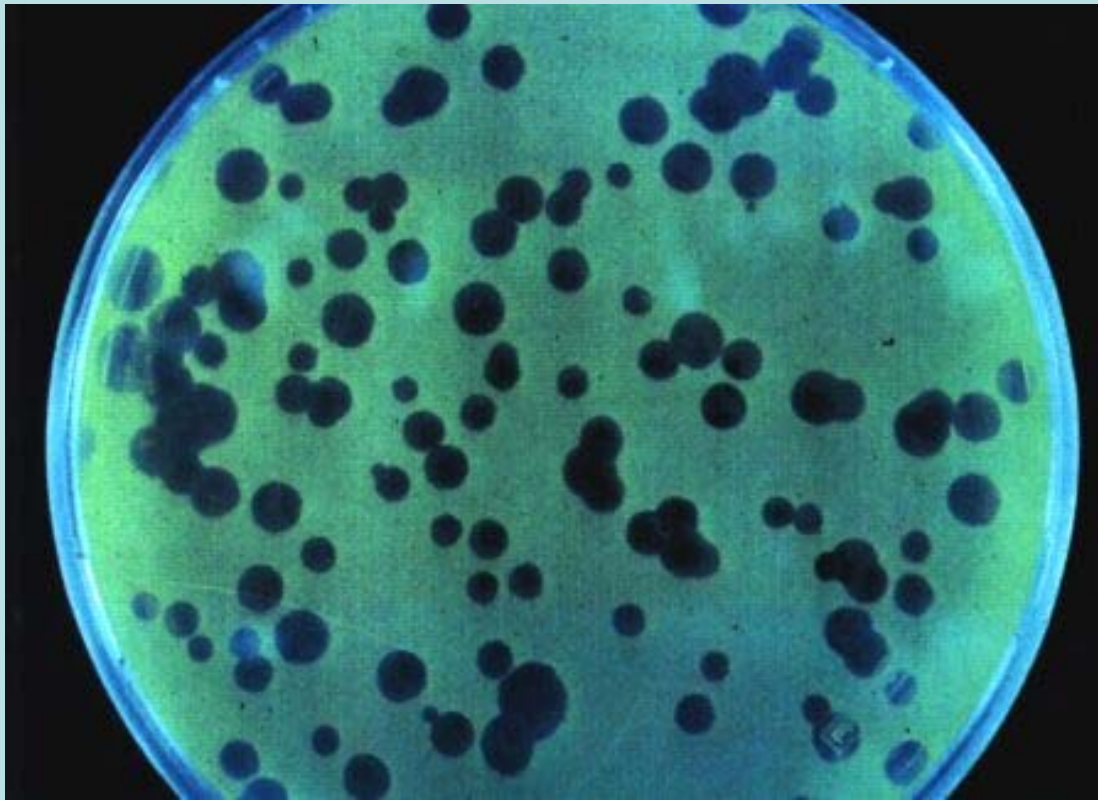
**3 ml of soft agar
0.2 ml host bacterium
0.1 ml sample (directly
or from dilution)**

**M
i
x
i
n
g**



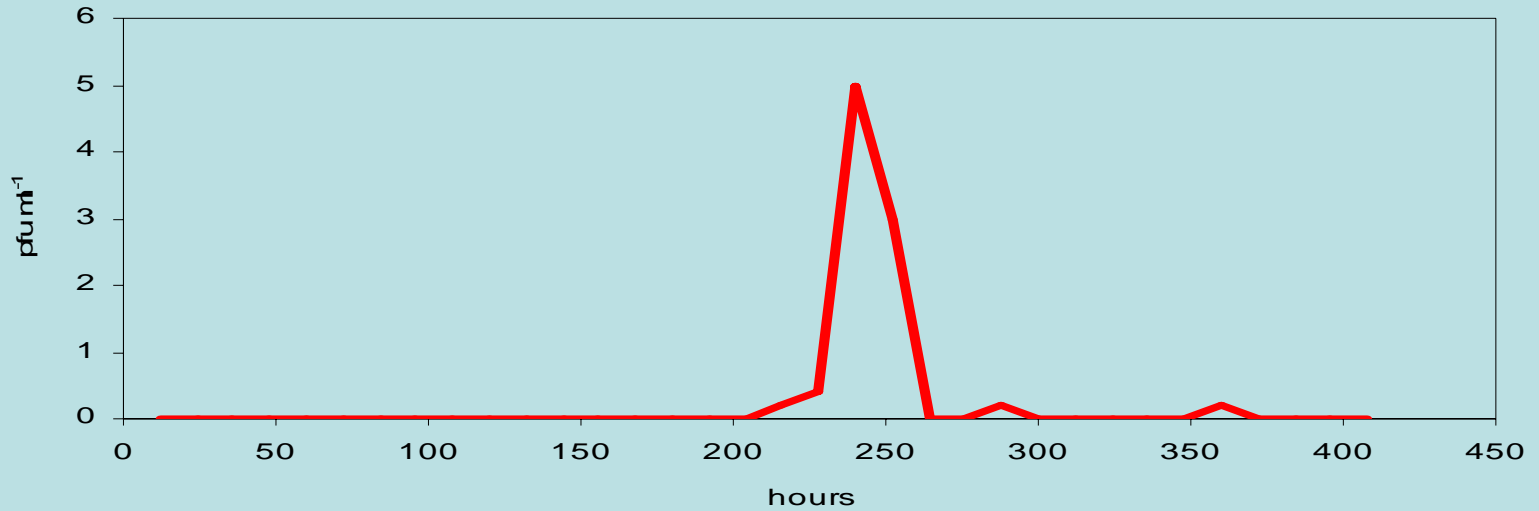


Petri dish



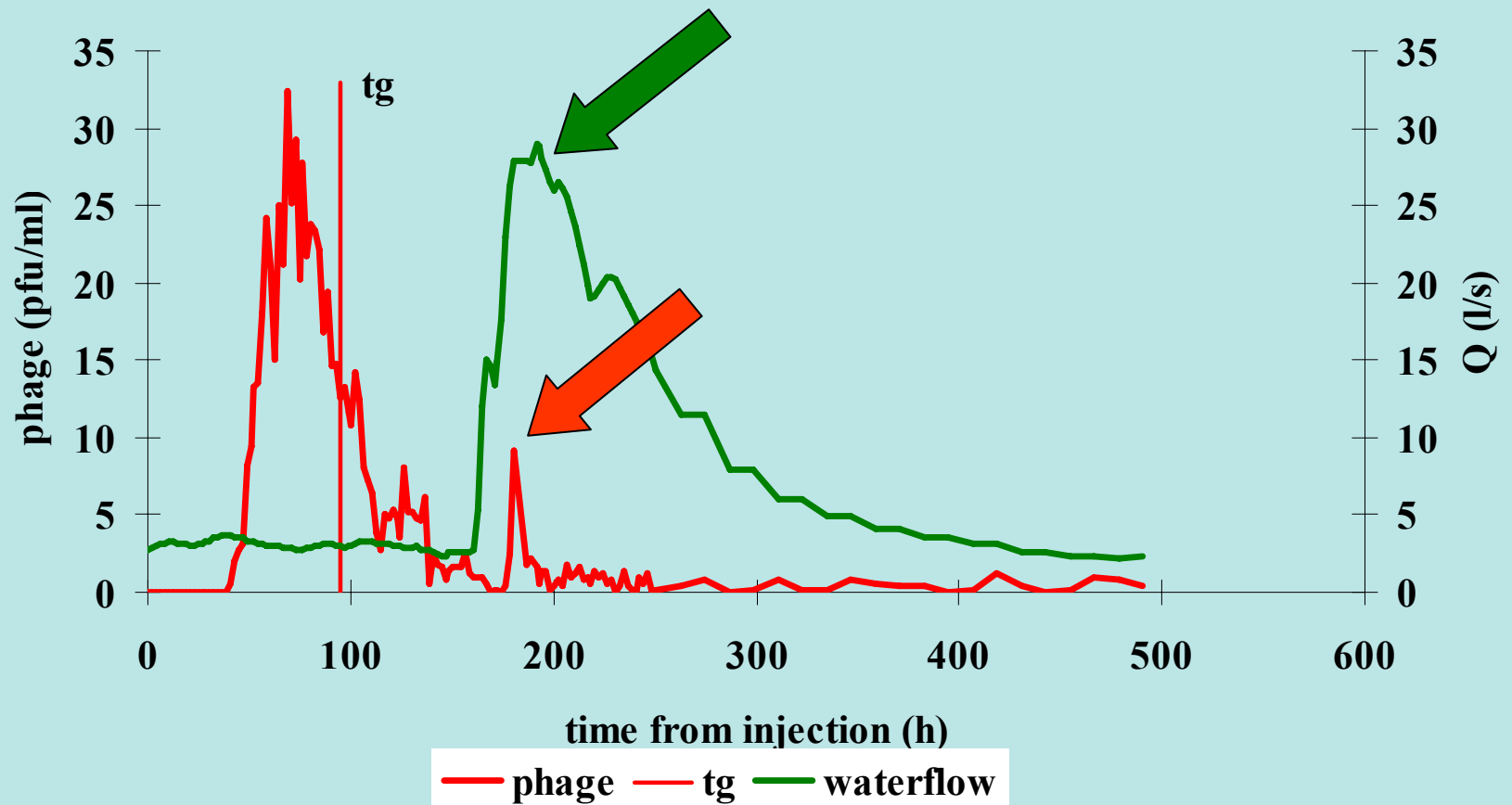
plaques

KAPSIA - KIVERI



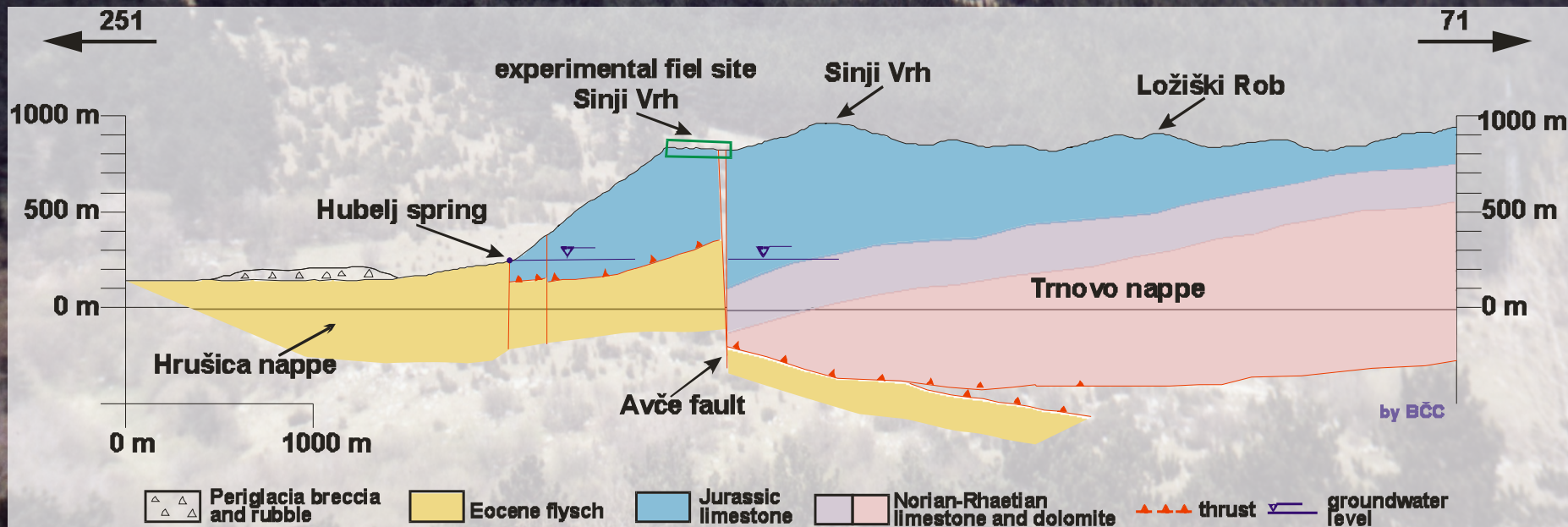
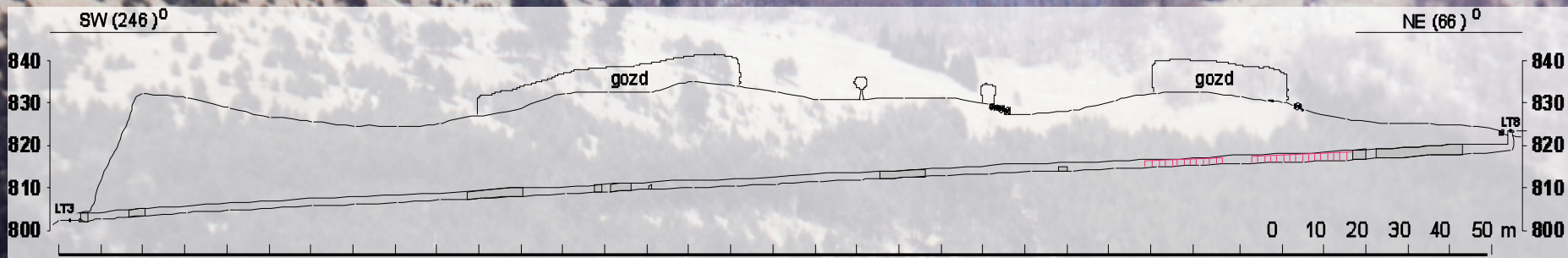
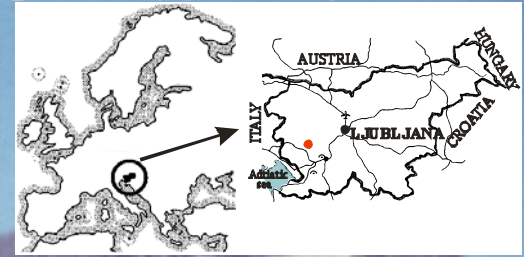
Injected quantity	Number of particles	Date of injection	Sinkhole	Distance	Spring
9.38 L	3.7×10^{14}	1. April 1984	Kapsia	39 km	Kiveri

t_g	Agar overlay	MPN	Concent.sample
hours	233	232	231

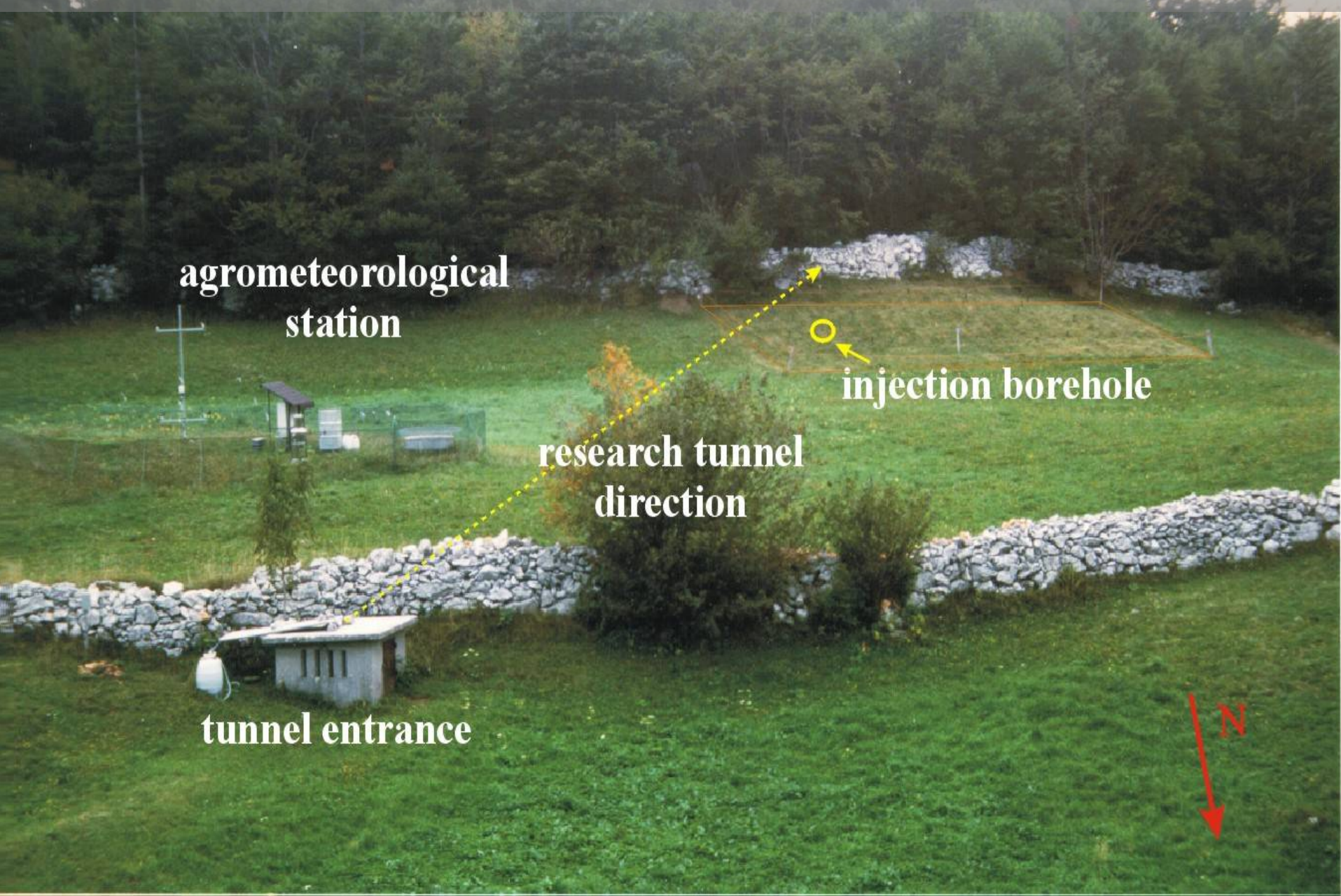


Tracing curve of phage P22H5 appearance in spring of Hubelj in October 1993

EXPERIMENTAL FIELD SITE SINJI VRH



EXPERIMENTAL FIELD SITE SINJI VRH



agrometeorological
station

injection borehole

research tunnel
direction

tunnel entrance

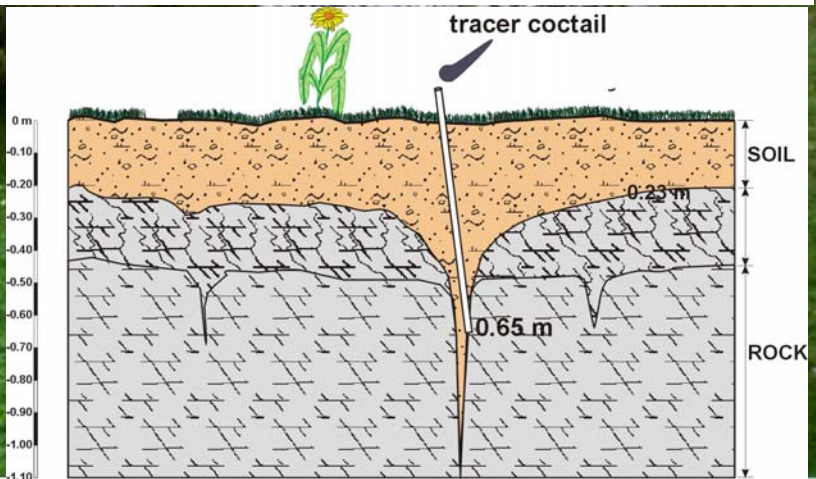
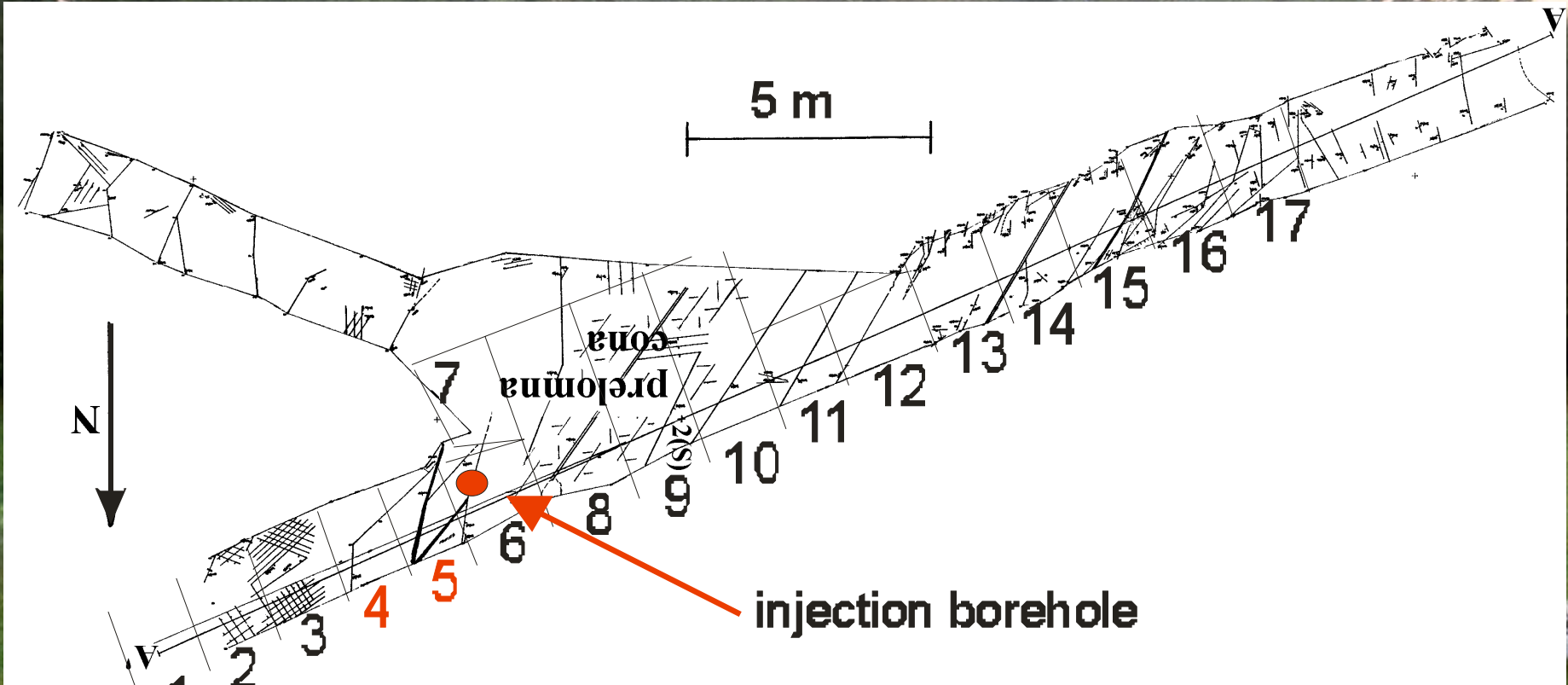
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EXPERIMENTAL FIELD SITE SINJI VRH



Tracer	Amount	Solution
lithium chloride	300 g	3.0 L
zinc sulphate	250 g	
sulfonic acid	10 mg	
pyranine	10 g	
naphthionate	20 g	
uranine	1.5 g	
sulforodamine B	5 g	
microspheres	10 mL	
potassium chloride	300 g	1.0 L
deuterium (90 %)		~ 0.2 L
bacteriophage P22H5	1.2 x 10¹⁵ pfu	4.0 L
rinsing water for the several tracers		2.2 L
	Σ	10.4 L

EXPERIMENTAL FIELD SITE SINJI VRH



EXPERIMENTAL FIELD SITE SINJI VRH system for water sampling

plastic tilt

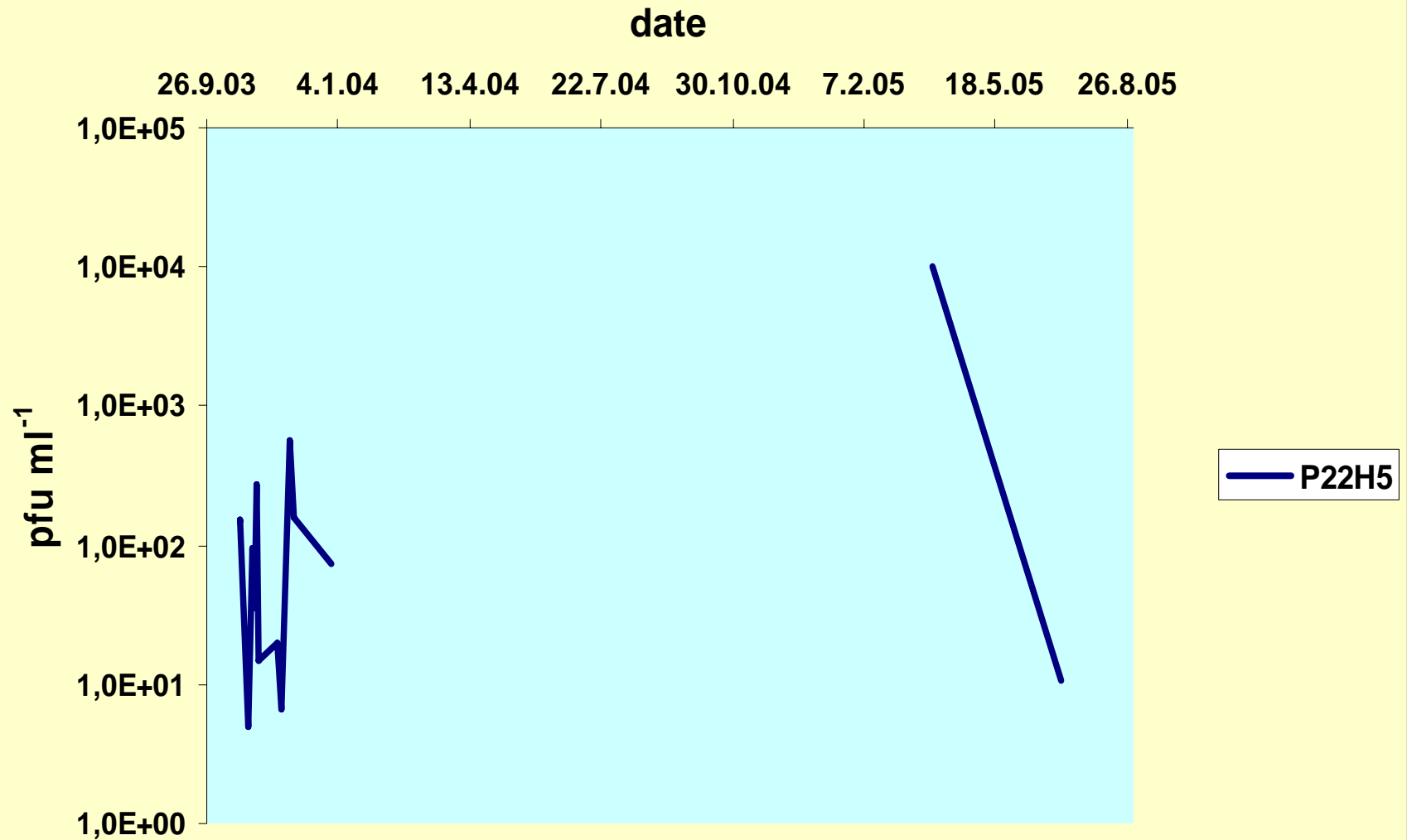
1.5m

2.2m²

- 10 segments
- $l=1.5$ m
- $A=2.2$ m²

18.11.2003

SP3 - P22H5 bacteriophage

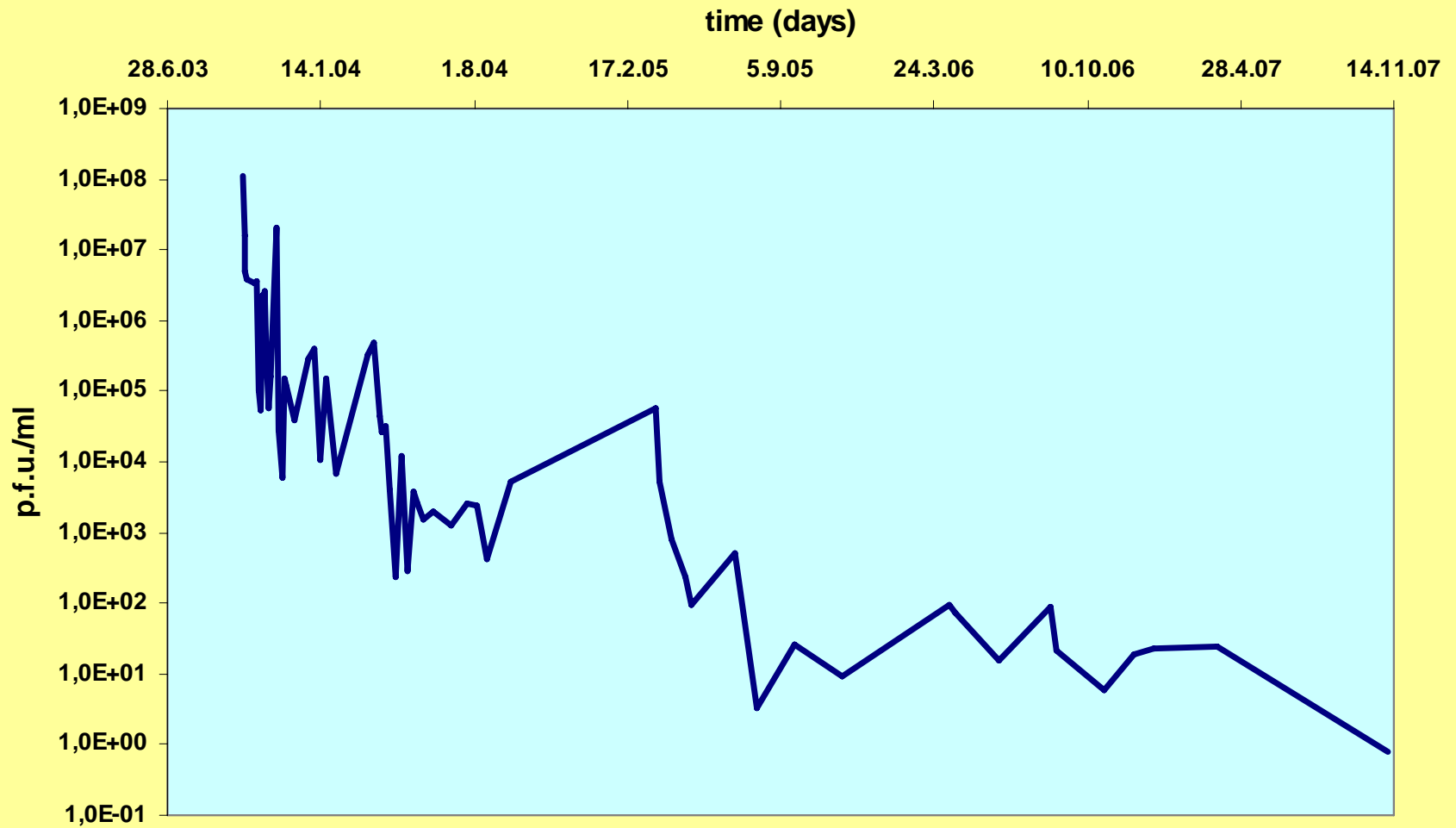


SP6 - P22H5 bacteriophage

days



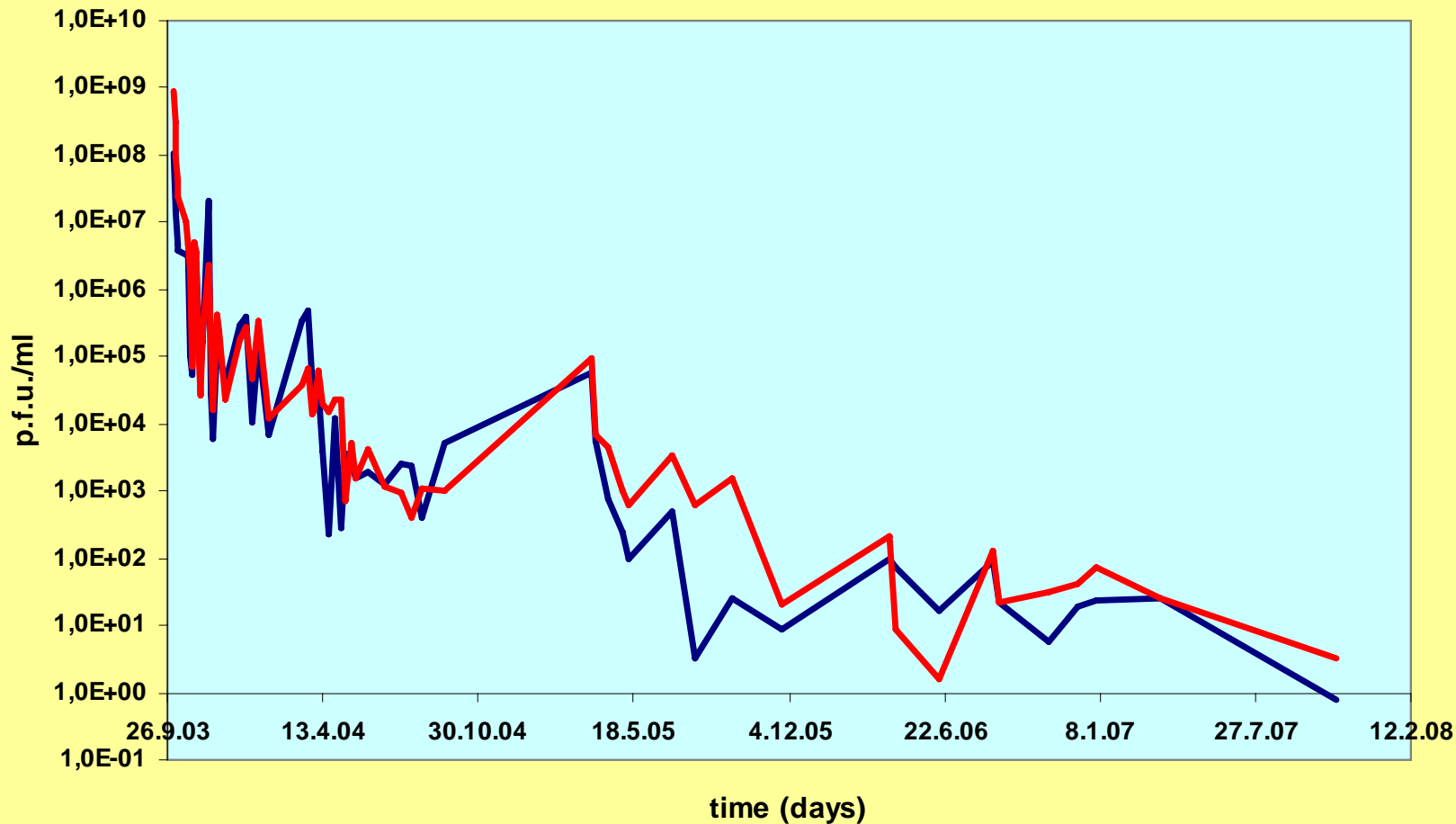
Bacteriophage P22H5 - Sampling point 4



Bacteriophage P22H5 - Sampling point 5



Bacteriophage P22H5 on SP 4 and 5

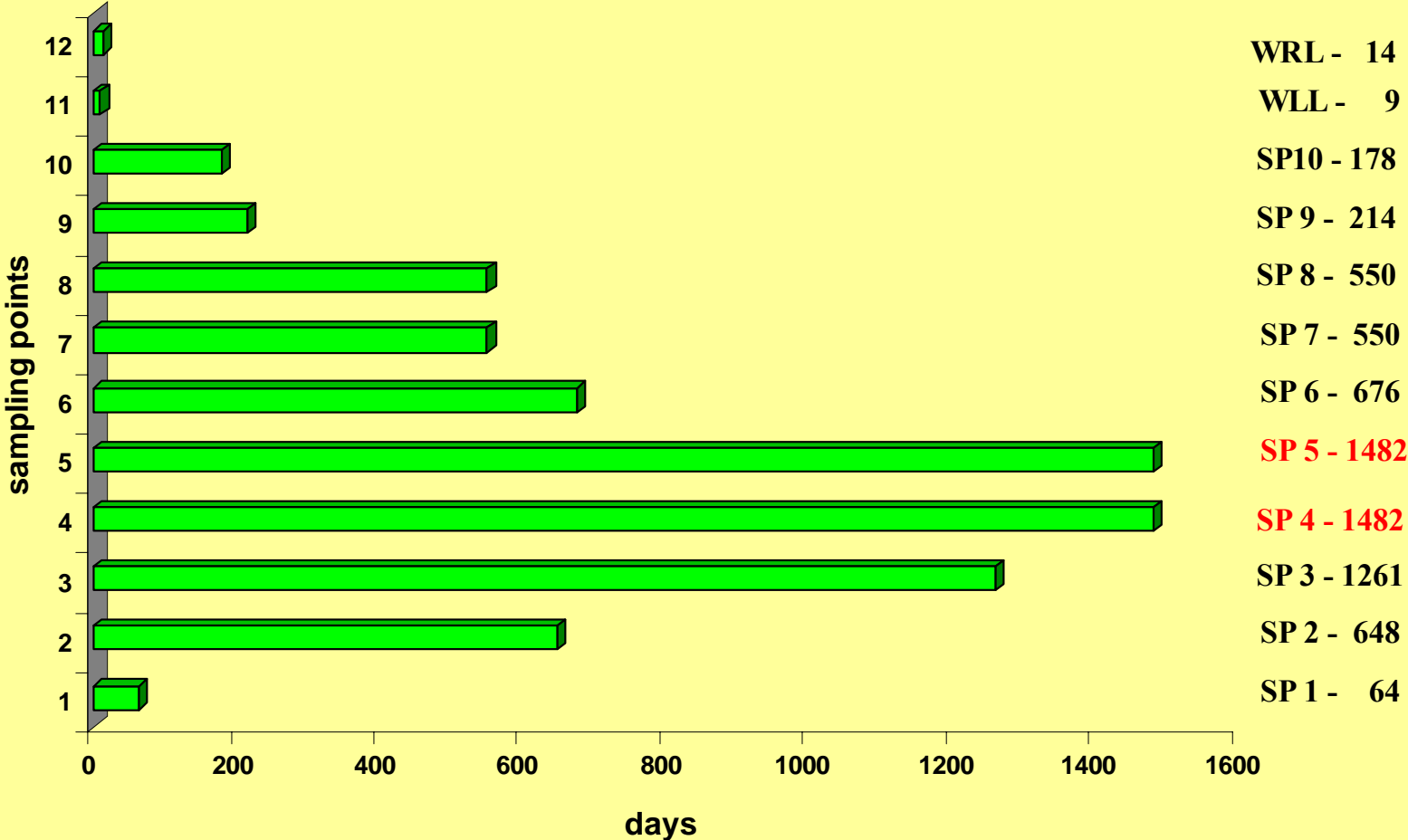


— Sampling point 4 — Sampling point 5

Sampling point	Appearance of tracer (days)	Max. value in pfu/ml	Appear. of max. value (days)	Last + result
1	7.0	2.1E+03	11.1	64
2	7.0	2.9E+02	7.0	648
3	22.1	5.7E+02	50.0	1261
4	5.0	1.1E+08	5.0	1482
5	4.1	3.1E+09	4.1	1482
6	8.0	4.3E+04	11.1	676
7	13.4	1.4E+04	30.9	550
8	7.0	6.5E+04	7.0	550
9	22.1	1.7E+03	24.9	214
10	8.0	4.9E+03	40.0	178

at 07.11.2007 (1482 days after injection) (SP 4) (SP5)
 pfu ml⁻¹ 8.0E-01 3.3E+00

The appearance of phage P22H5



CONCLUSIONS

BACTERIOPHAGES ARE APPROPRIATE PARTICULATE TRACERS FOR RESEARCH OF POLLUTANT TRANSPORT IN FRACTURED AND KARSTIFIED ROCKS

DATA OF COLUMN EXPERIMENTS WITH VARIOUS SUBSTRATES IN LABORATORY CONDITIONS CONFIRM THAT SOME HUMAN HEALTH HAZARDOUS VIRUSES, APPEARING IN POLLUTED WATER, ARE EQUALLY SENSITIVE TO ENVIRONMENTAL FACTORS AS BACTERIOPHAGES

COMPARATIVELY LONG-CONTINUED TIME OF BACTERIOPHAGE SURVIVAL IN FRACTURED AND KARSTIFIED ROCKS (> 4 YEARS) PROVE THAT THE KARST ENVIRONMENT COULD BE VERY VULNERABLE FOR POLLUTION WITH HUMAN HEALTH HAZARDOUS VIRUSES



THANK YOU!