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Comparative tracing experiments in a porous aquifer using bacteriophages and fluorescent dye on a test field located at Wilerwald (Switzerland) and simultaneously surveyed in detail on a local scale by radio-magneto-tellury (12–240 kHz)

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Abstract This article presents an example of a tracing experiment using two bacteriophages, T7 and f1, and a fluorescent dye (naphthionate), in a saturated porous environment. The test field was equipped with an injection borehole and 22 sampling piezometers set in three concentric half-circles. The distribution of permeabilities and the thickness of the aquifer were indirectly determined by Radio-Magneto-Tellury (RMT, 12–240 kHz). The results reveal a good correlation between the distribution of permeabilities obtained by RMT and the breakthrough curves and speed of migration of all three tracers. The restitution levels are far superior (by two to three orders of magnitude) in the more permeable zones, as opposed to those observed in th piezometers situated in less permeable areas. The speed of migration of the biological tracers is much greater than that of the naphthionate. In the most extreme case, the T7 bacteriophage migrated about 3.15 times faster than the chemical solution. These results indicate that bacteriophages are able to travel considerable distances along permeable gravel channels. They may be used as biological tracers and as models for the migration of pathogenic viruses. The simultaneous use of tracing techniques and appropriate geophysical methods leads to a better knowledge of the hydrogeological parameters of the underground terrain. This combination allows for a better interpretation both of the speeds of migration and of the maximal concentrations of the tracers, and thus considerably increases the interpretability of hydrogeological impact studies.

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Introduction

The transport and survival of pathogenic viruses in groundwaters is an important issue. In the United States, over 50% of the diseases transmitted by water are caused by contaminated aquifers. Of these cases, 65% are due to enteric viruses (Yates and others 1985). From 1971 to 1979, more than 50,000 people have been affected by a waterborne virus (Craun 1984).

Virus propagation and survival in aquifers has been investigated in depth in the laboratory, in batch or column experiments. The influence of saturated and unsaturated media, presence of organic material, and various physical factors such as temperature and pH have particularly been studied (Lance and others 1982; Lance and Gerba 1984; Powelson and others 1990; Straub and others 1992). Trials in natural environments are less numerous, and knowledge of porosity, permeability distributions, and boundary conditions is still insufficient to allow a precise relationships between the propagation of viruses and local underground hydrological conditions to be defined.

The use of bacteriophages as models for underground migration of pathogenic viruses started a few years ago (Goyal and Gerba 1979; Hurst and others 1980). This tracing method is based on the apparent similitude of the proteic structures of the bacteriophages (bacterial viruses) and of animal and plant viruses. Bacteriophages, also called phages, are obligatory viruses of bacteria (bacteriophage means "which feeds on bacteria"). Like all viruses they are incapable of reproducing on their own. They invade a specific host, diverting its metabolism to their own end: self-replication inside their host cell. Bacteriophages are constituted of a complex assembly of proteins enclosing the genetic information. It is intricately folded inside the phage's head, also called the capsid.

Phages vary in size from 45 to 230 nm. Their morphology is very diverse: there are phages with or without a tail, filamentous phages, and many others. Many also possess varied appendages (Ackermann and DuBow 1987).

The advantages of using phages for hydrological research are numerous:

- Their specificity towards their bacterial host is narrow. Several phages, each specific for a different bacterial species, can thus be used simultaneously during a tracing experiment and be detected individually.
- They are nontoxic and nonpathogenic for all organisms (human, animal and plant) other than their target bacterial species. 10^{15} phages weight about 1 g, so the organic pollution due to their introduction in an aquifer can be considered insignificant.
- Their persistence in the environment is limited to a few months. They do not accumulate; the background level in the environment remains negligible.
- The detection limit is low. In multiple serial analyses it is possible to detect one phage in 2–5 ml of water.

In karstic and fissured environments bacteriophages have been used as hydrogeological tracers in their own right since the 1970s (Fletcher and Myers 1974; Martin and Thomas 1974; Aragno and Müller 1982; Rossi and others 1992). Although many publications exist concerning bacteriophage migration in fissured environments, there are only a few on experiments conducted in areas of interstitial porosity (Schaub and Sorber 1977; Bales and others 1989).

This multidisciplinary project is a study of the migration and survival of several bacteriophages in a saturated porous terrain, on a site especially equipped for studying the propagation of artificial tracers in underground waters. Earlier experiments using different dye tracers had already revealed the existence of preferential channels at the Wilerwald test field (Sansoni and others 1988). The tracers followed rather complicated pathways that were difficult to interpret. To try to understand the situation better, a geophysical survey of the test field was made, using radio-magneto-tellury (RMT). This method uses frequency ranges of 200, 70, and 20 kHz. It gives very accurate values of the vertically integrated electrical resistivity of the geological formations at different depths.

This RMT method, calibrated on numerous borings in gravel and sand on the Swiss Plateau, allows an empirical relation to be established between the resistivity of the unconsolidated rock in saturated zones and its measured permeability. An image of the nature and thickness of the strata as well as of the distribution of permeabilities is thus obtained. The migration of tracers underground can then be interpreted in accordance with the spatial distribution of the hydrogeological parameters.

The aims of this research were: (1) to demonstrate the relation between the estimated permeabilities (established by the RMT method) and the behavior of the bacteriophages, and (2) to judge of their utility for hydrogeology as biological tracers in saturated porous environments.

Material and methods

Principles of the radio-magneto-telluric survey

Materials deposited in deltas and flood plains, such as at Wilerwald test field (Fig. 3 below), are characterized by numerous and rapid variations in their texture and mineral composition, from clean sand to silts and clays. The sand distribution is irregular and frequently cross-bedded. The lateral patterns are irregular too, and there may be both long and narrow or broad stretches of irregular patterns.

The best geophysical methods to explore these formations are those that do not homogenize the sediments at all (or do so only on a small scale), but also allow the detection of local heterogeneities to a great depth. The RMT electromagnetic method is perhaps the one best suited to explore these heterogeneous structures. Its good vertical selectivity and depth penetration, its high speed of performance, and the light weight and small size of the apparatus used are the main advantages of RMT prospecting (Turberg and others 1993; Mdaghri Alaoui and others 1993).

Radio waves generated by very low frequency (VLF) and low frequency (LF) emitters produce a primary magnetic field. This alternating magnetic field (between 12 and 240 kHz) also induces electric currents in the ground. The RMT receiver picks up the intensity of the magnetic field (Hy) with a coil and at the same time measures the potential difference (Ex) produced between two ground electrodes places 5 m apart. Thus, the apparent resistivity ($\mathrm{Rho_a}$) of the strata between the two electrodes can be obtained, given by the following equation:

$$Rho_a = \left(\frac{Ex}{Hy}\right)^2 \cdot \frac{1}{2\pi F \mu_0}$$

with: Rho_a, given in ohm meters (Ωm), Ex (V/m), the electric component of the electromagnetic field (measured value), Hy (A/m), the magnetic component of the electromagnetic field (measured value), F, the emitter's frequency, given in Hertz, set between 12 and 240 kHz, μ_0, the magnetic permeability of vacuum, given in Henry/m ($4\pi \cdot 10^{-7}$).

The parameters measured for each of the frequencies used are the apparent resistivity of the rock and the phase shift between the electric and the magnetic component of the electromagnetic field. These are sufficient to calculate the thickness and the true resistivity of each layer, using one-dimensional magnetotelluric inversion (Fischer and Le Quang 1981).

The depth D (m) of the sounding is given by the following equation:

$$D = 503\sqrt{Rho_a/F}$$

Geographical location and a brief hydrogeological description of the test field

The Wilerwald test field lies in the Emme valley (Fig. 1), 6 km south of Solothurn, on the edge of the forest that

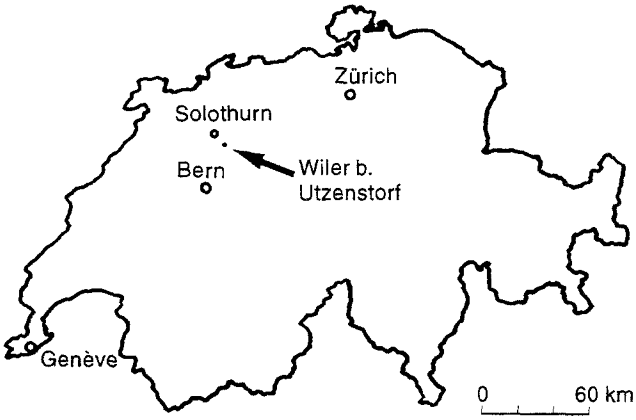

Fig. 1. Geographical situation of the test field of Wilerwald b. Utzensdorf (Switzerland)

surrounds Wiler b. Utzensdorf (BE, Switzerland). The aquifer is composed of postglacial sand and gravel and is covered by approximately 1.5 m of alluvial sediments (sandy silt) and humus. It is composed of 40–60 percent quartzite, 30–50 percent shales and calcareous sandstones, and 5–15 percent crystalline rocks. The thickness of this formation is quite variable and overlays a very irregular Miocene surface of molassic marls (Ledermann 1978). Lying between the Miocene rocks and the Holocene gravels are lacustrine sediments of silt and clay, very irregular in thickness, that function as an aquitard. The groundwater table lies between 1.5 and 3.5 m below the surface, with a gradient of about 0.4 percent in the northwest direction (Carvalho Dill 1993; Carvalho Dill and Müller 1992; Leibundgut and others 1992). Two dates were chosen to represent the configuration of the water table during the experiment. The isolines reflecting the different responses to rainfalls, a consequence of the heterogeneity of the field, can be seen in Fig. 2. Twenty-one observation holes set in three half-circles (C, D, F) and one injection well were bored on the site. They are 2.5 in. in diameter and descended through the entire thickness of the aquifer.

Injection method and tracers used

Two bacteriophages were chosen for their different morphologies. The T7 phage has an icosahedral head and a short noncontractile tail (fam. Podoviridae). The f1 phage is filamentous (fam. Inoviridae). Naphthionate (sodium amino-4-naphthalene sulfonate-1, MW = 254.24) was chosen as the fluorescent tracer. The chemical is frequently used in hydrogeology. It gives a good image of the speed of propagation of chemical substances in an aquifer.

The three tracers were injected simultaneously in piezometer B4, following the method given by Käss (1992, 1993). Water is pumped from a depth of 0.5 m below the water table. It is mixed with the different tracers aboveground and reinjected at a depth of 9 m. The operation lasted 20 min. The concentration of the tracers throughout the piezometer's water column is thus rapidly homogenized.

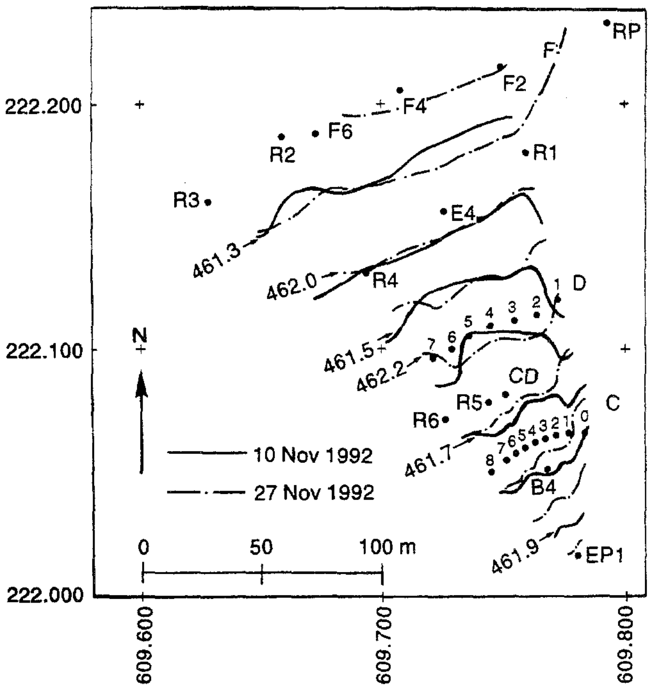

Fig. 2. Piezometric map of the Wilerwald test field (after Carvalho Dill and Müller 1992). (●) piezometers placed in concentric circles around the two injection wells B4 and EP1. The altitude of the water table, in meters above sea level, is shown by isolines: (–) on the day of injection and (–·–·) after 17 days

Tracers

Naphthionate: 1 kg, diluted first in 10 l of water; bacteriophage f1: $9.6 \cdot 10^{13}$ phages (total volume 960 ml); bacteriophage T7: $8.9 \cdot 10^{13}$ phages (total volume 2280 ml).

Sampling

Water samples were taken with 10-m-long weighted PVC tubes (1 cm ID). Each piezometer was equipped with a separate tube to avoid contaminations. The tube was connected to a small vacuum pump, and its weighted end lowered to 4 m belowground. As soon as the water level in the tube reached the head of the piezometer, the tube was lowered regularly by hand to maximum depth. The pump was then turned off and the tube removed and emptied into a plastic (polypropylen) container. The whole operation was repeated to rinse the apparatus and stir the water column before taking the sample. This is a rapid way of obtaining a homogeneous sample of the entire water column of a piezometer.

Phage analysis

Analysis of the samples is done following a modified method of phage enumeration on Petri dishes (Adams 1959). The modification consists of a division of the analysis into two stages. The first, upon receiving the samples, involves a simple presence/absence test using a single Petri dish per sample. In the second stage, positive samples are diluted if need be and analyzed on three Petri dishes. This method minimizes the analytical work necessary to treat the numerous samples of a multitracing experiment.

Naphthionate analysis

The samples were analyzed for naphthionate by W. Käss (Umkirch, D.). This was done by fixed-wavelength fluorescence ($\lambda_{Ex} = 322$ nm, $\lambda_{Em} = 420$ nm). The lower detection limit is 10^{-1} ppb.

Results and discussion

Geophysical map of Wilerwald site

The variability of the quaternary formation mentioned in the literature is also apparent on the local scale of the Wilerwald test field. In order to map it, the field was divided into 11 profiles (Fig. 3), totaling over 500 measuring points. Along each profile measures were made every 5–10 m. Three frequencies were used: 183, 60, and 19 kHz. The use of this frequency sounding allows the calculation of true resistivities and the local thickness of the underground layers by magnetotelluric inversion.

The contour map (Fig. 3) obtained from the 183 kHz apparent-resistivity data gives a reasonably good picture of the aquifer's structure. The higher *Rho*$_a$ values correspond to permeabilities (k) over 10^{-3} m/s. On the Fig. 3

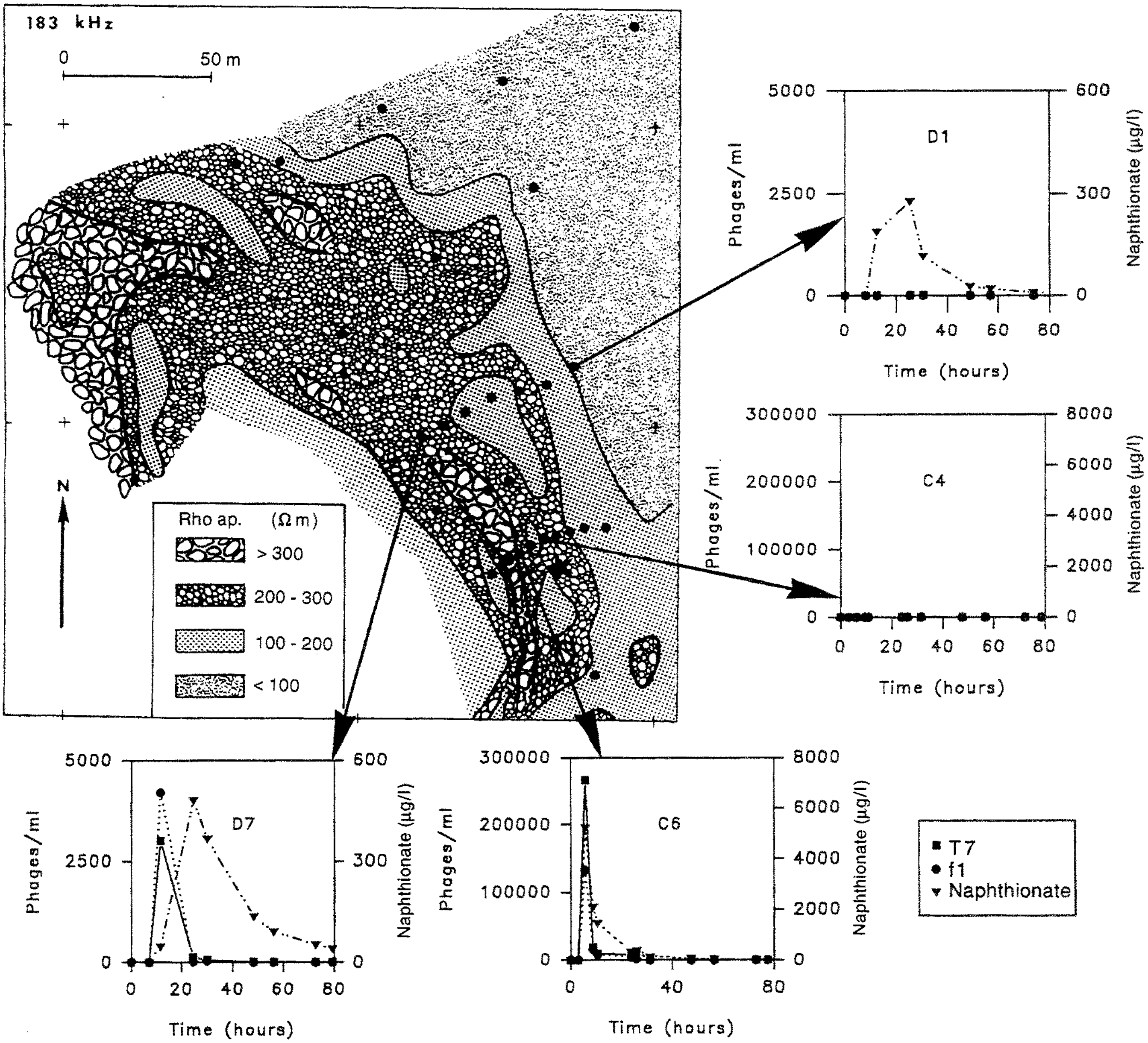

Fig. 3. Map of the Wilerwald test field showing the distribution of apparent resistivities determined by the RMT method and the breakthrough curves of the tracers for the piezometers C4, C6, D1, and D7 (after Carvalho Dill and Müller 1992). (●): piezometers; (★): injection point B4

they take the form of a channel running first northwards, then north-westward, and comprising piezometers C6 and C7, as well as D6 and D7. This paleochannel was formed during the last glacial retreat. It then probably formed a small affluent of the river Emme and was little by little filled up by coarser materials.

The smallest values recorded by RMT are of the order of 100 Ωm. They correspond to permeabilities of 10^{-5}–10^{-4} m/s. These much less permeable zones of restricted dimensions comprise piezometers C4, C5, D5, D4, and D1.

Influence of distribution of permeabilities on speeds of migration and breakthrough rates of various tracers

In addition to the geophysical map, Fig. 3 presents four breakthrough curves for the tracers injected at B4 (C4, C6, D1, D7). These four curves are redrawn in detail in Fig. 4–7. These clearly show that the maximal concentrations reached can be very different inside a single row of piezometers. For row C, the maxima were reached most rapidly in the piezometer C6 (see also Fig. 5). The highest overall concentrations of tracers were measured in this piezometer: $2.67 \cdot 10^5$ and $1.33 \cdot 10^5$ phages/ml for the phages T7 and f1, respectively, and 5220 µg/l for the naphthionate. Piezometer C4 only received a very small amount of all the tracers. This difference of several orders of magnitude can be explained by the very heterogeneous distribution of resistivities shown in Fig. 3. C4 is in the line of the current exactly facing the injection point B4, but situated in a zone of low permeability. Only a few meters away the influence of the high flow velocities in the paleochannel was considerable. The tracers were very rapidly and massively diverted northwestwards, towards piezometers C5 and C6.

This difference in the restitution rates is also very marked in the D row of piezometers (see Figs. 6 and 7). The highest concentrations of this series lying about 70 m from the injection site were found in the samples taken

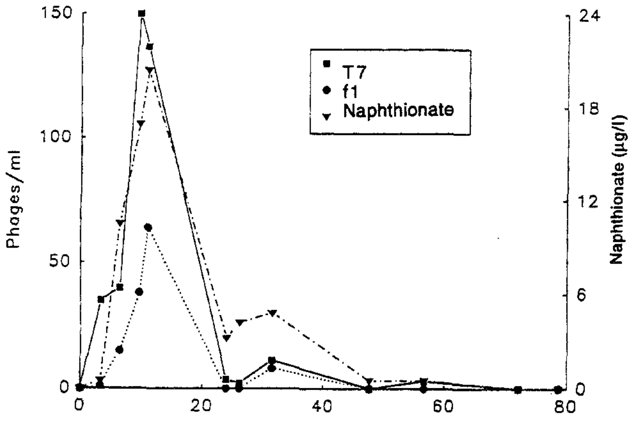

Fig. 4. Comparison of the breakthrough curves: naphthionate (Carvalho Dill 1993) and bacteriophages (Pierre Rossi) for piezometer C4

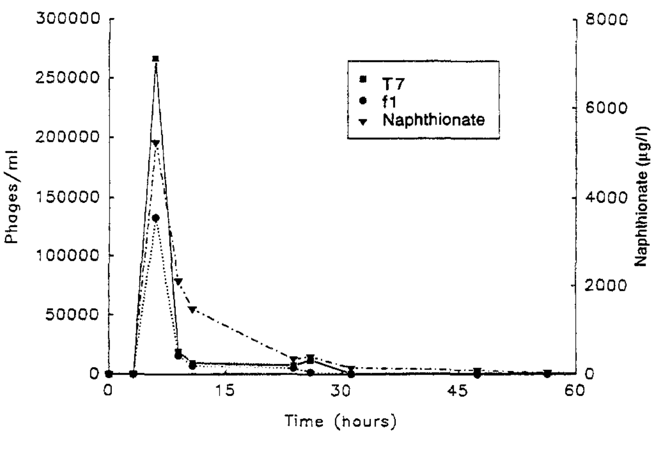

Fig. 5. Comparison of the breakthrough curves: naphthionate (Carvalho Dill 1993) and bacteriophages (Pierre Rossi) for piezometer C6

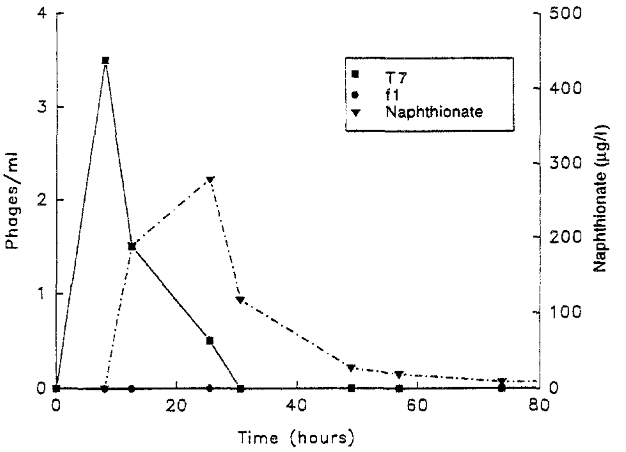

Fig. 6. Comparison of the breakthrough curves: naphthionate (Carvalho Dill 1993) and bacteriophages (Pierre Rossi) for piezometer D1. The scale of the y axis for the bacteriophage has been intentionally exaggerated (maximal concentration: 4 phages/ml!), by comparison with the one from Fig. 6, in order emphasize the speed of migration

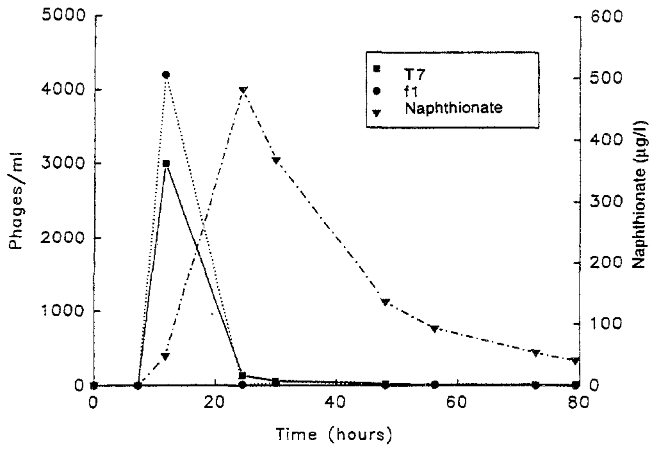

Fig. 7. Comparison of the breakthrough curves: naphthionate (Carvalho Dill 1993) and bacteriophages (Pierre Rossi) for piezometer D7

Table 1. Summary of the maximal concentrations (C_{max}) reached, their breakthrough times (T_{max}), and distances traveled by the tracers[a]

Piezometer	Distance from B4 (m)	Phage T7 C_{max} (P/ml)	Phage T7 T_{max} (h)	Phage f1 C_{max} (P/ml)	Phage f1 T_{max} (h)	Naphthionate C_{max} (μg/l)	Naphthionate T_{max} (h)	T_{max} ratios ($T_{max_{NAPH}}/T_{max_{PHAGE}}$)
C4	11.70	150	9.6	64	11	20.4	10.7	1.15
C6	14.32	$2.67 \cdot 10^5$	5.9	$1.33 \cdot 10^5$	5.9	5220	5.9	1
D1	69.18	9.5	8.1	0		278	25.5	3.15
D7	64.35	3000	11.7	4200	11.7	480	24.5	2.09
E4	112.5	22	243	1	243	N	N	N
F4	162.5	1	219	0		N	N	N

[a] N: not determined. P/ml: Bacteriophages per ml $T_{max_{NAPH}}/T_{max_{PHAGE}}$ ratio: ratio of breakthrough times of maximal concentrations

from D7, situated on the highly permeable channel. Generally all the piezometers of this series lying in a zone of low permeability only received minimal concentrations of the tracers.

In a same row of piezometers the differences between the maximal concentrations are better marked with the phages. For example, the D1 and D7 piezometers received 3.5 and 3000 phages T7/ml, and 278 and 480 μg/l of naphthionate, respectively. It thus seems that the phage concentrations are much more sensitive to variations in permeability, and they agree better than the naphthionate with the interpretation results of the RMT soundings.

The speed of migration of the tracer is also influenced by the distribution of heterogeneities within the aquifer. Over short distances, in permeable porous environments such as from B4 to C6 (14.32 m), the speeds of migration of the phages and the dye are identical. When the permeability decreases or the distance between the injection and sampling points increases, the phages move faster than the naphthionate. If both factors come into play, such as for the piezometer D1, the difference between the migration speeds of the phages and the chemical tracer becomes considerable. The $T_{max_{NAPH}}/T_{max_{PHAGE}}$ ratio, a good image of the relative migration rate of the tracers, is globally very high for the piezometers of the D series (data not shown). This ratio shows that at about 70 m from the injection site the phages have migrated 2–3.15 times faster than the fluorescent dye (Table 1). Furthermore, the ratios obtained are comparable to the permeability values given by the RMT soundings. However, D1 presented relatively high concentrations of naphthionate, showing that the hydraulic situation at the time of the experiment is another factor influencing solute transport. During the last experiment, daily observations of the piezometric level (Carvalho Dill 1993) showed the response of the aquifer to rainfall events. Piezometric observations are fundamental for the comprehension of solute transport. Higher amplitude variations and corresponding delays in reaching the maximal level seem to correspond with lower permeabilities and with the aquifer being confined or semiconfined.

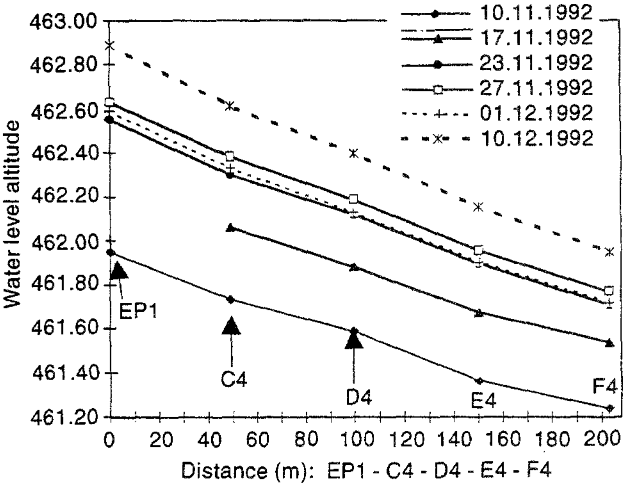

Fig. 8. Longitudinal profile along what was once considered to be the main direction of water flow, showing the variability of the water level (almost one meter) during the tracer experiment (after Carvalho Dill 1993)

On the other hand, it is clear that we are dealing with a complex phenomenon: Fig. 2 shows depressions in the west part of the field at sites with higher permeabilities (paleochannel), but simultaneously at the northeast part of the field (less permeable and confined situation) temporary depressions caused by the delay in response are also created. These are important as well, conditioning the direction of flow and transport, explaining the unexpectedly high concentration of naphthionate at the piezometer D1. Figure 8 shows the delta *h* of the observation wells situated on what was once considered to be the main direction of water flow (Sansoni and others 1987), showing the variability of the water level (almost 1 m) during the tracer experiment.

The speeds of migration of both the phages were identical for all the piezometers. The differences in phage morphology did not influence the rapidity with which these two phages traveled in the saturated porous environment. It seems, however, that it did strongly affect the restitution rates and the longest distance traveled. Phage T7 migrated in greater quantities than f1 in areas of low permeability. It was also recovered in the most distant piezometer that was reached (F4), covering a distance of 162.5 m in 219 h. The phage f1 migrated in greater quantities than T7 in permeable zones, as shown by the breakthrough curves of piezometer D7 (see Fig. 7), but did not reappear in the piezometers situated in areas of low permeabilities.

Semiquantitative interpretation of the tracer tests: modeling trials

RMT was used recently to obtain an image of the aquifer. This method is very accurate in locating lateral changes in resistivity (permeability) but does not give enough details of the vertical distribution of heteroge-neities. Figure 9 (after Carvalho Dill 1993) shows a 3-D representation of the distribution of true resistivities on the site. These were calculated using 1-D magnetotelluric inversion (Fischer and Le Quang 1981). Small block diagrams showing qualitative models of chosen RMT soundings complete the figure. The three vertical lines correspond to the depths of penetration of the frequencies used. This will also depend on the resistivity of the strata, so the lengths of the lines differ for each sounding. The lateral discontinuity of the strata is also illustrated.

The study of the single drill-core CD provided a detailed sedimentological description. Together with the indirect information given by the resistance to penetration registered during piezometer installation, the existence of local irregular lenses of finer material was made clear. These lenses seem to be irregularly distributed, indicating that it is erroneous to speak of continuous layers mainly in fluvioglacial sediments. Instead, the aquifer is composed of different pockets, containing varying proportions of finer sediments and of paleochannels of virtually clean gravels. One observes situations where the aquifer seems to be confined or semiconfined, and others of free nappe. Different and constantly changing hydraulic gradients exist within the site. The hydraulic mechanisms are therefore still far from understood. However, analytical models have been already tested (Leibundgut and others 1992) based on a hypothetical unidirectional mean flow, restricted to a simple three-layer situation, with a relatively small variation of the parameters.

The solution of an analytical model can only be formulated after some simplifying assumptions, in general very restrictive, have been made: it is usually necessary to postulate that the medium is homogeneous and isotropic, with a parallel flow of constant velocity, steady-state conditions and simple boundary conditions (infinite or semiinfinite media). A 3-D numerical model could be suitable, but it requires much more information about the structure and

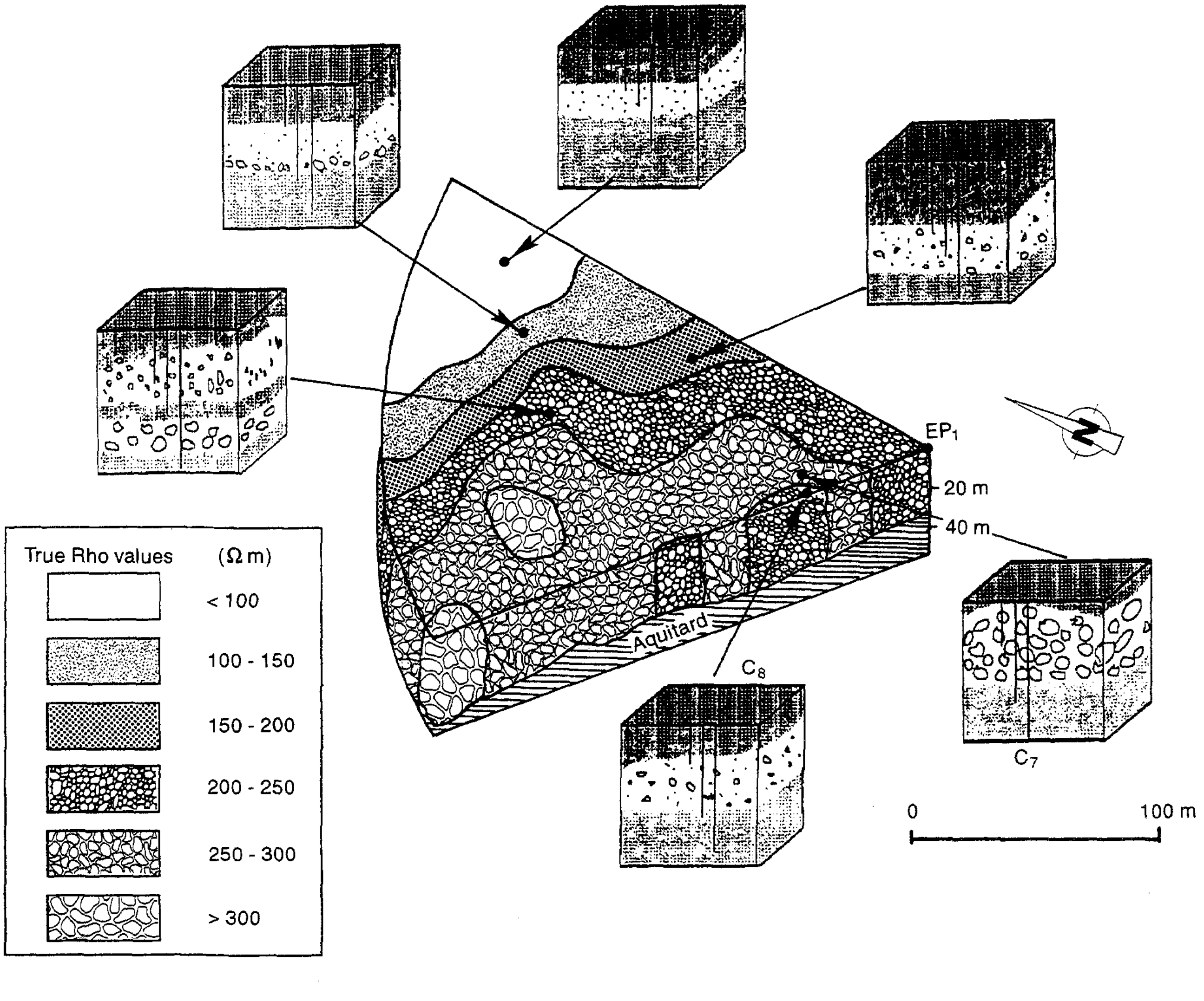

Fig. 9. 3-D representation of the distribution of true resistivities on the site calculated using 1-D magnetotelluric inversion. Small block diagrams show qualitative models of chosen punctual RMT soundings. The three vertical lines correspond to the depths of penetration of the frequencies used. For more details see text

boundary conditions. Unfortunately, not enough information about the vertical distribution of permeabilities was available. Besides, due to the narrow diameter of the observation wells, it was impossible to use other hydraulical techniques (such as flowmeters), which could have supplied useful information about the vertical distribution of permeabilities.

It is always possible to create a model from a set of data, but a model should be valid, i.e., it should have the capacity to simulate the aquifer's behavior and to describe a specific event. In a heterogeneous aquifer, the above investigations are essential and must be done before any attempt at modeling is made.

Conclusions

The radio-magneto-telluric method (RMT 12–240 kHz) offers new and very interesting possibilities for analysis on a very local scale of the nature and thickness of geological formations. It also allows a better understanding of the migration of tracers in porous environments. The values obtained reveal the complexity of the environment: paleochannels appear, filled with coarse gravel, whose permeability is much higher than that of the adjoining regions. They therefore determine the movements of the tracers. In light of this geophysical evidence, it becomes clear that the tracers bypass the obstacles represented by pockets of lower permeability and follow easier pathways through the more permeable areas. The breakthrough curves of the tracers will then be strongly influenced by the size and hierarchical organization of these channels.

In our experiment, the small permeable channel that cuts through the test field would be practically undetectable by classical hydrogeological methods. However, its existence explains the massive and rapid drainage of the tracers from the injection point to the C6 piezometer and then towards D6 and D7. T7 and f1, the two bacteriophages used in this trial, migrated faster than the naphthionate in all piezometers tested, independently of the distribution of permeabilities. It is only the maximal concentrations, both of the biological and chemical tracers, that can be said to be strongly influenced by the heterogeneity of the environment.

Bales and others (1989) also noted the rapid migration of viruses and bacteriophages in a sandy aquifer. According to these authors, the concentration of viruses along the flow shows evidence of dispersion but not of retardation. Their observations show that viruses and phages migrate 1.6–1.9 times faster than conventional fluorescent tracers, considered as conservative. Our results from Wilerwald test field confirm those of these authors. The two bacteriophages, T7 and f1, were injected simultaneously and migrated at the same speed, faster than the saline naphthionate solution. The ratio of the arrival times of the concentration maxima ($T_{\mathrm{max}_{\mathrm{NAPH}}}/T_{\mathrm{max}_{\mathrm{BACT}}}$) shows that the bacteriophages migrated up to 3.15 times faster than the conventional fluorescent tracer.

The tracing experiment on the Wilerwald test field reveals the interest of bacteriophages as biological tracers in environments of interstitial porosity. They could be used to simulate the propagation of pathogenic viruses in a porous aquifer, and this may lead to a review of the currently accepted values for maximal propagation speeds.

This trial also shows that using fluorescent tracers to define the size of protected areas around drinking-water pumping stations and sources is not always the best solution. The distribution of permeabilities should also be investigated by a geophysical method that does not homogenize the environment, so as to render possible the detection of favored channels and underground flow pathways. Interpretation of tracer breakthrough curves will then become much easier. Furthermore, this type of investigation is absolutely necessary to determine the appropriate injection and sampling points.

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