Doctoral School on Engineering Sciences
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Extended summary

Techniques and Methodologies for the hydrodynamic characterization of fissured aquifers

Curriculum: Ingegneria dei Materiali, delle Acque e dei Terreni

Author

Stefano Palpacelli

Tutor

Prof. Torquato Nanni

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Abstract

The research that has been developed during the PhD examined and refined methods which, have seen the use of both classical and next generation tracers in hydrogeology, to check for interactions/absorption with aquifer matrix of different geological domains of central Italy. In particular the use of a biotracer (DNA molecules), experienced in the scientific international until now only in the study of surface water, allowed to develop a new methodology in the study of groundwater.

This method was developed in a laboratory specifically designed to compare different tracers and different materials aquifers, then successfully applied in campaign in a hydrogeological structure of the Umbria-Marche carbonate ridge. A significant part of the research was finally given to the detailed reconstruction of the hydrogeological conceptual models and the hydraulic parameters of carbonate aquifers due to the geological-structural.

The results obtained from laboratory tests in column and batch of tests with different tracers are: the differences in arrival times of the peaks, at equal hydraulic conditions, are due to the different geometry of the columns; the values of effective porosity are very close to the values actually obtained by weighing the columns at the end of the tests; the values of actual velocity obtained with tracer tests are similar to those obtained in an indirect way.

Experimentation with the biotracer both in column and in field applications showed that is very useful for the study of karst aquifers characterized by conduits or dual porosity, but is less suitable in porous media if the fundamental objective of the field study is the determination of the effective porosity; in the column test biotracer showed almost exclusively convective flow; the adsorption of DNA on the limestone is negligible, so the biotracer behaves as a tracer conservative than carbonate materials; it has characteristics that can be safely used in the tests tracking.

Keywords

Hydrogeology tracers, column tests, batch tests, quantitative real-time PCR, synthetic DNA tracer, simultaneous tracing, Montelago, karst hydrogeology

1 Problem statement and objectives

The aim of this work is to find a new tracer with such characteristics that it can be easily used in the study of carbonate, karst and fissured aquifers, and at the same time compatible with the current regulation system.
Indeed the karst aquifers supply approximately 25% of the world population - even 50% in some European countries - with drinking water [1]. Meteoric water reaches the carbonate, karst and fissured aquifers by diffuse percolation via a thin soil cover or by infiltration in the epikarst via preferential infiltration areas such as ponors, sink-holes etc. In the fissured media, and in particular in the karstic ones, the processes of water circulation are sometimes extremely complex and difficult to outline and thus to model. This is the consequence of the high heterogeneity of the aquifer which origins conduits with different speed circulation, temporary water accumulation areas and, not frequently, real ground lakes. Therefore the procedures and return times of possible polluting substances are highly different from the ones that characterize porous aquifers. The karst and fissured aquifers are, infact, really vulnerable to the contamination due to their specific hydrogeological features, which allow an easy access for the surface contaminants, and the rapid diffusion of ground waters on wide distances, with limited attenuation processes (filtration and delay). Moreover it has been recently observed the appearance of Diclofenac, a human anti-inflammatory drug, in an apparently uncontamined Deutch karst system [2]. For this reason the authors have concluded that the intrakarstic biodegradation was of minor importance. Nevertheless, the conventional notion that the attenuation of a contaminant is inefficient in a karst conduit, generally due to the brief periods of immobility, has been recently criticized [3]. Some authors have observed the decrease in the concentrations of organic micropollutants between ponors and the source that cannot be explained by compounds diluition and delay. Thus, the almost elimination of some organophosphates was explained by biodegradation [3].
In this general description, contradictory at times, the use of innovative techniques for tracing in karstic masses seem fundamental for the evaluation of the water residency times, the vulnerability of said aquifers and for the definition of protection areas.
The combination of the information which comes from tracer tests toghether with the data of structural geology and other remarks can lead to improve the conceptual models of the conduits net system in an aquifer ([4]; [5]; [6]). However in a lot of locations, the routes of the conduits of the karst system are unknown. The artificial tracer tests, as a complementary instrument, allow a greater examination of the structure of the karst system and a direct evaluation of the speed of groundwaters.
Even if the theoretical aspect of the use of tracers is well known, there are many issues that need to be clarified/deepened on the use of tracers in fissured media. Infact, in the fissured media, the hydrodynamic characterization shows a set of problems since the methodology used in porous media cannot be applied equally in the study of fissured media.
One objective of this research was to identify a tracer that could be inject in karst and fractured aquifers in very limited quantities (microlitre or ml), and that could mark huge volumes of water, with high sensibility, detectable at very low concentrations, highly stable for the estimated times of tracing and at the same time biodegradable in such a time frame that should be nearly non- exsistent at the first moments of the picking up.

Unfortunately the use of the tracers, especially in Italy, is not controlled by specific regulations which should be reserved to graduate studies. Thus, every time a tracer test needs to be done, to obtain permissions is really difficult and the waiting periods are, most of the times, not compatible with the research schedule.
The main objective of this work has been the research of a new biologic tracer composed of synthetic single stranded DNA molecules (ssDNA) [7], never before used in the study of ground waters, that could eliminate this kind of problem, its applicability in field tests and the comparison with the electrolytic and fluorescent tracers known/commonly used.

2 Research planning and activities

In the current work a series of laboratory tests were performed with different artificial tracers employing several speed methods and the multitracer one (both with conservative and reactive tracers). These methodological approaches have been applied to various one-dimensional columns, with the aim to identify the ideal tracer to be used in a field test. The resulting information have been useful to set up and measure a series of deterministic transport models. A karst basin has been characterized both from an hydraulic point of view and a geomechanical point of view to outline the development of the heterogeneities inside the flow system. Finally, field tests have been conducted with the use of fluorescent tracers to verify the feasibility of the use of segments of synthetic DNA as an innovative tracer, both with single tracer tests and simultaneous insertion of two or more tracers.

In particular, the laboratory activity was divided in two phases.
In the first phase it was carried on a study to evaluate the absorption of tracers on porous media and on carbonate lithotypes, representative of the aquifers in different hydrogeologic places in central Italy. The aim of the study was, then, to establish the choice of the most efficient tracer to use for hydrogeologic objectives (measures with tracers in ground waters and the determination of the aquifer parameters). The absorption was analyzed through batch tests in the laboratory, using different traces and testing 4 different types of soil. Furthermore, several tests with laboratory columns of different dimensions were performed on them in order to examine the dimensional effect on the results obtained when using the tracers KCl, KBr, NaCl, fluorescein and rhodamine WT, through the instantaneous injection technique.
The second phase of the research activity in the laboratory consisted in the creation of PVC columns and plexiglas equipped with measurement instruments that measure continuously the rate, the temperature and the electrical conductivity. These columns were designed and created by the writer and filled with solid mineral granule mixtures collected directly on the site.

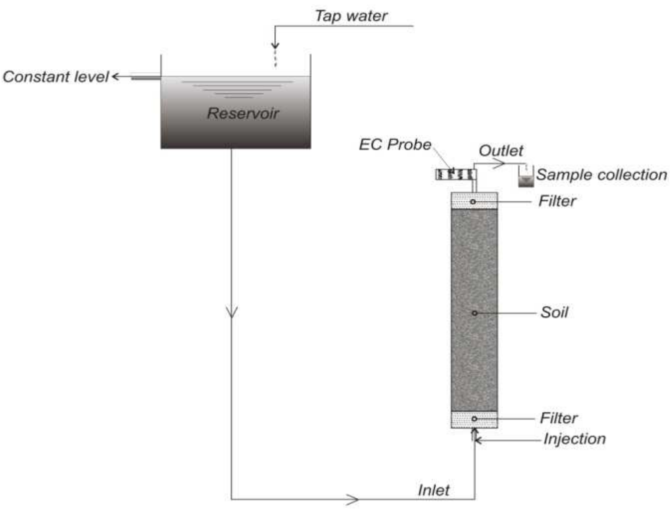

Figure 1. Schematic layout of the test apparatus.

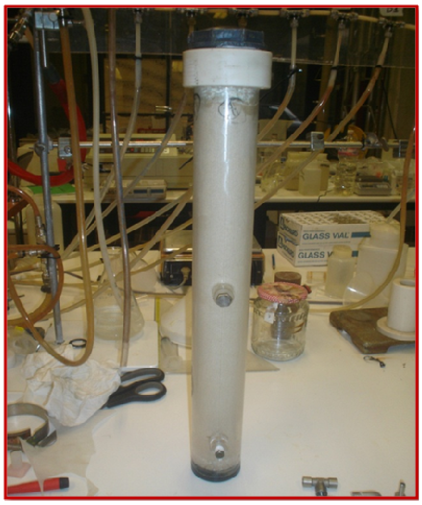
Figure 2. Close-up of the column.

This material represents the main aquifers in the Marche territory.
A tracer (electrochemical, fluorescent or biologic) was then injected inside the columns to examine the hydrodynamic behavior of the aquifers and to get, in a controlled situation, the main hydogeological parameters (transmissivity, dispersion, Darcy's law, effective speed of rate, effective porosity, dispersivity, absorption coefficient etc.). Furthermore, comparative tests have been carried out using different tracers in the same flow conditions and with the same materials, to find information about the reactivity of the porous media compared to a tracer data and thus to have useful information for the following experiments in site, ultimately to find the "ideal tracer". Currently the tracers used in the experiment are sodium chloride, potassium bromide, fluorescein, rhodamine WT, DNA sequences and the sample material is obtained by crushing down limestone formation of "Calcare Massiccio", which is the basal limit of the hydrogeological Apenninic system in Central Italy. Finally a preliminary field test was conducted to test the working principles of the synthetic DNA tracer and its potential limits.
The experiment proceeded, then, in the field with the analysis of a carbonate, fissured and karst ridge. The main aim of the study was to examine the hydrogeologic model of the hydrostructure, to identify the main flow patterns, the relationship with contiguous structures. For this reason it was necessary to perform a geologic-structural survey aimed to recreate of the hydrogeologic model of the macroanticline of Sefro-Montelago. This allowed to plan a first pilot tracer test with sodium fluorescein successfully fulfilled in January 2011, and then others with the application of several different tracers (fluorescein, tinopal), among them the DNA tracer (synthetic DNA sequences: biotracer) in the different nutrition condition of the karst system.

The application in site (realized in the karst ponor in Montelago, in the high Macerata territory) has allowed to establish a comparable filtration speed in the different tracers, and thus to obtain water volumes involved and the storage coefficient.

The development of this research is the application of tracers in other aquifers and other field tests, especially with the use of biotracers, in the different nutrition condition of the aquifer.

3 Analysis and discussion of main results

The first phase of the research in laboratory was aimed to the characterization of the absorption of the tracers on porous materials and on carbonate lythotypes, which represent the aquifers of different hydrogeologic domains in central Italy.

The results of the batch tests have proved that the tracer which gave the best results in terms of absorption is the Br. Even the fluorescein shows fairly contained absorptions, except for the more clayey soil. It is important to note a very low absorption for all the tracers when speaking about the carbonate material that constitutes one of the analyzed aquifer.

Table 1. Some results of the batch tests.

Soil	Tracer	Kd (cm^3/g)
X	K+	12.41
	Br-	0.04
	Rhodamine WT	8.95
Y	K+	4.72
	Fluorescein	7.2
Z	Br-	0.01
	Fluorescein	0.69
W	K+	0.69
	Br-	0.001
	Fluorescein	0.91
	Rhodamine WT	0.45

These experiments have two main implications. The first one is that, when referring to the carbonate aquifer (such as sand toghether with gravel used in the tests), all the tested tracers have shown contained absorption. The second implication is that, if we are in the presence of lens made of less rough material or aquitards, then the tracer which gave the best reaction is, without doubt, the Br.

As for the dimensions of the test equipment, it has been observed that in smaller columns the calculated effective porosity was strongly underestimated and, in some cases, missing its physical meaning. So the use of these columns is to be excluded or it can be only used on grain thin soils. Columns with a diameter larger than 5 cm show better results.

Table 2. Column features and test results.

Column name	Column length (cm)	Column diameter (cm)	Soil	Tracer	Total porosity	Effective porosity (calcu-lated)	Effective porosity (weighted)	Effective velocity (cm/s)
B	42.7	5.2	Sand	KCl	0.38	0.203	0.252	9.1×10^{-3}
10200A	184.5	9	Sand with gravel	R wt	0.28	0.262	0.251	1.6×10^{-3}
B2	18.4	1.8	Sand with gravel	R wt	0.26	0.213		7.6×10^{-2}
D1	27.2	5.2	Sand with gravel	NaCl	0.33	0.296		4.6×10^{-3}
A1	39.4	1.8	Sand with gravel	KBr	0.32	0.199		1.2×10^{-3}
B1new	19.7	1.8	Sand with gravel	KBr	0.32	0.132	0.213	9.7×10^{-3}
B2 new	20	1.8	Sand with gravel	KBr	0.25	0.001		6.1×10^{-4}
B2new	20	1.8	Sand with gravel	Fluo-rescein	0.25	0.051		1.9×10^{-3}
B2new	20	1.8	Sand with gravel	NaCl	0.25	0.814		4.1×10^{-3}
B	42.7	5.2	Sand	KBr	0.38	0.192	0.259	8.7×10^{-3}
PVC16	180	16	Sand with gravel	NaCl	0.25	0.233	0.221	4.6×10^{-3}
ST1	44.6	5.2	Sand with gravel	KCl	0.306	0.232	0.226	1.1×10^{-2}

The tracer BTC curves in different columns (different for lenght and diameter) has shown that for the smaller columns, even if with different diameter, the main peak is nearly instantaneous, this is most likely due to the pressure transfer caused by the injection. In general, the occurrence of secondary peaks in tracer experiments has been also related to the balance of the hydrodynamic conditions after the injection.

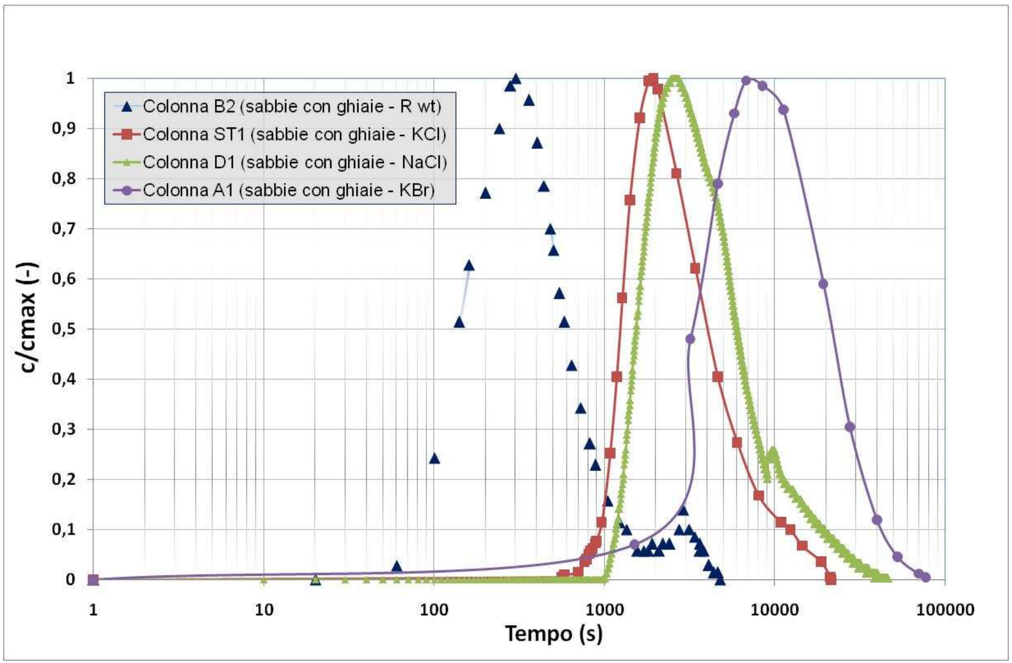

Figure 3. Comparison among different column test. BTC of the tracers.

Then, in the second phase of laboratory tests, a preliminary investigation of adsorption of KCl, fluorescein and DNA tracers to the studied aquifer material was conducted by performing batch equilibrium tests consisting in the agitation of mineral analyte samples in different bottles containing a relatively large volume of water solution containing the tracer under study at different concentrations.

These tests are known to simulate a completely dispersed particle system [8], thus allowing to investigate the kinetics of sorption. Batch tests performed with KCl, fluorescein and single−stranded DNA molecules revealed no significant adsorption of these tracers on limestone, confirming their suitability for tracing in fractured calcareous rock systems.

Column test studies were carried out using different equipment and applying several test conditions and configurations, to optimize the test and to evaluate the in laboratory optimal conditions (with the aim also being to compare laboratory to field conditions). In fact, the duration of the test is a basic element to be accurately investigated and taken into account both in laboratory and field tests, in order to properly plan the operations of installation. Contact time between aquifer material and water is known to play a significant role on sorption amount, therefore, to be more representative, the time duration of a column test should be similar (or at least of the same order of magnitude) to a field test. Moreover, the sample time span has to be taken into account (especially in perspective of preparing a field test), so as to provide the necessary equipment and plan the execution of the test

Different equipment and test configurations were therefore assayed to select a test setup that was as similar as possible to the actual situation of the investigated aquifers (especially limestone fractured and/or karst aquifers, often containing important springs) and depending on the optimal conditions obtained in the laboratory tests.

The presence of secondary peaks in the BTC of the smaller columns can be explained both with the balance of the hydrodynamic conditions after the injection [9], and also with the flow restriction and different channel lenght [10], typical of karst aquifers and fissured/fractured aquifers, where water had different velocity. Occurrence of flow regions, in which groundwater flow was slow or practically immobile, was probably responsible of long tail and skewness in most measured BTCs [10].

In the longer column, the DNA breakthrough curve showed a peak that preceded the peak of the KCl breakthrough curve, despite almost identical flow conditions. As clearly demonstrated [11], such behavior is due to size exclusion effects and selective flowpaths detected by the DNA tracer. The electrolytic tracer, on the other hand, affected almost the entire effective flow section. These findings suggest that in porous media, the DNA molecules travel more rapidly than ionic tracers, which are characterized by a more widely distributed flows. The rate of migration of linear DNA through porous media mainly depends on either the pore size or the length of the DNA molecules. During their transport, these molecules orient properly to “snake” through the pores. As the pore size decreases, the movement of the DNA molecules becomes impeded, thus explaining the preferential advancement of these molecules through the larger pores.

It has previously been demonstrated that the size of the DNA molecules largely affects their transport behavior, even through fractured rock systems [7]. Smaller DNA molecules are subjected to greater losses due to a more rapid diffusion into the relatively immobile water occurring within pores and fractures, whereas larger molecules enhance the retention processes in porous materials [7].

In terms of water flow and solute transport through the column, the difference between the KCl and the DNA tracer behavior is that the former shows a strong dispersive process.

The calculated pore water velocity is higher in the case of the DNA tracer (33.6 m/d); consequently, the effective porosity is very low (0.08). The KCl tracer gave an effective porosity close to the actual porosity, measured by weighing the saturated column at the beginning and at the end of the tracing test.

The longitudinal dispersion coefficient obtained from the DNA tracer tests was one order of magnitude lower than that from the KCl tracer test, which corresponded to a dispersivity of 0.23 cm against a value of 26.3 cm (calculated from the KCl tracer test). This last value confirms that the DNA tracer shows an unimportant longitudinal dispersion (of approximately zero).

The preliminary field test (performed with the aim to verify the test operations, the suitability of DNA tracer in groundwater and the tracer behavior in the subsurface environment) had a qualitative aim, as a preparatory action to detect if DNA tracer arrived at the sampling points. The results have underlined that there was a slight difference in the peak times of Fluorescein and DNA tracer, just observed in the column tests and also reported in bibliography [11]. Furthermore, it was probably due also to slight differences in the hydraulic and piezometric conditions between the two field tests. Such investigation was the first application of the DNA tracer for hydrogeological purposes at a real scale.

The BTC of the tracer test with fluorescein performed on 17th January 2010 in hydraulic conditions of temporary lake and a maximum load ponor showed the presence of only one peak after approximately 22 hours which proves a drainage dominant circulation. The BTCs referred to a second tracer test performed on 12th May 2011 in hydraulic conditions of no lake and the ponor in its final discharge phase, with the injection this time of 20 g. of Sodium Fluorescein and after 6 days of 1 ml of DNA biotracer solution containing 10^{16}

DNA molecules, showed an arrival of the tracer in multiple impulses with the main peak after 93-95 hours as regard the Fluorescein, and after 80-82 hours as regard the DNA. This can be explained with a drainage interdependent circulation.

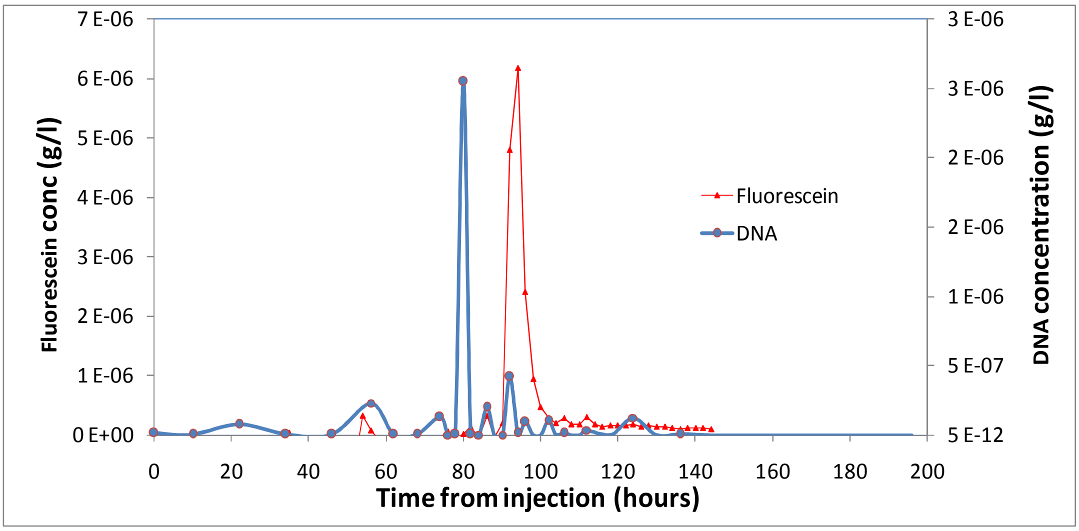

Figure 4. BTC curves of the Fluorescein and of the DNA tracer, related respectively to the tests on 12th May 2011and on 18th May 2011.

The laboratory tests have shown the compatibility of the tinopal cbs-x simultaneous tracer with the DNA tracer. On the contrary, the Fluorescein cannot be used toghether with the DNA. The first tracer tests have been performed at different times because of the fear of an interference both in the survey of the biologic tracer concentrations and of the fluorescent one, also because the biotracer behavior in field was not well known. The compatibility of the DNA tracer and of three fluorescent tracers commonly used in field tracer tests was previously evaluated with a laboratory test, aimed to observe possible interferences in the survey and/or quantification of the DNA tracer. These interferences could be linked to a overlapping in the excitation spectrum or emission of the SYBR Green I (a non specific fluorescent dye which intercalate the double-stranded DNA and for this reason used in the real-time PCR surveys to verify the amplification products) and of the fluorescen tracers analyzed.

The obtained results have proved what was said on the base of the excitation/emission spectrum above mentioned, clearly underlining the compatibility of the tinopal as the tracer to be used simultaneously with the DNA tracer.

After the compatibility laboratory tests it was decided to have another tracer test with the simultaneous injection of DNA tracer and Tinopal CBS-X.

The BTC referred to the multiple tracer test toghether with the fissured-karst circulation system in condition of low water (16th May 2012), with the simultaneous injection in the Montelago ponor of 50 g of Tinopal cbs-x and 1 ml of DNA biotracer solution containing 10^{16} molecules, showed an arrival of the tracer with multiple peaks typical of a drainage interdependent circulation. The main peak came after 55 hours. The analysis of the Tinopal shows an irregular behavior, with different consecutive peaks, probably due to the condition in which the test was held and to the quantity of the tracer used. This was a preliminary test to verify the behavior of the two tracers simultaneously.

The water spots recording the arrival of the tracer (for now this data are only related to the Tinopal CBS-X, because the quantitative determination of the DNA is still ongoing) were: apart from the karst spring of Grottone (San Giovanni head ditch), the Scarsito stream at the confluence of the San Giovanni ditch, Sorti, Potenza river, San Giovanni spring, Figareto spring. Even if with irregular samples, it has been noticed that the first arrival of the tracer was after approximately 9 days from the injection and a second and more weak signal after approximately 22 days.

The interpretation of the BTC with the Qtracer2 software [12] has underlined an excellent success of the tracer test. The Peclet numer has a value of 1276, coherently with the maximum velocity of the tracer > 150 m/per hour.

The analysis of the DNA tracer arrival curve in the test held on 16th May 2012, even if only qualitative (laboratory surveys are still ongoing to determine the effective quantity of the tracer in every processed sample), has confirmed the arrival of the biotracer in multiple impulses. Moreover, it was also confirmed with this test the little advancement of arrival of the DNA compared to the fluorescent tracers, as already seen in the tracer tests on 12th May 2011 and 18th May 2011.

Considering the obtained result it has been decided to do another series of DNA/Tinopal tests with different hydrogeological conditions, that is when the karst system is at the highest recharge and in the final phases of the exhaustation period.

As for the interpretation in an hydrogeologic key, the tracer tests carried out in Sefro-Montelago have confirmed and refined the conceptual hydrogeologic model, defining the limits of the hydrostructure, the volumes, the sampling points of the waters from Montelago plain, the main hydrodynamic parameters of the ponor karst system of Montelago-Grottone spring/San Giovanni ditch, and the different hydraulic behavior of the system in different nutrition/saturation conditions.

The hydrogeologic model defined by the macroanticline M. Primo - M. Torroncello, can thus be schematized with a superficial circuit guided by karsism and characterized by a rapid circulation that guides the waters, at least in the Western sector, of the Montelago ponor towards the Grottone spring. There is, then, a deeper circuit in which the base flow of the ridges seem strictly linked to the Appennine and anti-Appennine tectonic lines (pre and post orogeny). Therefore the greater water volumes are conveyed along the axial line of the hydrostructure and of the main tectonic formations both arranged towards the Appennine in order to be located in more depressed morphological areas and to sustain both linear and punctual springs.

The water circulation along the anti-Appennine lines is proved by the arrival of the tracer in the Figareto spring located in Selvazzano at the highest tectonic extreme marked by the thrust fault M. Primo - M. Cavallo.

4 Conclusions

The main aim of the work was to experiment non- toxic tracers detectable at very low concentrations, highly stable for the estimated time intervals in order to study the hydrogeology of the fissured and of the karst aquifers, compatible with the current regulation system and also used for the hydrodynamic parameterization in fissured and carbonate media.
The research activity was developed during the three years of PHD and it created new laboratory methodologies and refined some already known methodologies, in order to use new biologic tracers. The use of electrolyte and fluorescent tracers has allowed to verify possible interactions/absorption with characteristic materials of the aquifers of different hydrogeologic domains in central Italy and their hydrodynamic parameterization. The comparison with the tests held using an innovative biotracer (DNA molecules), never before used in the international scientific field to study underground waters, has allowed to experiment successfully a new methodology to trace underground waters. This methodology was carried out in a laboratory specifically designed for this purpose and it has underlined an high reliability and technique sensibility.
The experiment with the DNA biotracer continued, then, in the fields at a representative site for the hydrostructures of the carbonate Appennine in the Umbria and Marche regions. The Sefro-Montelago anticlinale (high Macerata territory) was chosen because of its advanced karstification with a typical circulation of fissured and karst aquifers.

The researches held in the laboratory have brought to these results:

- the column tests and the batch tests are useful as a preliminary study for the tracer behavior and to set up the test field conditions;
- the tested materials were representative of the aquifers of sediment alluvial terraces (clay slits, clay slits and sands, low varied sands, sands and gravel) and of colluvial deposits in the basin of the Aspio river. Moreover column tests with different dimensions were carried out, with samples of "calcare massiccio" crushed down toghether with a grained size selection of gravel sands. The mineralogy is representative of the aquifers belonging to the studied hydrostructure. Moreover the grain size was chosen so that, considering the range of hydraulic gradients applicable in the laboratory and the lenght of the columns, the tracer residency time could be of the same order of magnitude of the one expected for the entire duration of the test in the fissured carbonate and characterized by micro karst;
- the DNA tracer proved to be extremely useful to study the carbonate, fissured and karst aquifers, both with dominant drainage or with double porosity. However, it was less useful with porous media especially to determine the effective porosity;
- Laboratory tests have shown that the used DNA tracer, as regard its detectability, is compatible with the tinopal simultaneous tracer but not with the fluorescein.

The tracer field tests have been carried out on a carbonate, fissured and karst aquifer, preceded by a detailed reconstruction of the hydrogeologic model and, in particular, of the nutrition and circulation conditions of groundwaters and of the hydraulic connection with the spring manifestations taken into exam.

The obtained results have proven that the biotracer with segments of synthetic DNA gives quantitative results that are comparable, if not superior, with the fluorescent tracers, having better defined peaks.

Moreover the DNA tracer has such characteristics that it can be easily used in tracer tests. Infact it is not toxic, it is detectable at very low concentrations (detection limit: 10^5 molecules/ μL compared to an initial injection of 10^{16} molecules/ μL) and also stable for the estimated interval times useful to study the hydrogeological structures taken into exam.

Further field investigations are in progress to validate these methods for the study of groundwater circulation in karst and fissured rocks. Furthermore, laboratory tests are in progress to verify the efficacy of uptake systems of the DNA tracer based on the biotin/avidin (or streptavidin), for the future use in field tests.

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