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# Use of natural and artificial reactive tracers to investigate the transfer of solutes in karst systems

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by

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# IMPRIMATUR POUR LA THESE

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## Ludovic Savoy

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# Abstract

Numerous studies in unconsolidated aquifers have shown that natural attenuation, mainly due to biodegradation, can substantially reduce the contaminant mass in an aquifer. Much less is known about the relevance of natural attenuation in karst aquifers. This project investigated the potential for attenuation of dissolved compounds by biodegradation in the karst unsaturated zone. To what extent contaminants are removed during passage across the unsaturated zone strongly depends on the travel time of dissolved substances as well as on the biological activity in this zone. These two factors were addressed in a number of field studies at sites with different characteristics (saturated zone with more than 50 meter of unsaturated zone, conduits directly connected to the epikarst, percolating water from the low permeability volumes). The travel time of dissolved substances was investigated using a natural tracer approach which has the advantage that the reaction of the system to a substantial number of different rainfall events can be investigated at moderate effort. The approach relied on tracers that are naturally produced in the soil zone and are removed at different time scales in the unsaturated zone hence providing information about the residence of water and dissolved substance in the unsaturated zone. The tracers included $^{222}Rn$, dissolved organic carbon (DOC) and dissolved $CO_2$. $^{222}Rn$ originates from $^{226}Ra$ present in the soil zone and decays with a half-live of 3.8d. Dissolved organic matter (DOC) is released from soil organic matter and is removed by sorption and biodegradation at a longer less-well defined time scale than $^{222}Rn$. Finally, $CO_2$ is also produced in the soil zone and can be partly consumed for carbonate dissolution in the epikarst and unsaturated zone. In addition, the $CO_2$ production shows a large annual variation in the soil zone and hence water that infiltrates in summer or winter may have a substantially different $CO_2$ concentration than water in storage making it possible to trace freshly infiltrated water. These tracers were measured continuously at the Milandre site, with unsaturated zone more than 50 meters, to be able to assess the reaction of the system to rainfall events of different intensities likely leading to a different travel time distribution. In other test site with epikarst directly connected to conduits (Grand Bochat and Vers-Chez le Brandt) the tracers where measured during irrigation or rainfall events in order to evaluate the composition of the soil water.

During flood events, the $CO_2$ concentration increased at Milandre site. This pattern can be attributed to the mobilisation of water from the low permeability volumes (LPV) and epikarst with more elevated concentrations than in the saturated zone where CO2 was

degassed in the cave atmosphere. The $CO_2$ increase corresponds to a piston effect on the epikarst and the LPV. For small precipitation events, no increase in the $^{222}Rn$ concentration was observed indicating that the water resided for >20 days below the soil zone, the time necessary for ~95% of the $^{222}Rn$ to decay. In contrast, after larger rainfall events, a delayed response of $^{222}Rn$ is observed indicating a delayed contribution from the soil reservoir with a relatively short travel time. During irrigation experiments a similar behaviour of the two compounds was observed as for natural rainfall events. In contrast to $^{222}Rn$ that decays with a half-life of 3.8 days, the total organic carbon (TOC) is expected to persist longer especially in the epikarst close to the soil zone and hence provides information on a longer time scale. For small rainfall events a delayed TOC increase is observed indicating the arrival of water from the epikarst/soil zone. The significant delays of several days (much more than for the $^{222}Rn$ increase delay) suggest that the water has transited slowly through the fracture network rather than along vertical conduits. In contrast, during large rainfall event an immediate reaction of TOC is observed likely due to overflow of the epikarst and transfer of water along conduits.

In addition to the study of natural tracers, artificial tracing experiments were realised at Milandre test site. The goal of these experiments was to study the effect of the soil cover on conservative transport processes. These experiments demonstrated the high storage and buffer capacity of the soil and epikarst sub-systems and the occurrence of two types of flows in the unsaturated zone: quick flow in the conduits and seepage flow in the unsaturated low permeability volumes (LPV).

The natural tracing studies and the artificial tracing experiments demonstrated that storage occurs in the unsaturated zone for a prolonged period probably in the epikarst zone and the fractured LPV. To evaluate the relevance of storage in the unsaturated zone for the fate of organic contaminants, it is important to gain information on the microbial activity in this zone. To quantify the biological activity in situ, a new reactive tracer approach was developed. The approach was tested using degradable organic compounds. Two different types of analyses were performed to quantify biodegradation, the comparison of concentrations of reactive and conservative tracer and stable isotope analysis of the reactive compound. The reactive tracer experiments indicated a high biological activity in the unsaturated zone of karst systems.

In conclusion, the study indicates that in the absence of large precipitation events, a substantial biodegradation of organic contaminants can be expected in the unsaturated zone of karst aquifer during storage. However, during large rainfall events contaminants can likely breakthrough due to the short transit time in the unsaturated zone of some of the water (by-pass effect).

**Keywords**

Karst hydrology, Soil, Epikarst, $^{222}Rn$, $CO_2$, TOC, Unsaturated zone, Biodegradation

# Résumé

De nombreuses études concernant les aquifères poreux ont démontré que l'atténuation naturelle d'un contaminant, essentiellement attribuée à la biodégradation, peux considérablement réduire la masse de ce contaminant à l'intérieur de l'aquifère. Les processus d'atténuation naturelle d'un contaminant dans les aquifères karstiques sont par contre beaucoup moins connus. Ce projet a eu pour but d'étudier le potentiel d'atténuation par biodégradation de composés dissouts dans la zone non saturée des systèmes karstiques. La quantité de masse dégradée d'un contaminant dissout dans l'eau et traversant la zone non saturée dépend fortement du temps de transit de cette substance dissoute ainsi que de l'activité biologique au sein de cette même zone. Ces deux facteurs (temps de transit, activité biologique) ont été étudiés au sein de plusieurs sites karstiques présentant des caractéristiques hydrauliques différentes (zone saturée et zone non saturée de plus de 50 mètres, conduits directement connecté à l'epikarst, eau de percolation en provenance des volumes peu perméables non saturés). Le temps de transit de substances dissoutes dans le système karstique a été étudié en utilisant une approche basée sur les traceurs naturels. Cette approche présente l'avantage que la réaction hydraulique du système à un nombre élevé de précipitations de différentes intensités peut être étudiée relativement facilement. Des traceurs naturellement produits dans le sol et ensuite graduellement dégradé selon différentes échelles de temps dans la zone non saturée on été étudiés. Ces traceurs apportent ainsi des informations sur le temps de résidence de l'eau et des substances dissoutes dans la zone non saturée. Ces traceurs incluaient, le radon ($^{222}Rn$), le carbone organique total (COT) ainsi que le gaz carbonique dissout ($CO_2$). Le $^{222}Rn$ est produit par de la désintégration du $^{226}$Radium, naturellement présent dans le sol, et décroit selon une demi-vie de 3.8 jours. Le COT provient de la matière organique du sol et décroit par biodégradation avec une échelle de temps plus longue mais nettement moins bien définie et connue que pour le $^{222}Rn$. Finalement, le $CO_2$ est également produit dans le sol mais par la respiration des plantes et organismes. Il est partiellement consumé par la dissolution des carbonates dans l'epikarst et la zone non saturée. En outre, la production de $CO_2$ dans le sol montre une grande variation annuelle et par conséquent, l'eau qui s'infiltre en été ou en hiver présente une concentration différente en $CO_2$ par rapport à l'eau stockée dans la zone non saturée depuis une longue période. Ces traceurs ont été mesuré en continu à la grotte de Milandre, avec une zone non saturée de plus de 50 mètres, dans le but de pouvoir évaluer la réaction hydraulique du système karstique

suite à des précipitations de différentes intensités. Aux sites de Vers-Chez-le-Brandt et Grand-Bochat, ces traceurs ont été mesurés durant des précipitations naturelles ou artificielles afin d'évaluer la composition de l'eau du sol.

Suite à des précipitations modérées, une augmentation de la concentration en $CO_2$ a été observée. Ce comportement peut être attribué à la mobilisation de l'eau des volumes peu perméables (VPP) et de l'epikarst. L'eau de ces milieux présente une concentration en $CO_2$ plus élevée que pour la zone saturée ou le $CO_2$ est partiellement dégazé. L'augmentation de $CO_2$ correspond à un effet piston sur le VPP et l'épikarst. Lors de fortes précipitations, une réponse retardée du $^{222}Rn$ est observée indiquant une contribution retardée du réservoir lié au sol. Des expériences d'arrosage artificiel ont également été réalisées et un comportement semblable du $^{222}Rn$ est observé par rapport aux événements naturels.

Au contraire du $^{222}Rn$ qui décroit avec une demi-vie de 3.8 jours, le carbone organique total est supposé persister plus longtemps dans l'épikarst, plus particulièrement dans la zone proche du sol, et donc permet de fournir des informations sur le stockage de l'eau dans la zone non saturée sur une échelle de temps plus longue que le $^{222}Rn$. Pour de faibles précipitations, un pic retardé du COT est observé indiquant la contribution d'eau du sol et de l'epikarst au débit. Ce délai important suggère que l'eau a transité lentement à travers le réseau de fracture de la zone non saturée plutôt que le long de conduits bien développé. Par contraste, durant de fortes précipitations, une réaction immédiate du COT est observée vraisemblablement due à un débordement de l'epikarst dans les conduits.

En plus de l'étude de traceurs naturels, des expériences de traçage artificiel ont été réalisées. Ces expériences ont démonté les capacités de stockage et de tamponnage élevées du sol et de l'epikarst ainsi que la présence de deux types d'écoulement dans la zone non saturée : écoulement rapide dans les conduits et écoulement de suintement dans les volumes peu perméables de la zone non saturée.

L'étude des traceurs naturels et les expériences de traçage artificiel ont démontré qu'un stockage conséquent à lieu dans la zone non saturée pour des périodes prolongées, probablement dans l'épikarst et les volumes peu perméables. Afin d'évaluer l'importance du stockage dans la zone non saturée sur le comportement des contaminants il était important d'obtenir des informations sur l'activité microbienne dans cette zone. Afin de quantifier in situ cette activité microbienne, une nouvelle approche basée sur des traceurs réactifs a été développée. Cette approche a été testée en utilisant des composés organiques dégradables.

Deux différentes méthodes d'analyses ont été utilisées pour quantifier la biodégradation, la comparaison des concentrations entre traceurs réactifs et conservatifs et l'analyse des isotopes stables du composé réactif.

En conclusion, ces informations indiquent qu'en l'absence de précipitations importantes, une dégradation substantielle de contaminations organiques au sein de la zone non saturée d'aquifères karstique peut être attendue. Cependant, lors de fortes précipitations, les contaminants sont en partie expulsés du système.

**Mots Clés**

Hydrogéologie, karst, sol, epikarst, $^{222}Rn$, $CO_2$, COT, zone non-saturée, biodégradation

# Chapter 1

## Introduction

# 1. Introduction

Studies on transport of dissolved compounds in karst aquifers have so far mainly dealt with conservative transport of natural and artificial tracers and with transport of products from mineral dissolution. Only a few studies are available that investigate the behaviour of contaminants in karst systems. Furthermore, in contrast to porous aquifers, hardly anything is known about the importance of biodegradation in karst aquifers. In a review on contaminant transport in karst aquifers, it was stated that “the analysis of contaminant transport in karstic aquifers is just beginning” and that “contaminant transport in karst aquifers is perhaps the most important of the current generation of karst research subjects” (Vesper et al., 2001). Given that karst aquifers are generally well aerated and may be supplied with microorganisms and nutrients from overlaying soil zones, it can be expected that biodegradation processes could play a significant role in karst aquifers. However, significant biodegradation of dissolved compounds will only occurs if the transit time in the unsaturated zone is sufficiently long. Therefore, it is critical to obtain information about the transit time distribution when evaluating the role of biodegradation. This is why the BIOKARST project has investigated in a first part the transit time distribution of water in the different subsystems of the unsaturated zone (soil, epikarst, low permeability volumes). In a second part, the biodegradation of dissolved compounds in the karst infiltration system (soil, epikarst and low permeability volumes) was studied. The project has focused especially on the epikarst and vadose zone since biodegradation in this zone has a particularly high significance by possibly preventing contaminants from reaching the saturated zone. The aim of the project was:

(I) to develop and apply methods to investigate the transit time of dissolved solutes in the karst infiltration system under different hydrologic conditions, which determines the time available for biodegradation.

(II) to assess biological activity in the epikarst and unsaturated zone and investigate the biodegradation potential in the different subsystems using reactive tracers.

(III) to develop a conceptual model of biodegradation of organic compounds in the karst infiltration system by combining information on transit times in the different subsystems with degradation rates (Fig. 1.1) for each of the subsystems (soil, epikarst and low permeability volumes).

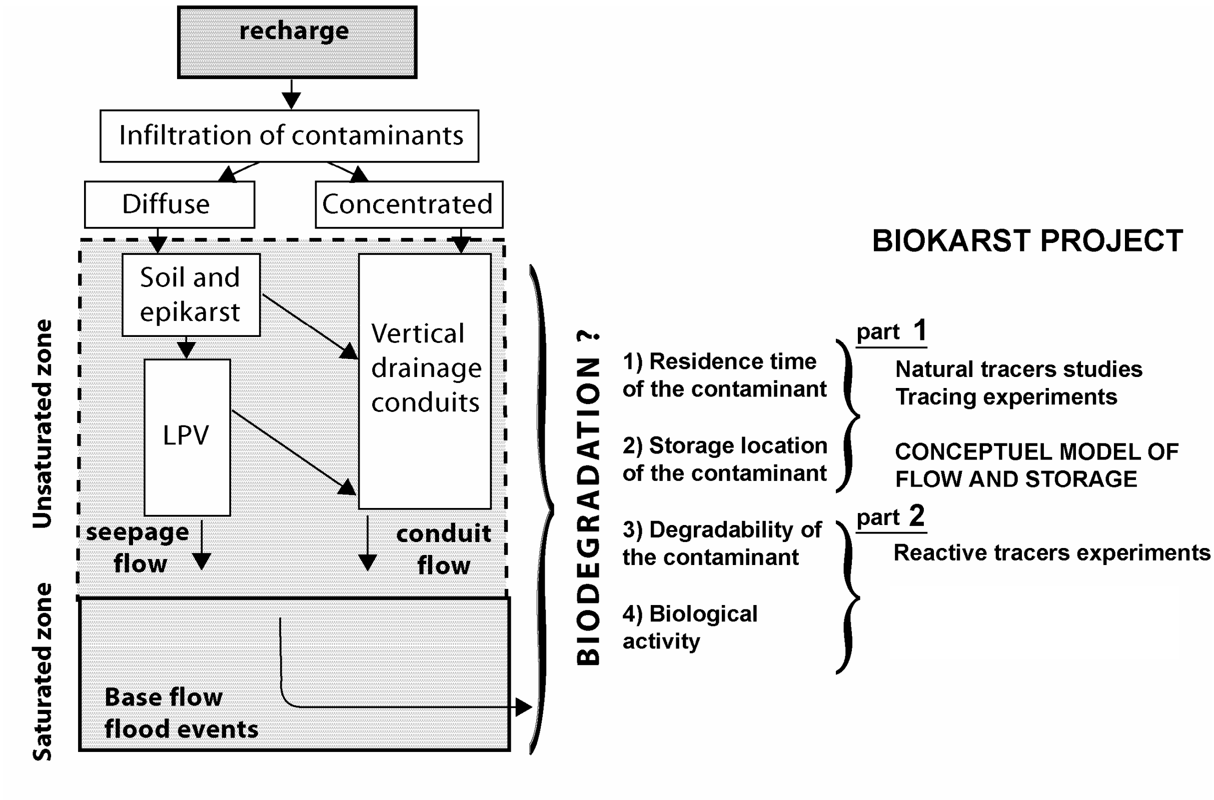


*Figure 1.1: Conceptual model of solute transport through karst unsaturated zone, factors that influence biodegradation of dissolved compounds and how they were investigated in the Biokarst project.*

The first part of the BIOKARST project, concerning the transit time of dissolved solutes in the karst infiltration system, has mainly focussed on the development of natural tracer methods to evaluate the transit time distribution. The methods rely on natural tracers that originate from the soil and decrease in the epikarst and vadose zone providing information on the transit time in these zones. The second part of the BIOKARST project focussed on the development of reactive tracer methods in order to evaluate the biodegradation of dissolved contaminants in the karst infiltration system.

## *1.1. Particularities of karst aquifers and implications for biodegradation*

The transport, storage and transformation of dissolved compounds through the unsaturated zone of karst aquifers strongly depend of their structure but also of the degradability and origin of the compounds. The following paragraphs describe the structures of karst aquifers (1.1.1), the existing conceptual models of karst aquifers (1.1.2) and their implication for biodegradation of different types of contaminants (1.1.3). Finally a review of contamination occurrences in karst systems is presented (1.1.4).

### 1.1.1. Structure and functioning of karst aquifers

As describe in Field (1999), a karst aquifer is “A body of soluble rock that conducts water principally via a connected network of tributary conduits, formed by the dissolution of the rock, which drain a groundwater basin and discharge to at least one perennial spring. The conduits may be partly or completely water filled. The karst aquifer may also have primary (intergranular) and secondary (fracture) porosity openings, which are saturated with water below the potentiometric surface (water table).”

The flow behaviour of karst systems is essentially characterized by variable recharge conditions (diffuse/concentrated), storage (vadose/phreatic), and flow (diffuse/concentrated) (Smart and Friederich, 1986; Ford and Williams, 1989; White, 1988). This duality of karst aquifers behaviour is a direct consequence of the geologic and geomorphologic structure of karst terrains. This specificity leads to a high variability of the flow and transit time with variable geochemical signature.

Different conceptual model of karst aquifers have been proposed for the 30 last year (Blavoux and Mudry, 1983; Drogue, 1992; Lee and Krothe, 2001; Mangin, 1975; Doerfliger et al., 1999; Perrin, 2004). These conceptual models generally separate the whole system in several sub-systems:

**The soil sub-system.**

The soil zone corresponds in this study to the unconsolidated pedologic cover of the limestone bedrock (Fig. 1.2). This pedologic cover is not necessary presents in the alpin karst systems for example. Perrin (2003) demonstrated that the soil zone in the karst infiltration system influences the infiltration rate in the unsaturated zone and the mixing of solutes. Rank et al. (2001) observed a mixing of added water with capillary water already stored in the unsaturated soil zone based on measurements done in a sandy soil under artificial controlled recharge conditions. Perrin (2003) observed that variable oxygen isotope ratios in precipitation were completely dampened in water arriving in the cave similarly as observed in other studies (Yonge et al., 1985; Chapman et al., 1992; Caballero et al., 1996). In case of large recharge events, some variations of the isotopic signal can be observed in the cave. These results where explained by an elevated storage of the infiltrated water in the soil zone and epikarst.

**The epikarst sub-system.**

This sub-system stores, mixes by horizontal drainage and distributes the infiltrated water towards the saturated zone (Fig. 1.2). Recent karst studies, based on natural tracers, have revealed the importance of the epikarst zone as buffer and storage subsystem in karst aquifers during rainfalls (Chapman, 1992; Caballero et al., 1996; Perrin, 2003; Vesper and White, 2004). Klimchouk, (2004) described the epikarst as the uppermost weathered zone of carbonate rocks with a substantially enhanced and homogeneously distributed porosity and permeability. The epikarst porosity was estimated between 1% to 10% by several authors (Smart and Friederich, 1986; Gouisset, 1981; Williams, 1985). Klimchouk (2000) summarized the conceptual model of the epikarst of several authors (Mangin, 1975; Gouisset, 1981; Williams, 1985; Smart and Friederich, 1986). These models describe a perched aquifer at the base of the epikarst zone resulting from a contrasted permeability between the epikarst zone and the underlying fractured low permeability volumes. The epikarst acts as a regulative subsystem that stores the water and splits it into several flow components (Klimchouk, 2004).

### The vadose zone

The vadose zone, also called unsaturated zone, connects the soil and the epikarst subsystem to the saturated zone by vertical drainage through a vertical network of fissures and conduits. In karst systems, groundwater flow is generally separated between concentrated quick flows in the drainage conduits and seepage flows in the fractured limestone and the matrix considered as low permeability volumes. Jeannin (1996) postulated the existence of a flow circulation across the low permeability volumes (LPV). “This flow may represent about 50% of the infiltrated water in the Bure test-field (Milandre). The epikarst appears to play an important role into the allotment of the infiltrated waters: Part of the infiltrated water is stored at the bottom of the epikarst and slowly flows through the low permeability volumes (LPV) contributing to base flow.” Jeannin (1996) described the phreatic zone as : ”a network of high permeability conduits, of low volume, leading to the spring, surrounded by a large volume of low permeability fissured rock (LPV), which is hydraulically connected to the conduits”.

Several studies have demonstrated a high spatial and temporal variability of the water arrivals in the unsaturated zone (Smart and Friederich, 1986; Destombes et al., 1997; Delannoy et al., 1999; Sanz and Lopez, 2000; Perrette et al. 2001; Perrin, 2003). Two types of hydraulic responses were identified; **conduit flow** leading to “nervous” hydraulic responses and **seepage flow** through the fractured LPV of the unsaturated zone leading to a dampened responses (Fig. 1.2). In karst aquifers the hydraulic responses at the spring correspond generally to a combination of the two types of flow. The repartition of water during infiltration between drainage conduits and fractured LPV zone was estimated for the Milandre karst system (Jeannin, 1996) with several methods (boreholes observations, base flow evolution, water balance). According to these calculations, between 50% and 70 % of the infiltrated water during rain events participate to the recharge of the fractured LPV. Moreover numerical simulations (Kiraly and Morel, 1976; Kiraly, 2002) indicated that 50% of the infiltrated water leaves the epikarst zone in "concentrated" form through vertical drainage conduits.

**The saturated zone**

The saturated zone is formed by a network of high permeability drainage conduits surrounded by low permeability volumes (LPV) with a high storage capacity. According to Worthington et al. (2000) and White (2005), the main portion of the storage takes place in the fractured limestones, although the main portion of the flow takes place in the drainage conduits (Fig. 1.2). In dense and low permeability limestones, the matrix flow is negligible (White, 2005) and discharge consists of a mixture of flow through the drainage conduits and the fractured LPV of the saturated zone. Charmoille (2005) demonstrated the importance of the fractured LPV in the Jura karst systems for the prolonged storage of groundwater and their possible participation to the discharge based on discharge and electrical conductivity measurements.

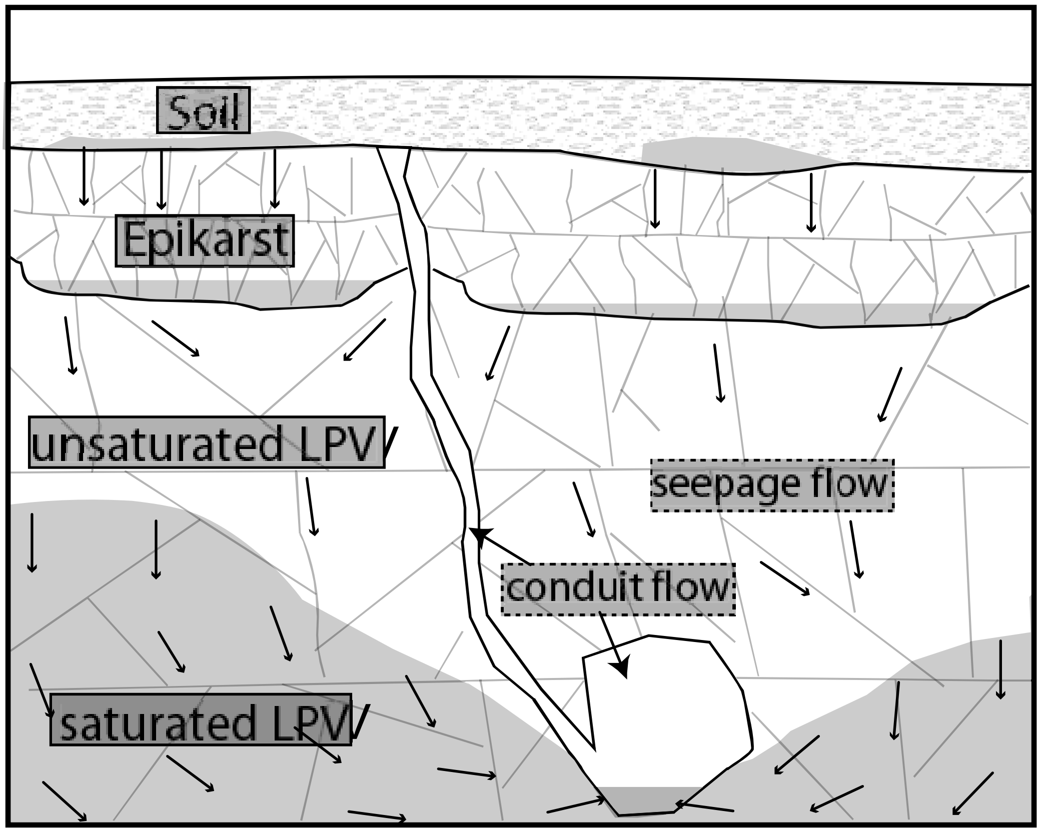


*Figure 1.2: Representation of the karst system with the different subsystem: Phreatic zone: Saturated LPV and conduits. Vadose zone: Unsaturated LPV , epikarst and soil.*

### 1.1.2. Conceptual models of karst aquifers

In his PhD thesis, Perrin (2003) compared a wide range of conceptual models of karst systems and observed that the major differences between these models are the following:

- The low permeability volumes in the saturated zone are represented either as volumes of fissured limestone (Drogue 1992, Lee and Krothe 2001) or as large cavities connected to the drain (Mangin 1975).

- Base flow is sustained either from the low permeability volume of the saturated zone (Drogue 1971, Mangin 1975, Kiraly and Mueller 1979) or from the epikarst storage (Williams 1983, Sauter 1992, Klimchouk 2000).

- Water contributing to discharge during flood events has a different origin: concentrated infiltration, diffuse infiltration and phreatic water stored in the low permeability volumes (LPV) (Vervier 1990) or phreatic water and fresh infiltrated water (Kiraly and Mueller 1979, Blavoux and Mudry 1983)or epikarst storage, conduit storage, and fresh infiltrated water (Williams 1983, Sauter 1992) or mixing of fresh infiltrated water, soil water, epikarst water, and phreatic water (Lee and Krothe 2001) or mixing of several tributaries (Hess and White 1988).

Perrin (2003) proposed a new conceptual model of flow and transport in a karst aquifer based on spatial and temporal variations of natural tracers (major ions and oxygene isotopes) at the Milandre test site. The main results of this conceptual model are summarized below.

The storage is mainly located in the soil and epikarst zones and the role of the saturated LPV is neglected, as it is not necessary for explaining the observed hydraulic responses. The unsaturated zone and the conduits are mainly seen as transmissive zones, with high flow velocity and limited storage. For a flood event, the following steps can be observed:

In steady-state conditions, the system is fed by waters stored in the epikarst. Discharge and chemistry are stable at the spring. Water is stored in the soil zone hold by capillary forces.

During step 1, after the beginning of rainfall, soil water is pushed into the epikarst reservoir. The hydraulic stress on the epikarst causes a discharge increase in the system. The system is fed by epikarst water mainly. Discharge rises at the spring, but chemistry does not change.

During step 2, rainfall continues, more soil water is pushed into the epikarst reservoir. A part of the soil water bypasses the epikarst reservoir and reaches directly the saturated zone. The system is fed by epikarst and soil water. Discharge still rises at the spring, but stable oxygene isotopes do not vary because soil water has a constant isotope signal.

During step 3, rainfall continues, the soil is entirely at field capacity. Some fresh water bypasses the soil reservoir and reaches the saturated zone. The system is fed by a mixing of fresh water, soil water, and epikarst water. Discharge is near maximum at the spring. Stable oxygene isotopes vary at the spring if the isotope signal in rainfall is significantly different from the system background.

During step 4, rainfall has stopped, the soil releases water into the epikarst reservoir. The system is mainly fed by epikarst water, but soil water can still be present. At the spring, the discharge decreases and the chemical parameters return to their pre-event concentrations (recession phase).

The described flood event corresponds to an strong recharge event. More frequent floods, with less recharge, will not reach phase 3 or even 2 and follow directly the recession phase.

The flow velocities are very different during base flow or flood events conditions (in Milandre test site):

- During base flow, no flow is present in the soil, flow velocity in the epikarst should be low, and velocity in the unsaturated and saturated zones is on the order of 100 m/h. The discharge is attributed to water stored in the epikarst.

- During flood events, flow velocity in the soil zone is about 0.5 m/h, 50 m/h in the unsaturated zone, and 400-500 m/h in the saturated zone.

The contributions of the different components during flood events are between 0-20 % for fresh water, up to 60-70 % for soil water, and the rest comes from the epikarst.

The conceptual model proposed by Perrin (2003) reduced drastically the role of the saturated LPV. Indeed the LPV are not necessary for explaining and simulating his observations. However several conceptual models described the contribution of the saturated LPV to the discharge:

Jeannin (1996) proposed a conceptual model of the Milandre aquifer based on hydrodynamic observations (discharges in the conduits, piezometers levels). This model postulates a significant contribution of water from the low permeability volumes (LPV) of the saturated zone to discharge: approximately 50% of the infiltration was expected to recharge the LPV and to be then slowly released into the conduits.

Charmoille (2005) highlighted an elevated water exchange between conduits and saturated LPV in the Fourbanne karst system based on hydrodynamic and hydrochemical observations. He proposed a conceptual model with a contribution of the saturated LPV to the discharge during flood event that can reached 20%. The base flow discharge is sustained by the saturated LPV, and the saturated LPV are recharged through the unsaturated zone by the diffuse infiltration. The contribution of the saturated LPV is generally observed at the beginning of the flood event as already noticed by Mudry (1987).

### 1.1.3. Contaminant transport and storage in karst aquifers

The behaviour of contaminants depends on the physical-chemical characteristics of the contaminants, their degradability, and also on the characteristics of the contaminant sources. Contaminants can originate from diffuse sources (e.g. agriculture) or concentrated sources (e.g. waste deposits) often also denoted as point sources. They may be released dissolved in water or as separate organic liquids. In the latter case, the behaviour of the contaminants depends on the solubility, density and vapour pressure of the organic liquid. In the following, different contamination scenarios are discussed based on Vesper (2001) and COST Action 620 (2004).

**Dissolved contaminants (inorganic or organic)**

Different types of contaminants can be found in groundwater due to concentrated or diffused contaminations. For example, inorganic soluble compounds can originate from anthropogenic sources (waste deposit, agriculture) and include nitrate, ammonia, chloride, suflate and cyanide. A lot of organic contaminants are also soluble in water (for example alcohols, phenols, agricultural chemicals).

These contaminants can transit through the unsaturated zone along different flow path depending on their point of entry and can be stored in different subsystems. Contaminants originating from diffuse sources (Fig. 1.3, 1) (e.g. agriculture) can be stored in soil and epikarst zones. Point source contaminations may frequently be located in sinkholes (Fig. 1.3, 2) and contaminations can rapidly reach the saturated zone.

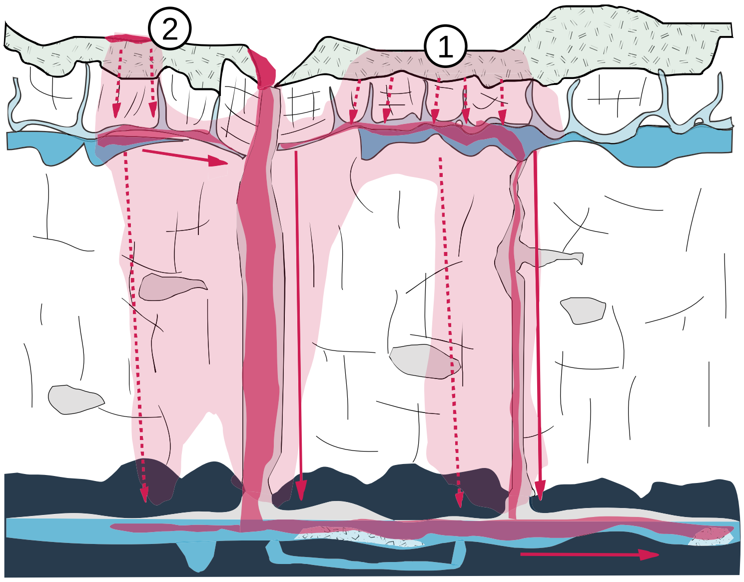


*Figure 1.3: Soluble contaminants transport and storage in the karst system. 1) Diffuse infiltration. 2) Concentrated infiltration.*

**Non aqueous phase liquids (NAPLs)**

Very few studies are available concerning LNAPLs and DNAPLs in karst systems. Kranjc (1999) observed that it can take tens of years before NAPL were pushed out the system. It is one of the main problem concerning NAPLs in karst system. They can be trapped in the conduits and act as a constant source of contamination by continuous dissolution in the water during very long time. Generally the exploited springs for water supply and contaminated by NAPLs are permanently abandoned (COST Action 620, 2004). NAPLs in the vadose zone can also volatilise (Field, 1990; Javandel, 1998) and vapours can reach the saturated zone of the surface.

- Light, non aqueous phase liquids (LNAPLs)

These compounds (e.g. gasoline, mineral oil and related petroleum hydrocarbons) have a smaller density than water and therefore float on the surface of water. Due to their small density, the LNAPLs will be entrapped in unsaturated conduits and fractures (Fig. 1.4, A), behind sumps and pocket in the ceiling of the drainage conduit (Fig. 1.4, B). The entrapped LNAPLs will evaporate in the unsaturated zone (Fig. 1.4, 1), and create gas contamination which could reach the surface with risk of explosion. The LNAPLs contain a number of compounds with variable solubility (Fig. 1.4, 2). Dissolved compounds such as BTEX can reach the saturated zone in the percolating water.

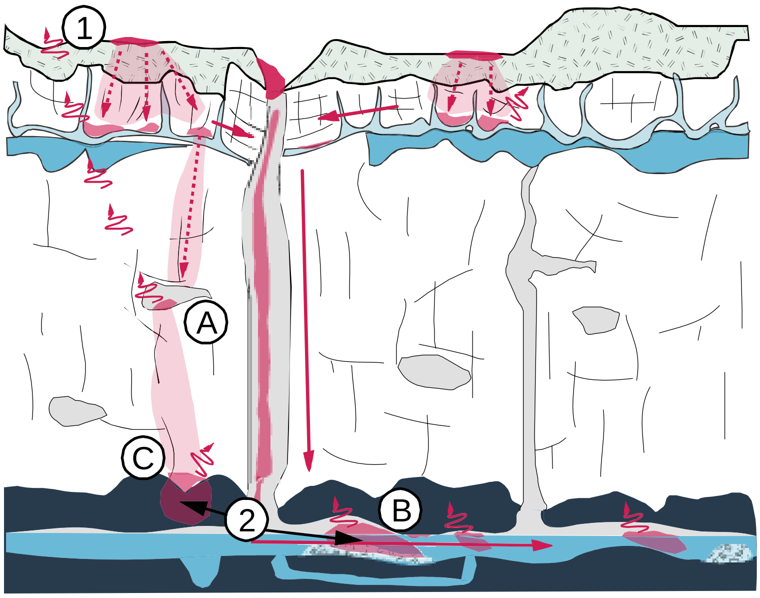


*Figure 1.4: LNAPLs transport and storage in the karst system. 1) Volatilization. 2) Dissolution. Entrapment A) in the conduits B) Behind sump C) at the surface of the water table.*

- Dense, non aqueous phases liquids (DNALPs)

These compounds have a higher density than water. Due to their wide range of volatility, these compounds can be divided in relatively volatile compounds like methylene chloride, trichloroethylene (TCE), perchloroethylene (PCE) and in non-volatile compounds, such as polychlorinated biphenyls (PCB). Transport of these contaminants do not necessary corresponds to the general flow path of water. They will generally migrate vertically. In the unsaturated zone they can become trapped in conduits and fractures (Fig. 1.5, A) and volatilised in the cave atmosphere (Fig. 1.5, 1). In the saturated zone, they can become trapped in the sediment (Fig. 1.5, B) or in the deeper part of the system (Fig. 1.5, C) and slowly dissolve in groundwater (Fig. 1.5, 2). During large flood events they can be remobilized by the increase of the discharge.

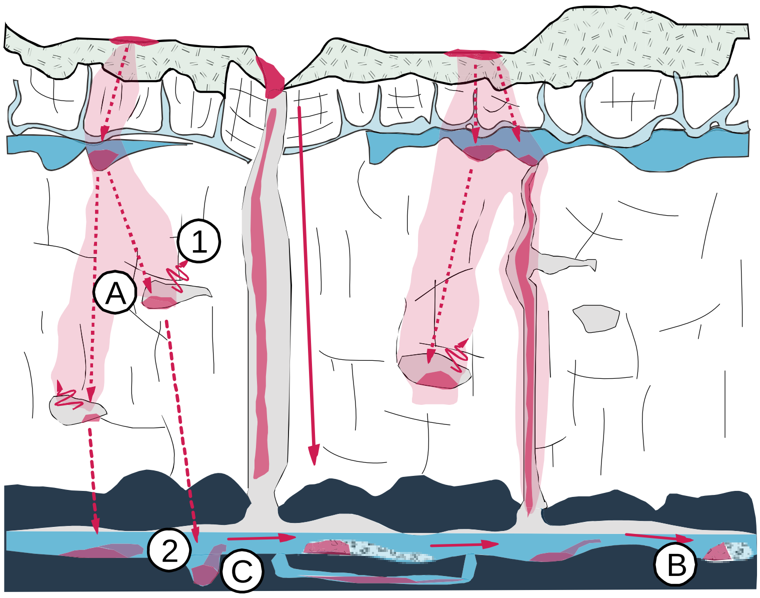


*Figure 1.5: DNAPLs transport and storage in the karst system. 1) Volatilization. 2) Dissolution. Entrapment A) in the conduits B) in the sediments C) in the deeper part of the saturated zone.*

This paragraph illustrates the many different scenarios for contaminant storage and transport in karst systems. This study focuses on the dissolved contaminants, because they have a similar behaviour as natural or artificial dissolved tracers. The transport of the dissolved contaminants corresponds generally to the general flow paths of water in contrary to NAPLs were (for example) vapour transport can play a significant role.

### 1.1.4. Biodegradation in karst aquifers

Large advances have been made in the understanding of biodegradation in porous aquifers in the last decade (e.g. Holliger and Zehnder, 1996; Hoyle and Arthur, 2000; Wiedemeier et al., 1999). Initially, studies have mainly focused on the saturated zone

(Baedecker et al., 1993; Barker et al., 1987; Bolliger et al., 1999; Eganhouse et al., 1996) but an increasing number of investigations of the unsaturated zone (Barker et al., 2000; Lahvis and Baehr, 1996; Lahvis et al., 1999; Ostendorf and Kampbell, 1991) have been performed since 15 years as well. In general, these studies have demonstrated that the potential for biological transformation is often larger than initially expected and it has been shown that natural attenuation can be effective to limit the migration of a large range of different contaminants (Wiedemeier et al., 1999). For example, it has been shown that compounds that were initially considered to be recalcitrant such as PCE or MTBE can be transformed relatively fast by naturally occurring microorganisms under suitable conditions (Bradley, 2000; Bradley et al., 2001; Landmeyer et al., 2001).

In contrary to porous aquifers, most of the studies dealing with reactive processes in karst aquifers have focused on mineral dissolution. A large number of laboratory and field studies on carbonate dissolution have been performed (e.g. Roques, 1969; Plummer and Wigley, 1976; White, 1977; Buhman and Dreybrodt, 1985a and b; Dreybrodt, 1988; Palmer, 1991; Groves and Howard, 1994a and b; Dreybrodt, 1996; Siemers and Dreybrodt, 1998; Zaihua and Dreybrodt, 1998). The background of many of these studies was to understand long-term dissolution processes or to infer some information about the structure of karst aquifers (e.g. Atkinson, 1977; Bakalowicz, 1979 and 1986; Friederich and Smart, 1982; Williams, 1983; Worthington, 1991). Furthermore, concentrations of ions that originate from water-rock interaction have been used as tracers to evaluate the origin of water and to estimate the transit time of water in the subsurface (Bakalowicz, 1979; Plagnes, 2000; Emblanch et al., 2003).

In contrast to studies on mineral dissolution, there are few studies that deal with chemical and microbial transformation of organic compounds and contaminants in karst aquifers. The studies performed so far have mainly focused on transformation of nitrogen compounds. The fate of monoaromatic hydrocarbons and ammonium that entered a karst aquifer due to infiltration of effluent of a wastewater treatment plant was investigated by Montandon et al. (1995). The study demonstrated that these contaminants are transformed during passage through the aquifer without providing information about the specific location of biodegradation. Evidence for denitrification based on $^{15}N$ isotope analysis was observed by Panno et al. (2001). In a study on the spatial variability of nitrate in Milandre karst aquifer

(Swiss Jura), the concentration distribution could be linked to differences in land use (Perrin et al., 2002). It was shown that the nitrate concentration at the spring was mainly controlled by mixing of water with different nitrate levels. Preliminary studies at the Milandre test site have demonstrated that pesticides are rarely detected in groundwater despite extensive use in the overlaying fields suggesting retention and transformation.

Little is known about the potential for degradation of organic compounds in karst aquifers and the biological activity in karst aquifers in general in contrast to unconsolidated/porous aquifers. Recent studies on variations of total organic carbon (TOC) concentrations in karstic streams or springs and speleothems suggest that substantial transformation of TOC occurs in karst aquifers (Albéric and Lepiller, 1998; Baker et al., 1997; Baker and Barnes, 1998; Baker and Genty, 1999; Baker et al., 1999; Baker, 2000; Mcgarry and Baker, 2000; Baker and Lamont-Black, 2001; Emblanch et al., 2001). Higher TOC concentrations were observed during peak discharge compared to baseflow (Batiot, 2002; Batiot et al., 2002). The high TOC concentrations were interpreted to indicate a rapid transfer of soil water to the spring while lower TOC concentrations during baseflow were explained by biodegradation. This interpretation was supported by a correlation between TOC concentration and parameters that indicate a long transit time such as Mg (Batiot, 2002).

Recently, the investigations of the fate of TOC in aquifers based on is natural fluorescence has found increasing interest. Fluorescence in natural waters is predominantly caused by organic acids (humic and fulvic) and amino-acid groups. The most common form of fluorescence of TOC is emission in the long wave ultra-violet and blue wavelengths (350–500 nm) after excitation by UV light (200–400 nm). Baker and Genty (1999) observed three fluorescence centres in cave groundwater; one at the excitation-emission pair of 290-340/395-430 nm, (humic, probably fulvic acid), one at 265-280/300-370 nm (protein) and a less defined region of high fluorescence at 230-280/310-420 nm (humic and/or protein). Time-series fluorescence analysis suggests that seasonal variations do occur and demonstrate the potential of using fluorescence wavelength variations in sourcing karst groundwater with different soil and vegetation covers.

Some information on the occurrence of biological transformation processes in karst aquifers can be derived from pesticide studies (Kozel and Garazi, 2001). The detection of pesticide degradation products in some spring waters (Börger and Poll, 1998) and the absence of pesticides in groundwater of poorly karstified areas (Goody et al., 2001) suggest that transformation and/or retention occur during transport across soil, epikarst and vadose zone. According to Novak (1999) specific configurations (high oxygen content, neutral pH, low organic carbon content and strong flow velocity variations) often present in karst systems can enhance the degradation processes of pesticides. Furthermore, mass balance calculations for a catchment area of a karst spring indicated that more than 99% of the applied pesticides are retained or degraded (Börger and Poll, 1998). However, little is known about where and to what extent transformation processes occur. Microcosm studies with material from the vadose zone of a chalk aquifer in England, demonstrated that microorganisms capable of pesticide transformation are present in the vadose zone (Johnson et al., 2000). Generally, it is assumed that pesticides may sorb or be transformed in LPV with a relatively low permeability and long water transit time while they migrate fast through conduits (Kozel and Garazi, 2001). However, this assumption has not been experimentally verified.

## *1.2. Methods to investigate conservative transport and reactive processes*

In order to study the transport and storage of dissolved compounds through the unsaturated zone of karst aquifers a wide range of natural or artificial tracers can be used. The most used methods are presented in paragraph 1.2.1. The methods used for study the biodegradation of contaminants are described in paragraph 1.2.2 with a particular emphasis to the selected method for this study.

### 1.2.1. Artificial and natural conservative tracers

The transit time of water between the surface and karst springs is frequently investigated using artificial tracers that show conservative behaviour (e.g. Kaess, 1998). While the method has the advantage that the input function is clearly defined, artificial tracers can generally only be applied over a small surface. Furthermore, it is difficult (memory effects) and time consuming to repeat a tracer test at the same location to evaluate the reaction of a system under different hydraulic conditions. An alternative approach is the use of environmental isotopes ($^{18}O$, $^{2}H$, $^{3}H$) as natural tracers that are “injected” over the whole catchment and show conservative behavior. The response of environmental isotopes to precipitation events has been investigated at the catchment scale by Maloszewski et al. (1992, 2002), Rank et al. (1992), Bakalowicz et al. (1974) Emblanch et al. (2003) and at local scale within the vadose zone by Harmon (1979), Yonge (1985), Chapman (1992), Caballero (1996) and Perrin et al. (2003). They were used to estimate mean transit times and to evaluate the respective contribution of different sources of water such as freshly infiltrated water and water stored in low permeability volumes or unsaturated zone ( Katz et al., 1998; Vervier, 1990; Blavoux and Mudry, 1983; Lakey and Krothe, 1996; Lastennet and Mudry, 1997; Lee and Krothe, 2001; Maloszewski et al., 2002).

In several studies, it was observed that the isotopic response ($^{18}O$, $^{2}H$) at water arrivals in caves is highly buffered compared to rainfall, although discharge varies substantially (Chapman, 1992; Caballero, 1996; Perrin, 2003). Similar results were observed at the catchment scale for different karstic systems by Bakalowicz et al. (1974), Stichler et al. (1997) and Maloszewski et al. (2002). From these observations, sometimes combined with chemical parameters, it was conclude that freshly infiltrated water is stored in the aquifer and only a part reaches the spring directly. While the method makes it possible to gain significant insight into the functioning of karst aquifers, environmental isotopes cannot be measured continuously and the input signals can be quite complex complicating the interpretation. Furthermore, the method does not make it possible to infer where storage takes place.

In most of the studies, breakthrough curves of tracers and concentrations of natural compounds are recorded at springs. However, due to the large heterogeneity of karst system and the resulting complex flow pattern, linking the observed behaviour of tracers to the structure of the aquifer can be difficult. Therefore, additional experiments and measurements are increasingly performed in smaller sub-systems to gain more detailed insight in the hydraulic behaviour of karst aquifers and the influence of various sub-systems on solute transport (Wicks, 1997; Groves, 1992; Mayer, 1999; Wicks et al., 1997; Vervier, 1990; Crandall et al., 1999; Chandler and Bisogni, 1999; Clemens et al., 1999; Tooth et al., 2003).

### 1.2.2. Reactive tracers

Different techniques can be used to investigate biological transformation in porous aquifers, including laboratory microcosm studies, detection of nucleic acids and other biomarkers to characterize microbial populations (Green and Scow, 2000; Madsen, 2000), in situ microcosm studies (Gillham et al., 1990), investigation of spatial variations of concentration and isotope ratios of naturally occurring compounds and contaminants (Eganhouse et al., 1996; Hunkeler et al., 1999), and injection of a combination of reactive and conservative tracers (Barker et al., 1987; Sandrin et al., 2003). The knowledge gained on biodegradation processes in porous aquifers cannot be directly extrapolated to karst aquifers since the geological structure and flow pattern of karst aquifers are very different from those in porous aquifers. Moreover most of the methods used in porous aquifers can not be applied in karst due to the elevated heterogeneity of such aquifers and specific methods have to be developed. The elevated heterogeneity and the complexity of water sampling and monitoring in the unsaturated zone required to use a method that was for long time tested with success in karst systems. A method based on the use of reactive tracers (e.g. Barker et al., 1987; French et al., 2001; Sandrin et al., 2003) that can be easily injected in the karst system was used for this study.

While conservative tracers have been used for decades to study transport processes, the use of reactive tracers is a relatively new research area. Reactive tracers are compounds that are injected into the subsurface to study transformation processes. Transformation processes can be assessed based on the production of a characteristic stable intermediate. If the reactive tracers are transformed to non-unique products, they are injected in combination with conservative compounds. Reactive tracers have been used to gain insight into the general biological activity in porous aquifers or to evaluate if specific contaminants are degraded (e.g. French et al., 2001; Sandrin et al., 2003). In the latter case, studies may involve injection of contaminants or non-toxic substrates with a similar structure as contaminants. Examples for these types of application is the injection of chloride, benzene and toluene into a non-contaminated aquifer to evaluate the potential of biodegradation for these compounds (Barker et al., 1987), the injection of deuterium labelled aromatic hydrocarbons into a gasoline contaminated aquifer to study biodegradation of specific aromatic hydrocarbons (Thierrin et al., 1995) or the injection sodium benzoate, ethanol, hexanol and pentanol to study the spatial variability of in-situ microbial activity in a sandy aquifer (Sandrin et al., 2003). Field experiments with injection of propyleneglycol, potassium acetate and non-reactive tracers were also performed (French et al., 2001) in order to study the transport and degradation of these compounds under natural conditions.

## 1.3. Concept of the Biokarst project

The starting point of the BIOKARST project were the recent findings that water can reside over considerable time in the karst infiltration system before reaching the saturated zone (see above). This insight led to the hypothesis that substantial attenuation and biodegradation of organic contaminants may occur before water reaches the saturated zone, from were transport to the spring is frequently fast. The main aim of the project was to verify this hypothesis by means of field studies at the local scale. The studies focus on the karst infiltration systems (soil, epikarst, unsaturated LPV) in regions where diffuse infiltration occurs and thus attenuation processes are more likely to be relevant.

While previous studies have demonstrated storage in the karst infiltration system, they did not make it possible to conclude where storage took place, which is important to know for biodegradation studies as rates are expected to be different in different subsystems. Therefore, the first part of the BIOKARST project involved studies investigating where storage takes place and transit times of solutes in the unsaturated zone of karst systems, before proceeding to biodegradation studies. Storage of water with dissolved compounds could be investigated using artificial tracers or natural conservative tracers. However, while they make it possible to draw conclusions on how long it takes until conservative solutes arrive in the saturated zone, no conclusions can be drawn with respect to the transit time in each subsystem. Therefore, it was decided to investigate alternative methods based on natural tracers. These have also the advantage that they are generally “injected” over a larger area, that the natural hydrodynamic conditions are not disturbed and that they are less time consuming and thus more events with different hydrological conditions can be investigated at the same cost. They can have the disadvantage that the input function is less well constrained than for artificial tracers. The concept of the new natural tracing approach is to analyse substances that originate from the soil zone and then decrease below the soil zone (epikarst, unsaturated LPV). Thus they should make it possible to draw conclusions on how long the water containing solutes has resided in the karst infiltration system below the soil zone. Several natural tracers with different characteristics were selected and their responses compared to evaluate if they provide similar or complementary information. A particular emphasis was given to $^{222}Rn$, which is produced in the soil zone and then decays with constant half-life of 3.8 days, which corresponds to the time scale of interest. A second natural tracer, $CO_2$, also produced in the soil zone and decreasing in the unsaturated zone was studied in parallel to $^{222}Rn$. For comparison with $^{222}Rn$ and $CO_2$, total organic carbon (TOC), which was recently proposed by other research groups (Emblanch et al., 2001; Batiot, 2002; Batiot et al., 2002; Emblanch et al., 2003) was used. TOC concentration decrease by biodegradation and other attenuation processes once TOC has left the soil zone. The kinetics of decrease for this natural tracer is less well known than for $^{222}Rn$. In order to measure these natural tracers, specific measurement systems were developed. A $^{222}Rn$ – $CO_2$ detector was constructed and test by Dr. Heinz Surbeck at the Centre of Hydrogeology (CHYN) and a field fluorometer with a light source of 370 nm (UV LED) was used with successed for the TOC measurements. These systems were installed in differents test sites during prolonged periods and allowed to investigate flood events with different hydrological conditions at high temporal resolution with relatively little cost.

The aim of the second part of the BIOKARST project was to develop and test a method to evaluate if the degradation in the unsaturated zone of karst aquifers can be demonstrated and if so, what minimal rates can be detected. For that, a specific method was developed, based on the use of reactive tracers. This method consisted to use a similar injection procedure of the reactive tracers as for the injection of conventional conservative tracers that have been used successfully in karst systems for a long time.

The occurrence of biodegradation was investigated at several tests sites using reactive tracers. After characterization of the hydraulic behaviour of the test locations, water percolating through the unsaturated zone was sampled during specific tracing experiments making it possible to study processes during passage through soil, epikarst and unsaturated LPV. Experimental sites with a different thickness of the unsaturated zone and with a different distribution of the transit time of the water in the epikarst and unsaturated LPV were used to evaluate the effect of the structure of the unsaturated zone on biodegradation processes. Including the transit time of water in the different sub-systems of karst aquifer, a conceptual model of biodegradation in the unsaturated zone was developed

The BIOKARST project represented a fundamental research in an area where little is known so far and has a high significance for water-quality problems in karst aquifers.

## 1.4. Field sites and experiments

### 1.4.1. Milandre cave

The Milandre cave is located in the Swiss Jura, 8 km N-W of Porrentruy (Fig. 1.6). The Milandre cave (JU, Switzerland) is a CHYN's major test site. Measuring devices have been installed between 1989 and 2006 at the springs and at various locations within the karst systems: underground river (caves), tributaries, boreholes, various inlets, epikarst features, etc. It has been intensively studied for the last 10 years (Gretillat, 1996; Turberg, 1993; Jeannin, 1996; Kovacs, 2003; Perrin, 2003). The catchment area, estimated to be on the order of 13 km2, is drained by the Milandre karstic network (Fig. 1.6). The catchment is divided in four sub-systems corresponding to the three most important underground tributaries (Milandrine upstream, Droite tributary and Bure tributary) and la Font watershed (unknown part of the cave system). The Milandre karstic network is developed in the Rauracian limestone overlying the impermeable Oxfordian marls. The system is characterized by diffuse infiltration on the entire watershed. The unsaturated zone is about 40 to 80 meters thick (Jeannin, 1996). It is strongly heterogeneous with variable soils thickness, a well developed epikarst, and an important fractures network draining water from the surface to the saturated zone. The saturated zone is thin near the principal drains (a few meters) but can reach 30 to 40 meters in the fractured limestone. Access to the cave is possible by two deep shafts (20 and 8 meters). The entrance of the cave is close by a door avoiding air circulation. Milandrine groundwater is sampled and monitored directly in the underground river, in the saturated zone 55 m below the ground surface. The discharge consists of a mixture of flows through the drainage conduits and the fractured LPV of the saturated zone.

This site was ideal to study the detailed characterization of conservative transport and storage processes of solutes across the unsaturated zone at the local scale. During the BIOKARST project continuous $^{222}Rn$, $CO_2$ and TOC measurements at the underground river were realised with conventional measurements (discharge, electrical conductivity, temperature, Fig. 1.7). In parallel, $^{222}Rn$ and $CO_2$ measurements were realized in soil and epikarst boreholes located in the catchment area of the Milandrine tributary. Furthermore, during construction of the TransJurane highway during winter 2003, the soil was removed to some locations, which make possible to study the effect of the soil cover on conservative and reactive transport processes through 50 m unsaturated zone.

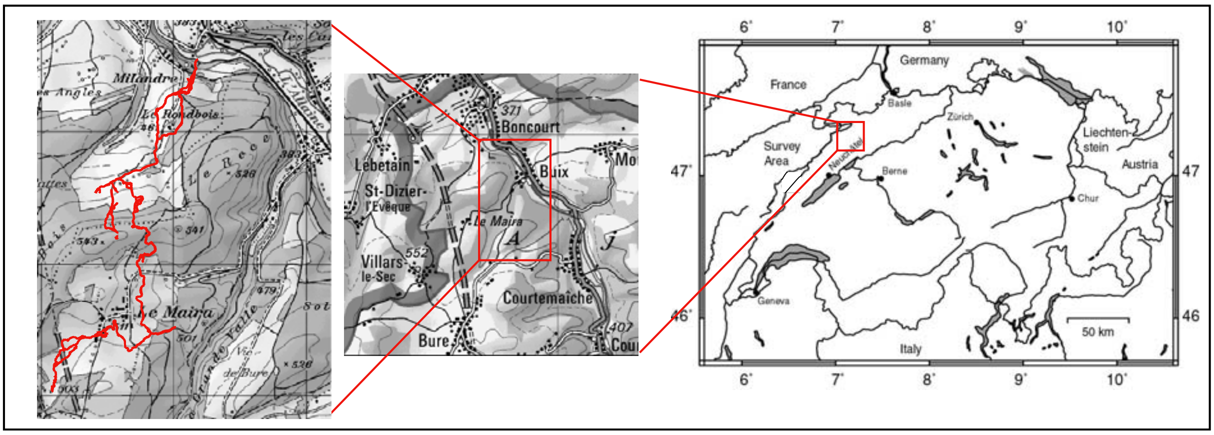


*Figure 1.6: Location of the Milandre cave in the Swiss Jura and Milandre karstic network.*

*Figure 1.7: Picture of the measurement station in the Milandrine river.*

### 1.4.2. Gänsbrunnen galleries

The artificial galleries (Fig. 1.8) in the Weissenstein Mountain near Gänsbrunnen (SO, Switzerland) provide an ideal site to study transport processes through karst infiltration systems. The geology of the unsaturated zone above the galleries has been characterized in great detail and the hydraulic boundary conditions are well defined due to the location on the top of a hill. Measuring devices have been installed during summer 2003 in order to study the hydraulic and chemical behaviour of several locations to gain insight into the spatial and temporal variability of flow and transport processes in this location. Tracer experiments have revealed a homogenous hydraulic behaviour with small differences in transit times on a small scale, which was linked to the location of faults and orientation of bedding planes. Specific tracing experiment using reactive tracers were performed in order to estimate first order biodegradation rates.

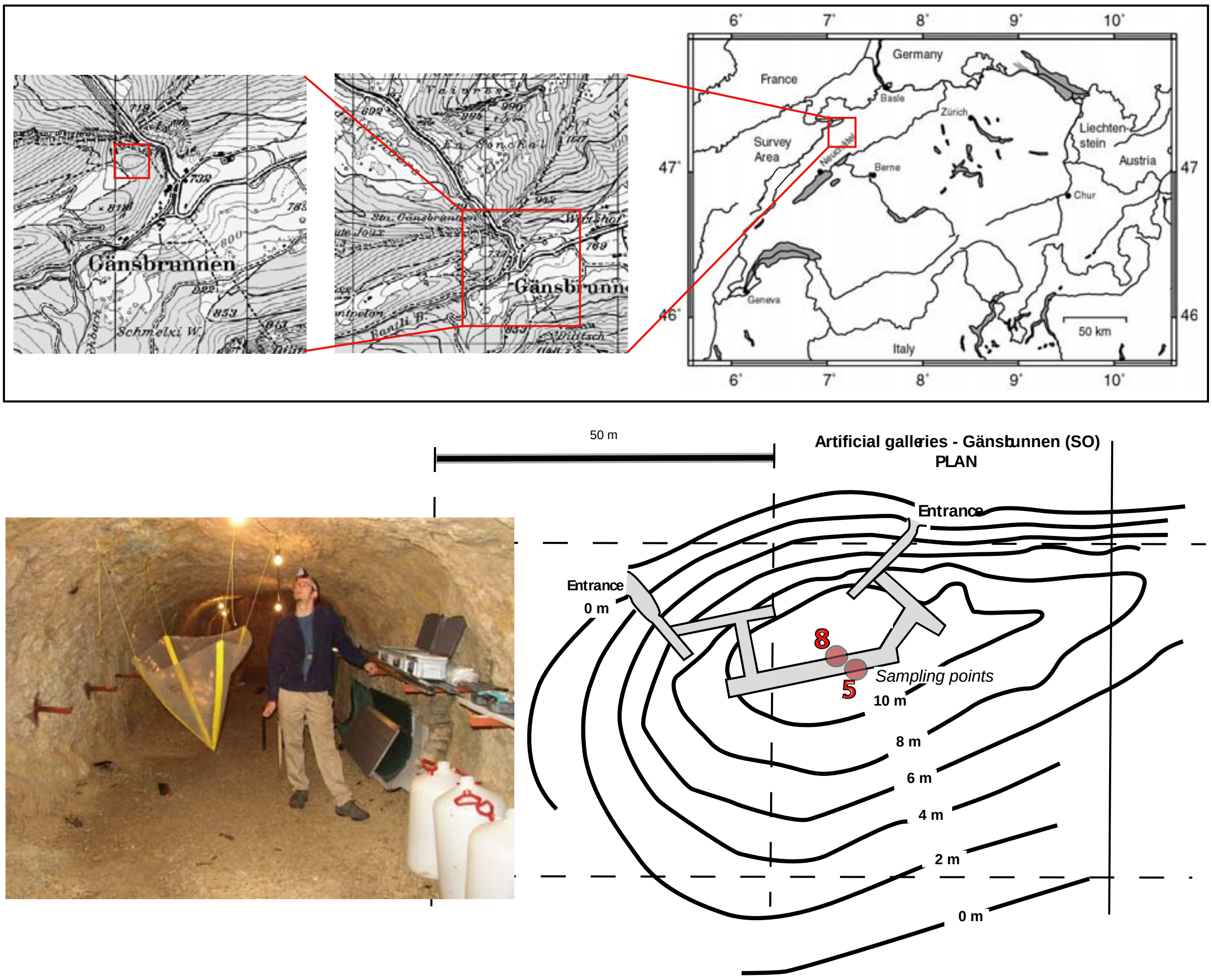


*Figure 1.8: Location and map of the Gänsbrunnen artificial galleries. Red points 5 and 8 represent the sampling locations.*

### 1.4.3. Grand-Bochat and Vers-Chez-le-Brandt caves

The Grand-Bochat (Fig. 1.10) and Vers-Chez-le-Brandt (Fig. 1.9 and 1.11) caves test-sites (NE, Switzerland) have been extensively used (Puech et Bourret, 1998; Madec, 1999; Perrin, 2003) within the last four years for characterization of conservative transport in the karst infiltration system. They are small caves with respectively 15 and 30 meter unsaturated zone. Measuring devices have been installed on a unique outlet in each test-site to gain insight into the spatial and temporal variability of flow and transport processes in this karst system. Watering experiments have been conducted in order to discriminate water coming from the soil reservoir, the epikarst or freshly infiltrating rainwater, to estimate the time and the volume of water storage in the soil and the epikarst and to calculate first order degradation rate.

*Figure 1.9: Vers-Chez-le-Brandt test site, cave and surface.*

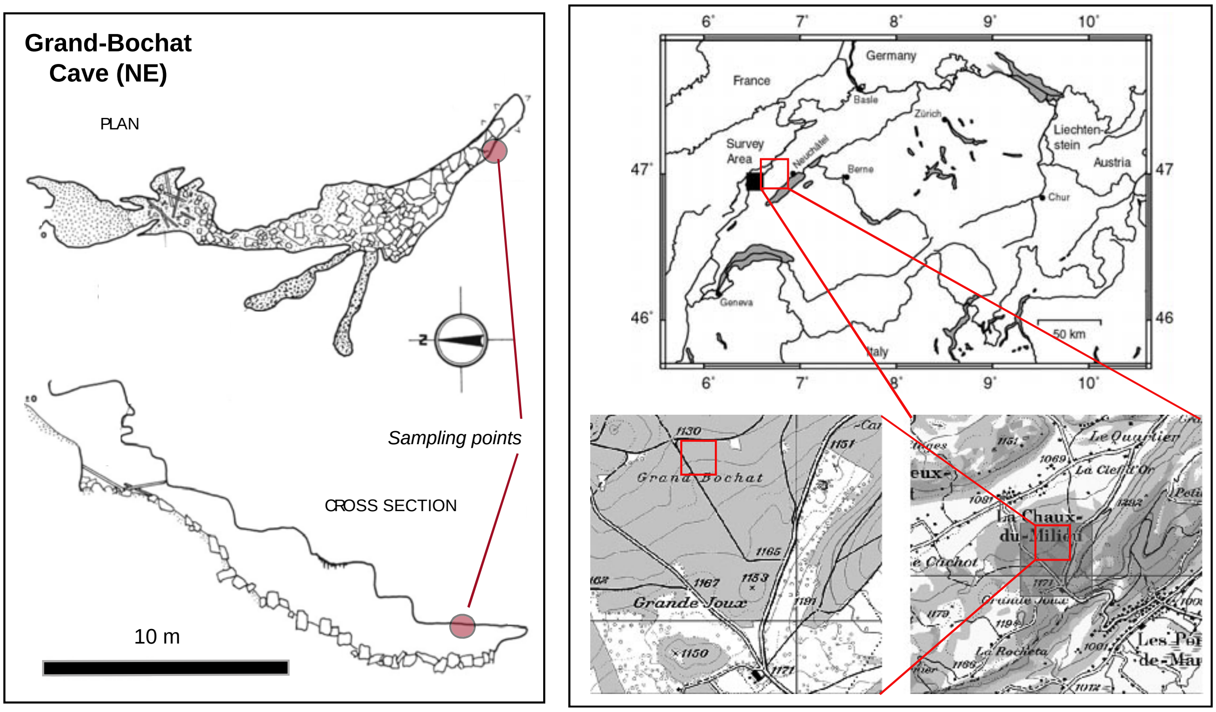


*Figure 1.10: Location and map of the Grand-Bochat cave (topography, Gigon, 1976)*

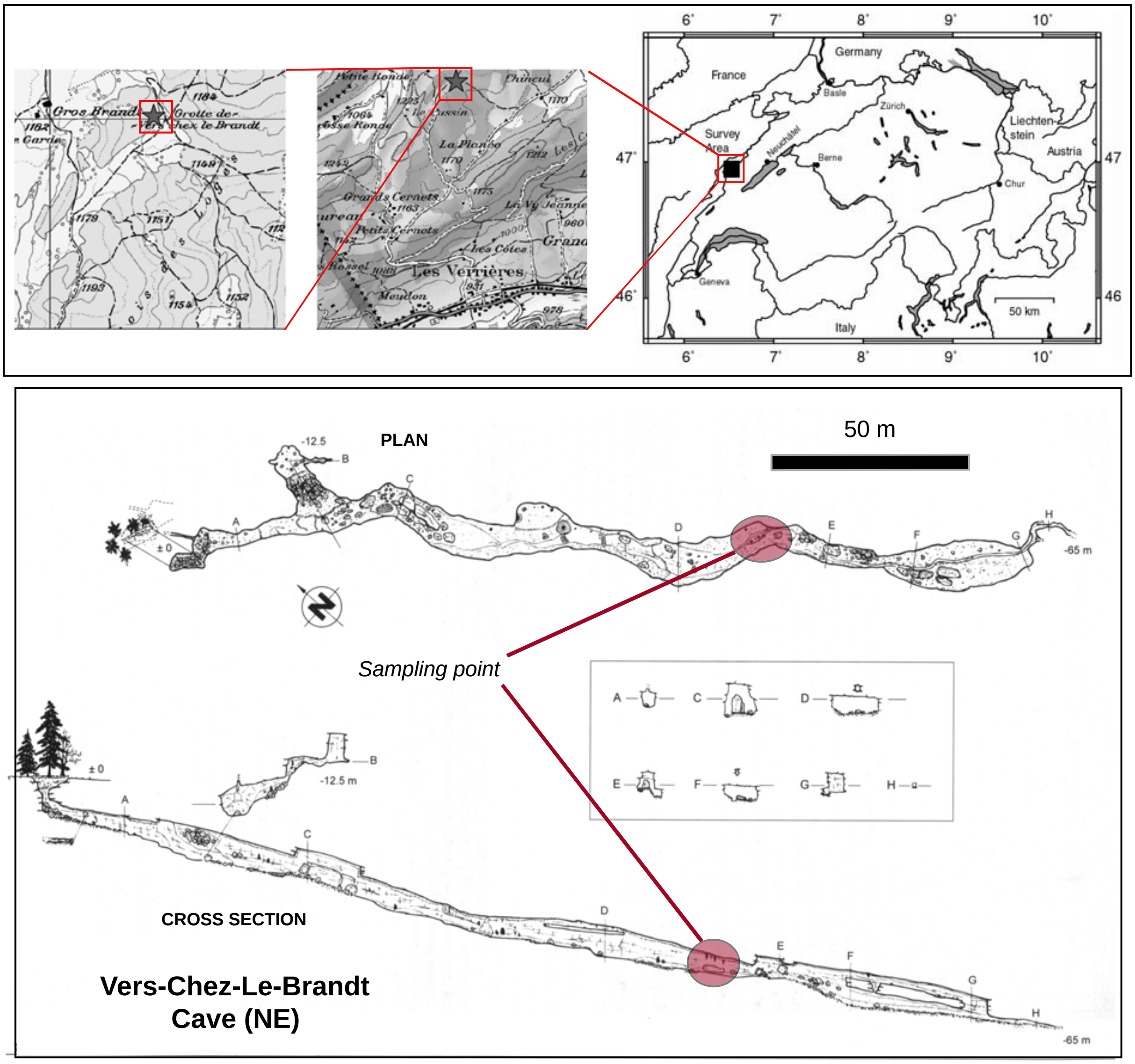


*Figure 1.11: Location and map of the Vers-Chez-le-Brandt cave (topography, Gigon, 1976).*

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# Chapter 2

# Storage, location and flow path of water in the unsaturated zone of karst aquifers

# 2. Storage location, transit time and flow paths of water in the unsaturated zone of karst aquifers

As explain in the chapter 1, to study the biodegradation of contaminants in a karst system, a detailed knowledge of the hydraulic behaviour of the unsaturated zone under variable hydrogeological conditions is needed.

The location of the storage, the transit time and the flow paths of dissolved contaminants through the unsaturated zone of the karst system are three hydrogeological factors that influence the fate of contaminants in the karst systems. These factors are dependent on the water recharge intensity and to the geological structure of the karst system. In order to estimate the location of the storage, the transit time distribution and the different flow paths of groundwater in the unsaturated zone, several field studies including artificial tracing experiment or monitoring of natural tracer during different hydrological conditions were realised

This part of the study focussed mainly on the behaviour of natural tracers ($^{222}$Rn, $CO_2$ and TOC) that originate from the soil zone and diminish in deeper parts of the unsaturated zone hence providing information about the dynamics of solute transfer across the unsaturated zone as discussed in detail in the following paragraphs. In an initially phase, methods for continuous measurement of the parameters were tested that are indispensable to assess the reaction of the system to flood events. Continuous measurement methods were preferred since only very dense sampling intervals provide sufficient information to understand the dynamics of karst systems. Methods for continuous $^{222}$Rn, $CO_2$ and TOC measurement were developed by Dr. H. Surbeck (Surbeck, 1996) and Dr. P.A. Schnegg, University of Neuchâtel. The monitoring devices have the advantage of being small in size and thus can be installed in caves for local scale studies. For comparison with continuous monitoring of natural tracers under natural hydrological conditions, artificial tracing experiments were carried out as well.

In the Milandre cave, the experiments focused on the monitoring of $^{222}$Rn, $CO_2$ and TOC coupled with electrical conductivity and discharge in the underground river (saturated zone) for more than three years under natural hydrological conditions. Complementary artificial tracing experiments and discharge measurements in the unsaturated zone were carried out in order to evaluate the buffer effect of the soil zone, the epikarst and the LPV.

During winter 2003, the soil was removed at some locations, which made it possible to study the effect of the soil cover on conservative transport processes. In order to study the transport of the $^{222}Rn$ enriched soil water in the karst system at a local scale and to identify the origin of water in the cave under artificial recharge conditions, irrigation experiments were performed at Grand-Bochat site. The Grand Bochat site is a smaller karst system compared to the Milandre site and artificial irrigation experiments allowed to study the transport of the $^{222}Rn$ enriched soil water in the karst system at a local scale. Indeed the sampling location in the Grand-Bochat cave is well connected to the epikarst and soil zones and the influence of the unsaturated LVP to the discharge is low compared to the Milandre site where moreover an influence of the saturated zone to the discharge is observed. In order to study the transport of TOC enriched soil water in the karst system at a local scale, continuous monitoring of TOC concentration was also conduct at Vers-Chez-le-Brandt. As for the Grand-Bochat site, the Vers-Chez-le-Brandt site is covered by a thin soil layer less than 0.5 meters and the sampled location in the cave well connected to the epikarst and soil zone without significant contribution of the saturated LPV to the discharge.

The major results of these measurements have been synthesised in three papers modified for the thesis and presented below: *$^{222}Rn$ and $CO_2$ as natural tracers for flow and storage in the unsaturated zone of karst systems* for continuous measurement of $^{222}Rn$ and $CO_2$ under natural conditions at Milandre cave (paragraph 2.2); *TOC as natural tracer for flow separation in the unsaturated zone of karst systems systems* for continuous measurement of TOC under natural conditions at Milandre cave and Vers-Chez-le-Brandt cave (paragraph 2.3) and *Characterisation of flow in karst unsaturated zone. The case-study of A16 freeway and its impact on Milandre cave* for results of tracing experiment at Milandre cave (paragraph 2.4).

## 2.1. $^{222}Rn$ and $CO_2$ under natural recharge conditions[1]

**Abstract**

The combined measurement of radon ($^{222}Rn$) and carbon dioxide ($CO_2$) with common hydrochemical parameters makes it possible to get a detailed picture of the origin and storage of water during baseflow and flood events. $^{222}Rn$ provides information on the location of storage because $^{222}Rn$ production is much larger in soil than in the limestone matrix and because $^{222}Rn$ decay with a half-life of 3.8 days. In the saturated zone, the $CO_2$ level during baseflow varies relatively little on an annual basis, and an immediate increase of the concentration is observed during flood events indicating a contribution of the saturated low permeability volumes to the discharge. In a karst aquifer characterized by diffuse infiltration and thick soil cover, continuous measurements showed for small rainfall an increase in $CO_2$ without variation in $^{222}Rn$. The constant $^{222}Rn$ level suggests that the water was stored at least 20 days (5 times half-life of $^{222}Rn$) below the soil zone. For stronger rainfall a delayed increase in $^{222}Rn$ occurred, indicating a significant delayed contribution of the soil zone to the discharge possibly due to an overflow of the epikarst. In some cases a strong decrease of the electrical conductivity indicates that freshly infiltrated water contributing to the discharge. These results indicate that the soil and epikarst sub-systems have an high buffer and storage capacity. The study demonstrates the potential of $^{222}Rn$ and $CO_2$ as natural tracer. $^{222}Rn$ and $CO_2$ have the advantage that they can be continuously measured and more hydrological events can be investigated at less cost.

**Keywords**

Karst hydrology, Soil, Epikarst, $^{222}Rn$, $CO_2$, Unsaturated zone.

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[1] **$^{222}Rn$ and $CO_2$ as natural tracers for flow and storage in the unsaturated zone of karst systems**

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*Paper prepared for Journal of Hydrology*

### 2.1.1 Introduction

The transit time of water between the surface and karst springs is frequently investigated using artificial tracers (e.g. Kaess, 1998). While the method has the advantage that the input function is well defined, artificial tracers can generally only be applied over a small surface. Furthermore, it is difficult (persistent background concentrations) and time consuming to repeat a tracer test at the same location to evaluate the reaction of a system under different hydraulic conditions. An alternative approach is the use of environmental isotopes ($^{18}$O, $^{2}$H, $^{3}$H) that are naturally spread with rainwater over the whole catchment and show conservative behaviour. The response of environmental isotopes to precipitation events has been investigated at the catchment scale (e.g. Bakalowicz et al., 1974; Rank et al., 1992; Maloszewski et al., 1992; Maloszewski et al., 2002; Emblanch et al., 2003) and at local scale within the unsaturated zone (e.g. Harmon, 1979; Yonge, 1985; Chapman, 1992; Caballero et al., 1996; Perrin et al., 2003b). These tracers were used to estimate mean transit times and to evaluate the respective contribution of different sources of water such as freshly infiltrated water and water stored in low permeability volumes or the unsaturated zone (Blavoux and Mudry, 1983; Vervier, 1990; Lakey and Krothe, 1996; Lastennet and Mudry, 1997; Katz et al., 1998; Lee and Krothe, 2001; Maloszewski et al., 2002). In several studies, it was observed that the isotopic response ($^{18}$O, $^{2}$H) at water arrival points in caves is highly buffered compared to rainfall, although discharge varies substantially (Chapman, 1992; Caballero et al., 1996; Perrin, 2003). Similar results were obtained at the catchment scale for different karstic systems (Bakalowicz et al., 1974; Stichler et al., 1997; Maloszewski et al., 2002). From these observations, sometimes combined with chemical parameters, it was concluded that during rainfall, freshly infiltrated water is stored in the aquifer and only a part reaches the spring directly. While the method makes it possible to gain insight into the functioning of karstic aquifers, environmental isotopes cannot be measured continuously and the input signal can be quite complex complicating data interpretation. Furthermore, the method does not make it possible to infer where storage takes place.

An alternative approach is the use of hydrochemical parameters to gain insight into the dynamics of karst flow systems such as parameters related to limestone dissolution (e.g. Bakalowicz, 1979; Mudry, 1987; Plagnes, 1997; Batiot, 2002; Maloszewski et al., 2002;

Mudry et al., 2002; White, 2002; Celle-Jeanton et al., 2003) or more recently dissolved organic carbon (e.g. Bakalowicz, 1979; Plagnes, 1997; Emblanch et al., 2003; Batiot, 2002; Pronk et al., 2006).

This study focuses on the use of $^{222}Rn$ and $CO_2$ as natural tracers. These two substances are potentially sensitive indicators for water storage in different subsystems (soil, epikarst, low permeability volumes) of the unsaturated zone. They have also the advantage that they can be continuously measured. Thus events with different hydrological conditions can be investigated at high temporal resolution with relatively little cost.

$^{222}Rn$ is produced in the soil zone by α-decay of $^{226}Ra$ and then decays with a half-life of 3.8 days (Avrorin et al., 1982). The half-life of $^{222}Rn$ corresponds to the time scale of the rapid flow component in karst aquifers, which is often of particular interest to assess the vulnerability of karst systems. At 10°C, the Ostwald Coefficient (ratio of the $^{222}Rn$ concentration (Bq/l) in water versus the $^{222}Rn$ concentration (Bq/l) in the gas phase) is about 0.35 (Clever, 1985) and hence the compound is relatively well soluble in water. In the western Jura, high $^{226}Ra$ activities have been found in many soil samples (Medici, 1992; Surbeck, 1992) despite the low $^{226}Ra$ concentration in the limestone bedrock and the lack of alpine material from last glaciations (Pochon, 1978) with possible higher activities. In such soil, the high enrichment of $^{226}Ra$ are mainly found on grain surfaces of organic matter, hydroxides (e.g., ferrihydrites), and oxides (e.g., goethite). According to Von Gunten et al. (1996), this high enrichment of $^{226}Ra$ originates from weathering of calcite releasing uranium and its decay products. Elevated $^{222}Rn$ concentrations are observed in the Jura Mountains, which can reach 50 kBq/m$^3$ in water and 150 kBq/m$^3$ in the soil air (Surbeck, 1990). In a study of the Areuse spring, Switzerland, Eisenlohr and Surbeck (1995) have observed that the $^{222}Rn$ concentration increases after the discharge increase indicating a $^{222}Rn$ production in the infiltration zone rather than in the low permeability volumes (LPV).

$CO_2$ is mainly produced by the soil biological activity (Bourges et al., 2001). The $CO_2$ concentration can decrease below the soil zone by dissolution of carbonate minerals (under closed conditions) (Stumm and Morgan, 1996). Several studies have discussed the origin and behaviour of $CO_2$ in the atmosphere of karstic systems (e.g. Orgnac cave, France, Bourges et al., 2001; Ballynamintra cave, Ireland, Baldini et al., 2006) and in soils covering karst systems (Clemens et al., 1997, Zaihua et al., 1997) where seasonal variations are observed due to the seasonal variability of the microbial activity. These studies found concentrations varying between 2 and 5 % vol. $CO_2$. In a karst spring in the USA (Fort Campbell springs, Kentucky - Tennessee), Vesper and White (2004) have observed that the $CO_2$ partial pressure continues to rise after maximum discharge was reached during storm flow. They explain this result by dispersed infiltration through $CO_2$-rich soils lagging behind quickflow from a sinkhole.

In this study, $^{222}Rn$ and $CO_2$ concentrations were measured continuously at one field sites simultaneously with classical parameters including discharge, electrical conductivity and temperature. The study site (Milandre cave) is situated in the Swiss Jura, and is characterized by diffuse infiltration, a 40 to 80 meters thick unsaturated zone, a well developed epikarst and an thick soil cover. At this site, $^{222}Rn$ and $CO_2$ concentration in the soil and unsaturated zone was measured as well to characterize the source of these compounds.

### 2.1.2 Study area

#### *Milandre Cave*

The Milandre cave test site is situated in the Swiss Jura, 8 km N-W of Porrentruy. It has been intensively studied for the last 10 years (Gretillat, 1996; Turberg, 1993; Jeannin, 1996; Kovacs, 2003; Perrin, 2003). The catchment area, estimated to be on the order of 13 km$^2$, is drained by the Milandre karstic network (Fig. 2.1). The springs of the system consist of the Saivu, with a discharge between 20 to 200 l/s, the Bame temporary spring with a discharge reaching 3000 l/s and the La Font intra-alluvial spring (Grasso and Jeannin, 1994). The catchment is divided in four sub-systems corresponding to the three most important underground tributaries (Milandrine upstream, Droite tributary and Bure tributary) and la Font watershed (unknown part of the cave system). The Milandre karstic network is developed in the Rauracian limestone overlying the impermeable Oxfordian marls. The system is characterized by diffuse infiltration on the entire watershed. The unsaturated zone is about 40 to 80 meters thick (Jeannin, 1996). It is strongly heterogeneous with variable soils thickness, a well developed epikarst, and a well developed fractures network draining water from the surface to the saturated zone. The saturated zone is thin near the principal drains (a few meters) but can reach 30 to 40 meters in the low permeability volumes (LPV). Access to the cave is possible by two shafts (20 and 8 meters). The entrance of the cave is close by a door avoiding air circulation.

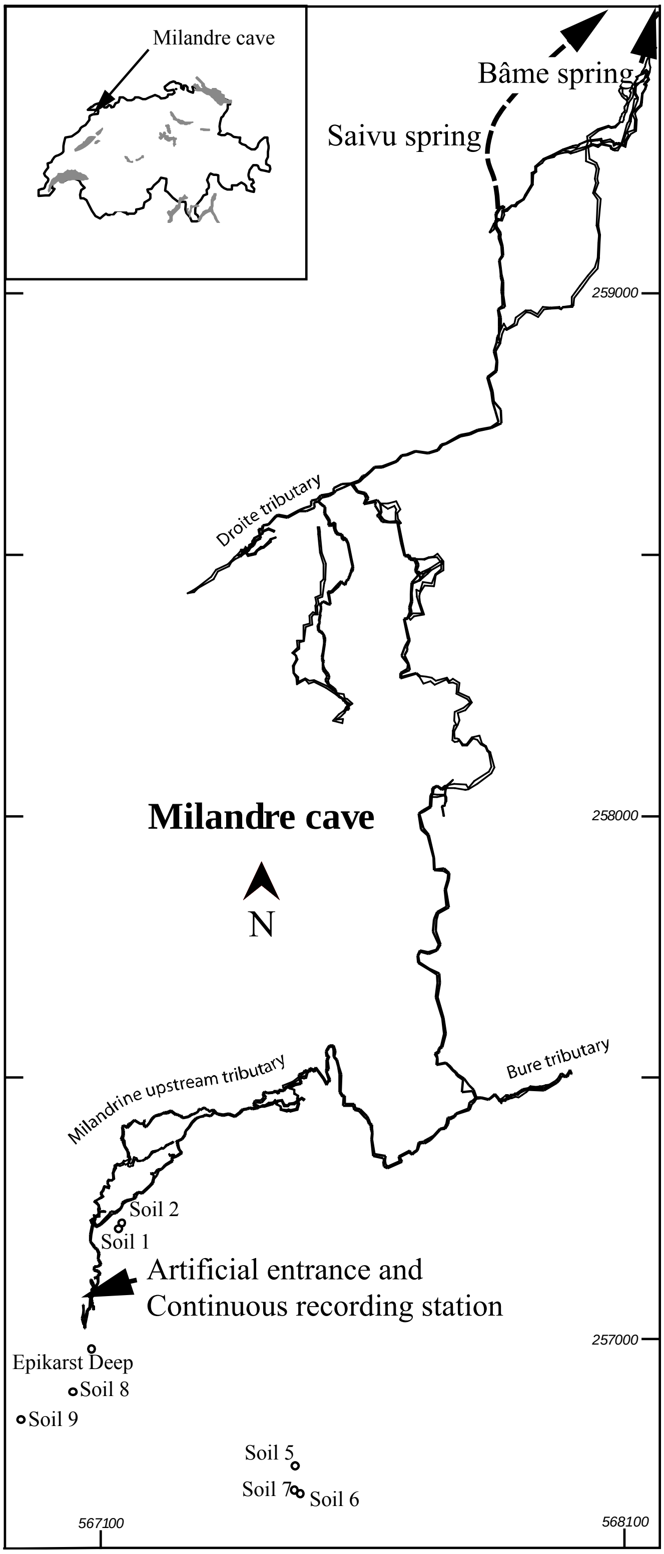


*Figure 2.1: Situation of Milandre cave spring with detailed map of the Milandre cave and localisation of boreholes and continuous recording station for discharge, conductivity, $^{222}Rn$ and $CO_2$ concentration.*

### 2.1.3 Sampling and Data acquisition

To investigate the $^{222}Rn$ and $CO_2$ distribution in the infiltration zone of the Milandre site, 7 soil boreholes and 1 epikarst borehole were drilled during Perrin PhD project (Perrin, 2003) in fields with different land use (pasture or forest) (Fig. 2.1). The depth of the soil boreholes depends on the soil thickness and the epikarst alteration and varies between 1,6 m and 3.7 m. The soil borehole diameter is 10 cm and they are filled with successive 30 cm layers of gravel and bentonite. Each gravel layer is connected to the surface with an aluminium tube (∅ = 6 mm) and closed with rubber stoppers when no measurements are taken. The epikarst borehole is located in a forest with less than 30 cm soil and reaches a depth of 15 meters. It has two separate screened intervals, 0-5 meters (Epikarst shallow) and 5-15 meters (Epikarst deep). Each part of the borehole is connected to the surface with an aluminium tubes (∅ = 6 mm). Monthly soil $CO_2$ measurements were carried out between June 2001 and August 2002. Because it is a very time consuming procedure, $^{222}Rn$ concentration measurements in the soil were only done one time (August 2002) for each borehole.

For measurement of $^{222}Rn$ in the soil and epikarst boreholes, a portable Lucas-cell based instrument (RDA-200, Scintrex, Canada) was used. The aluminium tubing was purged with a peristaltic pump. The replaced air corresponded to 3 times the volume of the air in the tube. After purging, 600 ml of gas was pumped to purge and fill the Lucas cell (180 ml). After 5 minutes delay to allow for production of $^{222}Rn$ daughters and decay of external luminescence, the $^{222}Rn$ was measured during 10 minutes. $CO_2$ concentrations were determined by IR absorption using an Anagas CD 98 HR (Environmental Instrument, England) with integrated pump. Gas was pumped until a stable $CO_2$ concentration was observed for more than one minute.

Between September 2002 and December of 2004, the most upstream part of the Milandrine underground river (Fig. 2.1) was equipped with a continuous recording station measuring $^{222}Rn$ and $CO_2$ concentrations in parallel with discharge, electrical conductivity and water temperature, with measurement interruption for $^{222}Rn$ and $CO_2$ between November 2002 and June 2003 (see annex 1 for complete chronicles). The measurements in the soil zone and in the underground river were not carried out during the same year due to logistic reasons. Continuous water level and electrical conductivity measurements were carried out with pressure and electrical conductivity probes. The discharge was calculated from the water level using the water level-discharge relationship obtained from punctual gauging experiments. For continuous $^{222}Rn$ measurement in water, a closed circuit of air-filled semipermeable (polypropylene) tubing was immerged (Fig. 2.2 a) directly into the water of the river (Surbeck, 1996). Every 30 minutes (time necessary for equilibrium with dissolved gases in water) the gas in the tube was pumped (200 ml/min) through the detector. A Lucas-cell coupled to a photomultiplier detector (Fig. 2.2 b and c) was used to measure the $^{222}Rn$ concentration in the air circuit. A concentration of 1 Bq/l in the water typically leads to 50 counts/min. For an integration time of 30 min, the standard deviation is 3% at 1 Bq/l and the detection limit is at 0.1 Bq/l. The calibration uncertainty for the $^{222}Rn$ value is 5%. $CO_2$ is determined in the same closed air circuit by IR absorption, in series with the $^{222}Rn$ detector (Fig. 2.2 a and c). The standard deviation is 0.05 % vol. at 1% vol. The absolute calibration uncertainty for the $CO_2$ sensor is 20%.

The $^{222}Rn$ detector and the $CO_2$ sensor were packed together with the pump and electronics in a small watertight box (Fig. 2.2c). Thanks to the power dissipated and a styrofoam insulation, the temperature in the box stays slightly above the outside temperature (10°C +/- 0.3°C). As long as the outside temperature does not fall considerably below the water temperature there is no risk of condensation inside the box. In a cave where air temperature and water temperature are very similar, there is thus no need for a desiccant in the closed air loop. To calibrate the continuous measurement system, batch samples were taken in the river during different discharge conditions to cover the variations of $^{222}Rn$ and $CO_2$ levels. Batch samples were taken in 20 ml glass vials (PTFE/silicone septum cap). In the laboratory, $^{222}Rn$ was extracted with an organic solvent/scintillator cocktail (Maxilight, Hidex Oy, Finland) and after 3 h, $^{222}Rn$ and $^{222}Rn$ decay products are determined by liquid scintillation counting (LSC). The LSC-instrument used (Triathler, Hidex Oy, Finland) allows for an efficient alpha/beta separation, leading to a low background count and thus to a low detection limit of 0.2 Bq/l for a measuring time of 1000 s. $CO_2$ in batch samples was determined by bubbling air through a 20 ml sample and measuring the $CO_2$ concentration in a closed air circuit by IR absorption (Texas Instruments Sensor, USA).

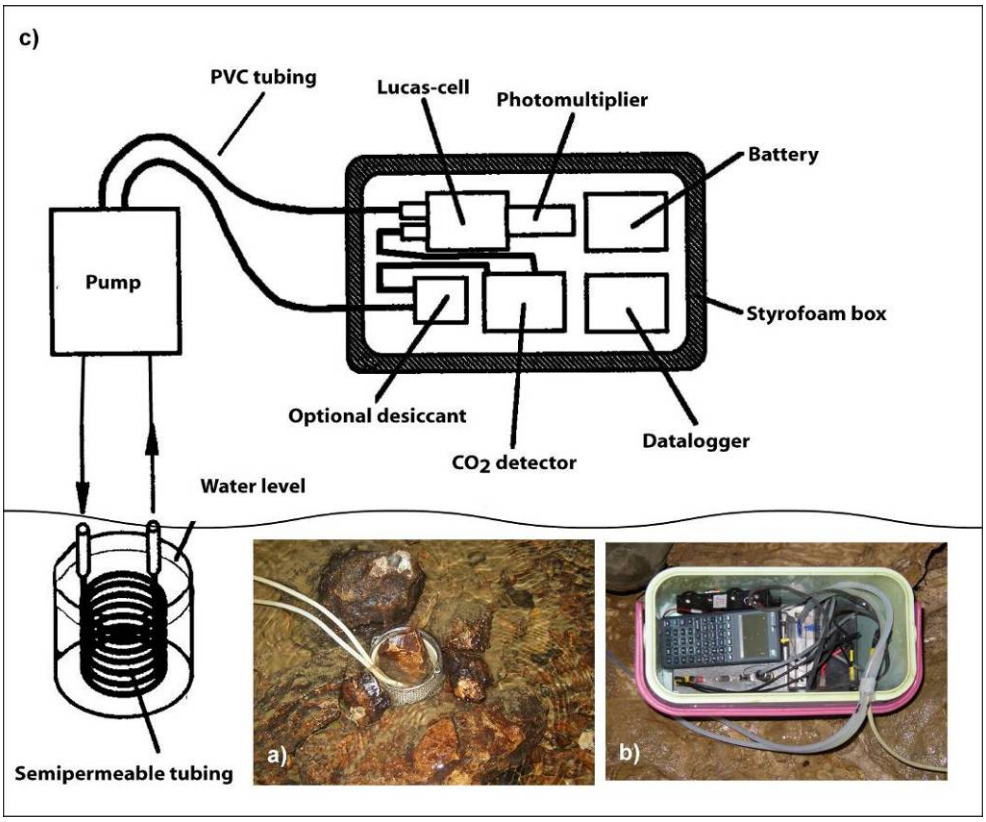


*Figure 2.2: a) The $^{222}$Rn detector and the $CO_2$ sensor packed together with electronics in a small styrofoam box b) the closed circuit of air-filled semipermeable (polypropylene) tubing immerged directly into the water of the river. c) Schematic representation of the $^{222}$Rn detector - $CO_2$ sensor and the semipermeable tubing*

## 2.1.4 Results and Discussion

### *$^{222}$Rn and $CO_2$ in soils*

In the 7 soil boreholes and the epikarst borehole, $^{222}$Rn concentrations vary between 0.3 Bq/l and 74.3 Bq/l (Fig. 2.3). For each soil borehole, $^{222}$Rn concentrations increase with depth and the maximum values tend to occur at the soil/epikarst interface (Fig. 2.3). The low concentrations near the soil surface are due to gas loss to the atmosphere by diffusion (Climent, 1996). In the epikarst boreholes, smaller concentrations than in the soil were measured with 5.5 Bq/l for Epikarst shallow and 1.2 Bq/l for Epikarst deep, respectively. These data demonstrate that $^{222}$Rn production is higher in the soil than in the epikarst, likely because $^{226}$Ra is enriched in the soil and because the specific surface area is higher in the soil than in the epikarst. Hence the soil zone has to be considered as the major source of $^{222}$Rn.

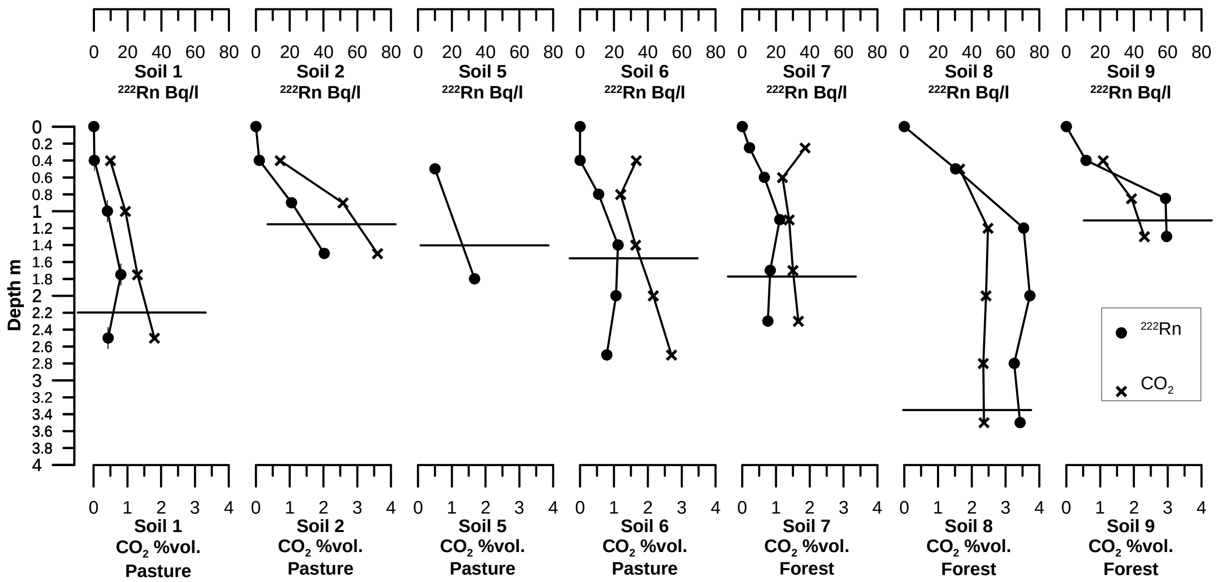


*Figure 2.3: $^{222}$Rn and $CO_2$ measurement results (August 2002) for the 7 soil boreholes. The horizontal lines represent the soil-epikarst limit for the different boreholes.*

Monthly $CO_2$ sampling campaigns were carried out in the same boreholes as for $^{222}$Rn measurement during more than one year. In all soil boreholes (Fig. 2.3), the $CO_2$ concentration generally increases with depth. The $CO_2$ concentrations show strong annual variations with the highest levels at the end of the summer and the lowest at the end of the winter (Fig. 2.4a, Table 2.1). The annual mean $CO_2$ concentration varies between 1.73 and 3.49 % vol. with no apparent link to landuse (Table 2.1). Minimal values observed during the end of winter vary between 0.31 and 1.1 % vol. and maximal values (end of summer) between 2.73 and 5.10 % vol. (Table 2.1). The amplitude of $CO_2$ concentration variations lies between 1.70 and 4.44 % vol. For the Epikarst deep borehole, the annual mean $CO_2$ concentration is 2.66 % vol. and corresponds to the average annual value for soil boreholes (2.42 % vol.) but with a lower amplitude (2.02 % vol. instead of 3.01 % vol., Fig. 2.4a). The minimal value observed in Epikarst deep during the end of winter is 1.58 % vol. and the maximal value (end of summer) 3.60 % vol. The seasonal variation of the $CO_2$ concentration in the soil zone is linked to temperature variations with lower temperatures in winter leading to lower $CO_2$ concentrations due to a lower microorganisms activity, less roots respiration and less degradation of natural organic matter (Zaihua et al., 1997). The smaller amplitude of the $CO_2$ concentration in Epikarst deep suggests that the seasonal variations are buffered with depth. Regarding the $CO_2$ fluxes in the saturated zone, the maximal levels are observed during winter due to a more elevated base flow discharge in the river than during summer, although minimal concentrations are observed in the soil zone during winter. The information contained in the $CO_2$ concentration signal is completely masked by the discharge.

*Table 2.1. Results of annual $CO_2$ (% vol.) measurements (2001 - 2002) in soil boreholes and deep epikarst borehole for different land use. Mean, maximal, minimal values and amplitude are represented in % vol. * For logistic reasons, the measurements in the underground river (2003) were not carried out during the same year as for boreholes.*

| Boreholes | Soil 1 | Soil 2 | Soil 5 | Soil 6 | Soil 7 | Soil 8 | Soil 9 | mean | EPI deep | River * |
|---|---|---|---|---|---|---|---|---|---|---|
| Land use | Pasture | Pasture | Pasture | Pasture | Forest | Forest | Forest | | | |
| Depth of borehole, river (m) | 2.90 | 1.60 | 1.90 | 2.70 | 2.30 | 3.80 | 1.40 | | 15.00 | 50.00 |
| Depth of represented data (m) | 2.2 | 1.2 | 1.4 | 1.6 | 1.8 | 3.5 | 1.2 | | | |
| Mean (annual) | 1.73 | 3.49 | 2.81 | 2.36 | 1.48 | 2.18 | 2.88 | 2.42 | 2.66 | 1.90 |
| min (annual) | 0.31 | 0.66 | 0.87 | 0.77 | 0.36 | 1.10 | 0.68 | 0.68 | 1.58 | 2.30 |
| max (annual) | 2.83 | 5.10 | 4.50 | 3.32 | 2.73 | 2.80 | 4.50 | 3.68 | 3.60 | 2.17 |
| amplitude | 2.52 | 4.44 | 3.63 | 2.56 | 2.37 | 1.70 | 3.82 | 3.01 | 2.02 | 0.41 |

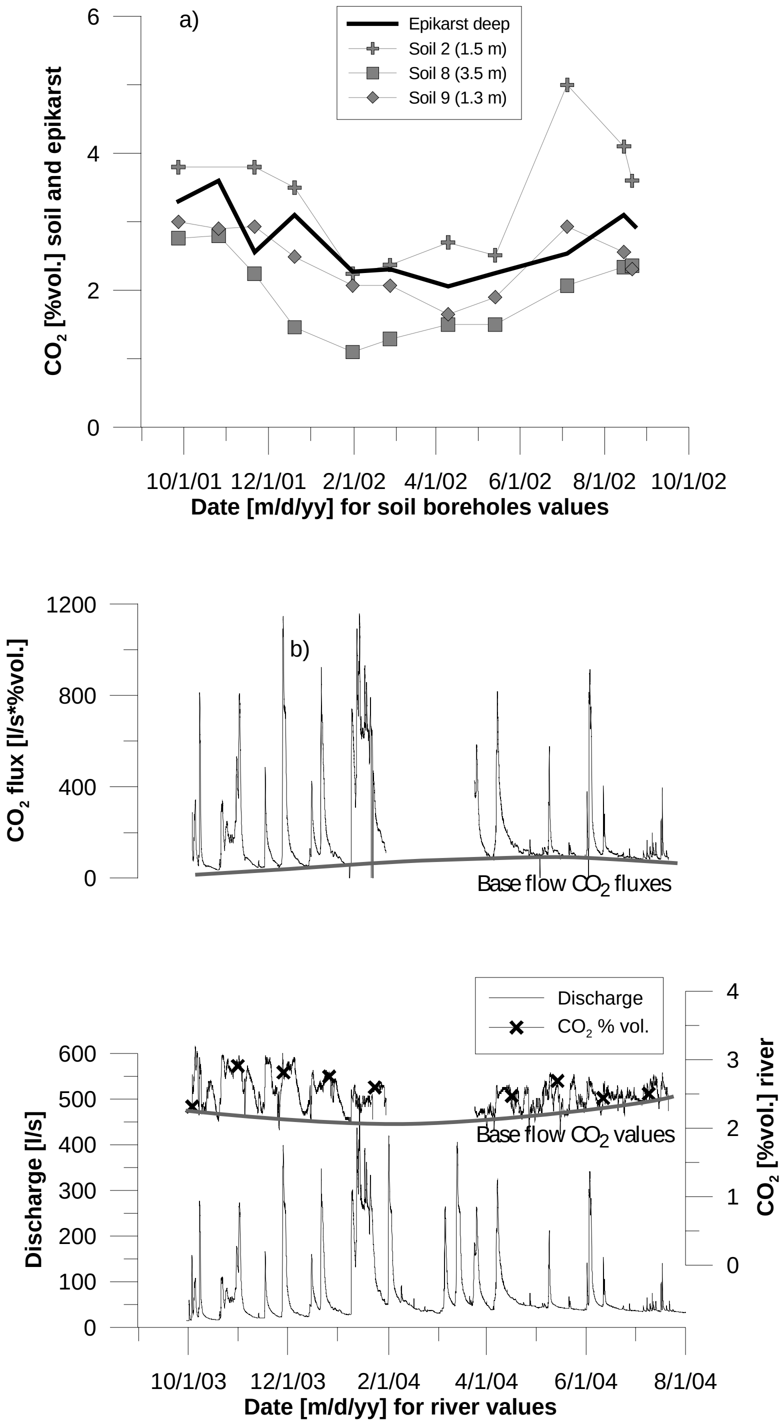


*Figure 2.4a. Soil at the soil/epikarst interface and deep epikarst boreholes CO2 concentrations. b) $CO_2$ concentration in the Milandrine river (black line with crosses) and $CO_2$ flux, discharge and base flow minimal values for $CO_2$ concentration (grey line).*

### $^{222}$*Rn and $CO_2$ during baseflow and flood events*

The results were separate in 3 different groups depending on discharge: 1) baseflow and small flood events with discharge less than 150 l/s; 2) medium flood events with discharge comprise between 150 l/s and 400 l/s; 3) large flood events with a discharge above 400 l/s (see annex 1 for complete chronicles).

**Baseflow**

During June and September 2003 (Fig. 2.5a), a long dry period without elevated rainfalls was observed (105 mm in 65 days). In the Milandre river, the flow was very low (mean 16.7 l/s) and some small floods events with maximum discharge less than 60 l/s were recorded. A mean $^{222}$Rn concentration of 3.2 Bq/l (min 2.9Bq/l and max 4 Bq/l) and a mean $CO_2$ concentration of 2.2 % vol. (min 1.9 % vol. and max 2.6 % vol.) was measured (Fig. 2.5a).

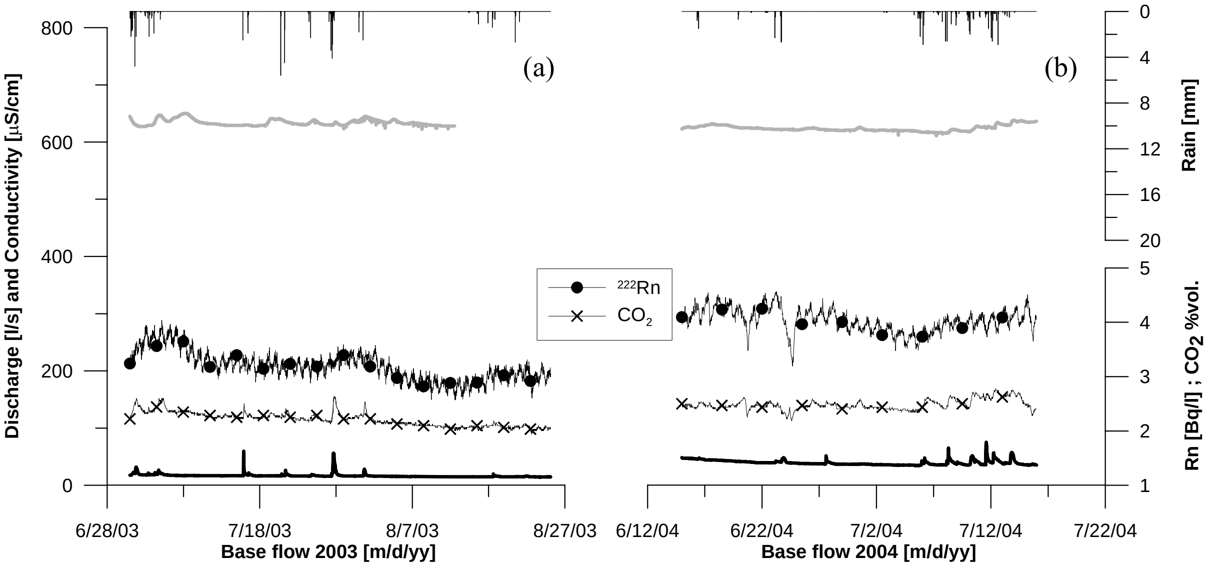


*Figure 2.5a. Summer 2003 (between June and September) and b. Summer 2004 (June and July) base flow continuous measurement. Black line corresponds to discharge and grey line to conductivity. The thin black lines correspond to radon (points) and CO2 (crosses) with one symbol every 7 days.*

During June and July 2004 (Fig. 2.5b), there was also a period of low rainfall (55.9 mm in 30 days) with mean $^{222}Rn$ concentration of 4 Bq/l (min 3.2 Bq/l, max 4.6 Bq/l) and mean $CO_2$ concentration of 2.5 % vol. (2.2 % vol., 2.8 % vol.). During both periods, the electrical conductivity was constant at about 630 µS/cm. The sustained presence of $^{222}Rn$ during baseflow despite its short half-life indicates that some production of $^{222}Rn$ occurs in the limestone as well and probably in the sediments deposed in the river bed. It is unlikely that $^{222}Rn$ during baseflow originates from the soil zone because a continuous decrease of the $^{222}Rn$ concentration during the baseflow period would be expected, which is not observed. Furthermore, in the absence of precipitation little transfer of water from the soil zone to the cave is expected.

Using the baseflow measurements, the annual trend of the $CO_2$ concentration in the river can be characterized (Fig. 2.4b). Similarly as in Epikarst deep borehole (Fig. 2.4a) during 2002, a seasonal trend is observed with a minimal concentration in February. However, the amplitude is even lower than in the deep epikarst borehole (Table 2.1). Thus the amplitude of the $CO_2$ concentration variations seems to decrease from soil to epikarst to river, which can be explained by mixing of water in the saturated LPV leading to a $CO_2$ concentration in the saturated LPV during baseflow close to the annual average. In addition, dissolution of carbonates in the saturated LPV (under closed conditions) may contribute to a smoothing of the $CO_2$ concentrations compared to the values measured in the epikarst and soil zones and degassing of the $CO_2$ in the cave atmosphere may also contribute to a lower $CO_2$ concentration in the river compared to the saturated LPV. In summary, the $^{222}Rn$ and $CO_2$ measurement in groundwater during baseflow indicate a water contribution of the saturated LPV to the discharge (decay of $^{222}Rn$ and buffered $CO_2$ values) and do not reflect a direct contribution of water from the soil zone.

**Small flood events**

On July 27$^{th}$ 2003, a flood event was observed after 15.9 mm rainfall during the morning (Fig. 2.6a). The flood occurred after a sunny period of 25 days with little rainfall (30 mm). The maximum discharge (55 l/s) of the Milandrine River was observed about 8 hours after the maximum rain intensity. The $^{222}Rn$ concentration showed no significant changes (constant level at 3.2 Bq/l) but the $CO_2$ concentration increased by 0.5 % vol. from 2.16 % vol. to 2.63 % vol. The electrical conductivity is about 630 µS/cm with no variations.

On October 9th 2004, a flood was measured after 25 mm rainfall (Fig. 2.6b). The discharge of the Milandrine river increased about 4 hours after the start of the rainfall. The discharge reached 95 l/s, the $^{222}Rn$ level was stable (mean 4.0 ± 0.2 Bq/l) and $CO_2$ concentration increased by 0.7 % vol. from 2.7 % vol. to 3.4 % vol. The electrical conductivity is about 630 μS/cm and a small electrical conductivity increase by 20 μS/cm is observed 1 hour after the discharge increase.

The constant $^{222}Rn$ concentration (3 - 3.5 Bq/l) corresponding to baseflow levels indicates that the soil reservoir does not significantly contribute to the discharge during small rainfall events. The immediate $CO_2$ increase with increasing discharge is due to the fast contribution of water from the saturated LPV reservoir. During baseflow, the $CO_2$ concentration in the river is lower than in the saturated LVP due to a degassing of the $CO_2$ to the cave atmosphere. During small flood events, the fast contribution of the saturated LPV to the discharge with less time for degassing leads to a small $CO_2$ increase. The water of the two small flood events was likely stored for more than 20 days below the soil zone otherwise $^{222}Rn$ concentrations should be higher than baseflow values. The constant electrical conductivity indicates that water contribution is the same than during base flow periods. In contrary to the model developed by Perrin (2003), the base flow and small flood discharge is attributed to the saturated LPV as highlighted by the immediate $CO_2$ increase and seasonal trend. Indeed the discharge can not be attributed to the epikarst otherwise the $CO_2$ increase should be delayed compared to the discharge.

In order to evaluate the degassing of the $CO_2$ in the cave atmosphere, continuous measurements of the $CO_2$ concentration in the cave atmosphere was conducted between July 1st and July 15th 2004 (Fig. 2.6c) in the Milandrine cave at the same location as the measurement station for the $CO_2$ concentration in the water. The $CO_2$ concentrations in the atmosphere are systematically lower than the values measured for the water making degassing of the dissolved $CO_2$ from the water to the cave atmosphere possible. Hence $CO_2$ values in the saturated LPV are likely higher compared to the river explaining the increase of the $CO_2$ concentration during small flood events. Moreover a small delay of the $CO_2$ increase in the cave atmosphere is generally observed compared to the $CO_2$ increase in the river consistant with a degassing of $CO_2$ from the water to the cave air. However the small lag between the two increases (1/2 hours) corresponds to the time between two measurements. In order to get more informations regarding the degassing of the $CO_2$ in the cave atmosphere, more detailed measurements with shorter time resolution should be carried out.

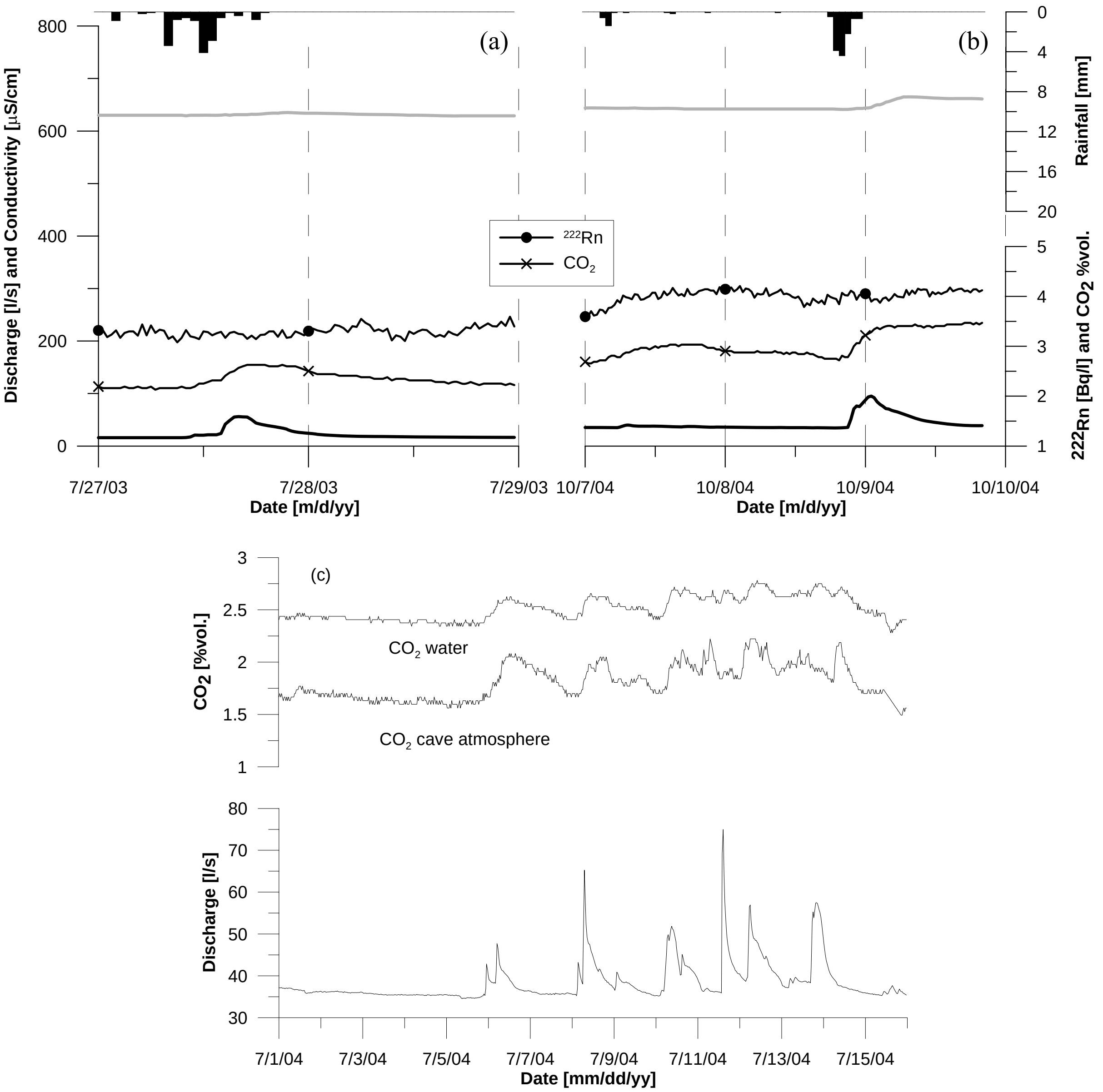


*Figure 2.6. Discharge (black line), electrical conductivity (grey line), $^{222}$Rn (line with points) and $CO_2$ (line with crosses) during small flood events in July 2003(a) and October 2004(b). $CO_2$ concentrations in the river and in the cave atmosphere (c)*

**Medium flood events**

On September 11th 2002, a flood event started after 21.2 mm rainfall during 3 hours and a second discharge peak was observed after 11.8 mm rain during 12 hours (Fig. 2.7a). Two days before the flood, 8.1 mm rainfall was measured but no flood was observed. The maximum discharge for the first peak (229 l/s) was observed about 9 hours after the maximum rain intensity. The $^{222}Rn$ concentration showed a significant increase of 0.9 Bq/l and the $CO_2$ concentration an increase by 0.9 % vol. from 2.5 % vol. to 3.4 % vol. The $CO_2$ increase coincides with the discharge increase. The $^{222}Rn$ increase was observed 8 hours after the beginning of the flood and the $^{222}Rn$ maximum 13 hours after the maximum flow of the second peak, during decreasing discharge.

On June 1st 2004 an increased in discharge by 100 l/s was observed after 26.3 mm rainfall throughout the day. 10 hours later a stronger rainfall (44.1 mm) led to a second increase of the discharge to a maximum value of 341 l/s (Fig. 2.7b). The first discharge increase was observed about 7 hours after the start of the first rainfall and the second discharge increase 6 hours after the second rainfall. During the first discharge increase a small $CO_2$ increase (2.4 to 2.7 % vol.) was observed. 9 hours after the beginning of the second flood, a $^{222}Rn$ increase is observed. The maximum $^{222}Rn$ concentration (4.94 Bq/l) was observed 16 hours after the maximum discharge of the second peak. Again the $CO_2$ increase coincides with the first discharge increase. The electrical conductivity is about 630 µS/cm and increases with the discharge.

In these two cases, with flow varying between 200 and 400 l/s, an immediate increase of the $CO_2$ concentration was observed with increasing discharge which also becomes apparent when $CO_2$ concentrations are plotted versus discharge (Fig. 2.7c and e). The increase in the $CO_2$ concentration was smaller in the June flood than in the September flood, which is likely due to higher $CO_2$ levels in soil and epikarst in September than in June. In contrast to the immediate $CO_2$ increase, the $^{222}Rn$ increase was delayed (Fig. 2.7d and f), indicating a delayed soil reservoir contribution to groundwater discharge during the flood. The simultaneous increase in electrical conductivity (Fig. 2.7b) with the $^{222}Rn$ during the discharge peaks suggests that the water received a higher mineralization during storage in the soil zone than base flow water in the saturated LPV, maybe due to carbonates dissolution in the soil zone. As for the model developed by Perrin (2003), during medium flood events a contribution of the soil and the epikarst to the discharge was observed

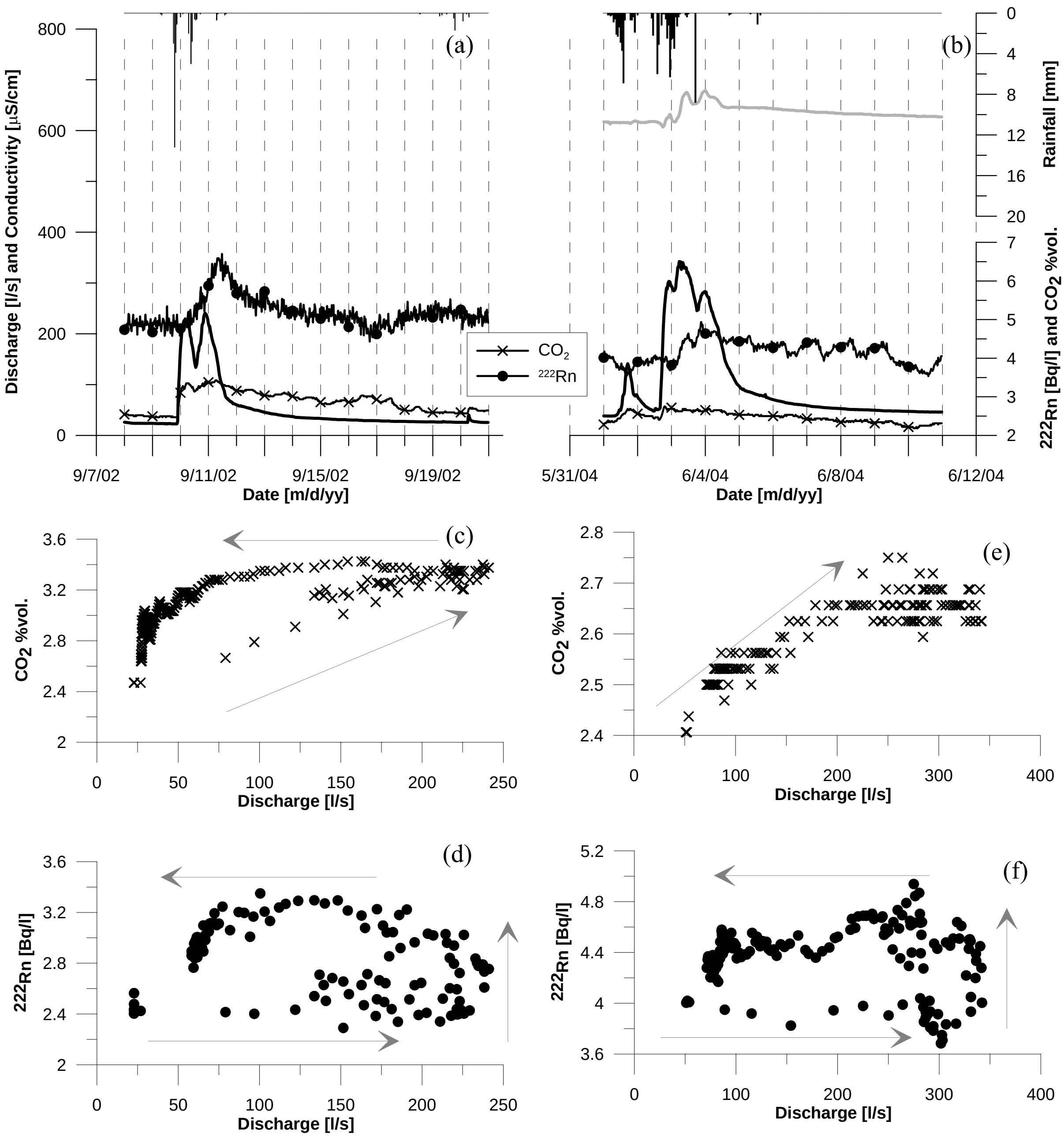


*Figure 2.7. Discharge (black line), electrical conductivity (grey line), $^{222}$Rn (line with points) and $CO_2$ (line with crosses) during medium flood events in September 2002 (a) and June 2004 (b). Hysteresis curve of discharge and $CO_2$ concentration for September 2002 (c) and July 2004 (e) flood events. Hysteresis curve of discharge and $^{222}$Rn concentration for September 2002 (d) and July 2004 (f) flood events.*

**Large flood events**

The October 16th 2002 flood event corresponds to a rainfall of 104.8 mm during 7 days (Fig. 2.8a). This event can be separated in four successive floods of increasing magnitude. The first discharge peak starts after 14.4 mm rainfall during 24 hours and reaches 172 l/s. A small $CO_2$ increase by 0.6 % vol. from 3.3 to 3.9 % vol. was observed while no $^{222}Rn$ increase occurred similarly to the small flood events described above. After 19 hours without rainfall, a series of three larger discharge peaks followed (max 515 l/s, 478l/s and 579l/s, respectively), caused by 90.4 mm of rainfall. Each of the three flood peaks was accompanied by an increase in the $^{222}Rn$ concentration, which occurred with a delay of 4-8 hours. The $CO_2$ increased by 0.2 % vol. (3.6 to 3.8 % vol.) immediately after beginning of the first large flood peak and then decreased again. The electrical conductivity decreased during the flood peaks from 625 µS/cm to 490 µS/cm.

The October 26th 2004 flood event corresponds to 84 mm rainfall during 2 days (Fig. 2.8b). This event can be separated in two successive floods of increasing magnitude each linked to a rainfall event. The first small rainfall event let to a slight $CO_2$ increase of 0.7 % vol. (3.1 to % vol.) but no $^{222}Rn$ increase is observed. During the second discharge peak, the $^{222}Rn$ concentration started to increase (from 3.3 to 10.1 Bq/l) 9 hours after the beginning of the flood and the maximum value (10.2 Bq/l) was reach 10 hours after the discharge maximum. Similarly to the October 2002 event, the electrical conductivity decreases during the flood event from 668 µS/cm to 488 µS/cm.

In both flood events, the $CO_2$ concentrations immediately increased during the flood event, while the reaction of $^{222}Rn$ lagged behind, indicating a delayed arrival of water from the soil zone similar as in the medium flood event. The $^{222}Rn$ remained elevated during the flood event indicating a continued contribution of soil water to flow. In contrast to the medium flood event, the electrical conductivity decreased during the flood events. For both events, minimal values were observed before the $^{222}Rn$ peak was reached. The decrease of the electrical conductivity, although a $^{222}Rn$ increase is observed, is likely due to a mixing of a small percentage of rainwater (low electrical conductivity and very small $^{222}Rn$ concentration) with rich $^{222}Rn$ soil water. Indeed considering the Ostwald coefficient (0.35) for the $^{222}Rn$ (see paragraph 2.1.1) and a mean $^{222}Rn$ value between 20 and 40 Bq/l in the gas phase of the soil zone (Fig. 2.3), the concentration of $^{222}Rn$ in soilwater should be around 7 and 14 Bq/l, near concentrations observed during the flood. Moreover, for both floods, the contribution of fresh infiltrated rainwater to the discharge can be evaluated between 20 to 25 % with:

$$EC_{min} = Q_{rain}*EC_{rain} + (1- Qr_{ain})*EC_{max}$$

Where:

$EC_{min}$ = minimal observed conductivity during the flood event

$Q_{rain}$ (%) = Contribution of fresh infiltrated rainwater to the discharge in percent

$EC_{rain}$ = electrical conductivity of the rainwater ~50 µS/cm

$EC_{max}$ = electrical conductivity measured before the flood

Rapid dissolution of 222Rn in fresh infiltrated rainwater during transit through the soil zone is also possible (Heinz Surbeck, personal communication) and can explain the decrease of the conductivity with the $^{222}$Rn increase.

Similar results of rainwater contribution (20-25%) to the discharge during large flood events were already observed by Perrin (2003).

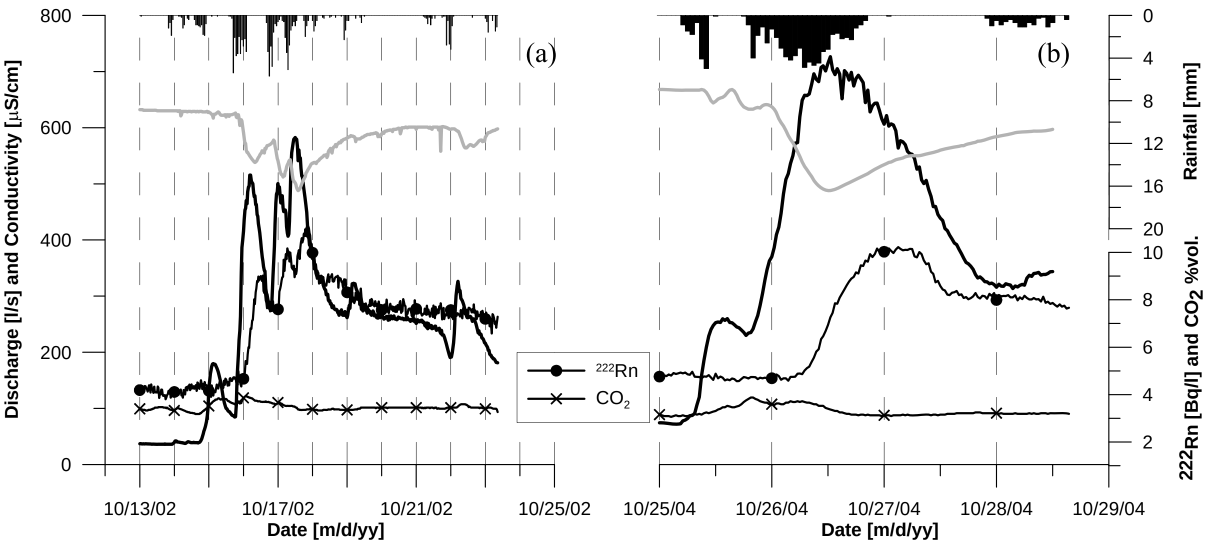


*Figure 2.8. Discharge (black line), electrical conductivity (grey line), $^{222}$Rn (line with points) and $CO_2$ (line with crosses) during strong large events in October 2002 (a) and October 2004 (b).*

### 2.1.5 Conclusions

The inclusion of $^{222}Rn$ and $CO_2$ measurements along with common hydrochemical parameters makes it possible to get a more detailed picture of the origin of water during baseflow and flood events. While classical parameters such as electrical conductivity are usually used to distinguish between rain and pre-event water arriving at a sampling point, $^{222}Rn$ and $CO_2$ values make is possible to characteristics the origin of the pre-event water in more detail. An increase in $CO_2$ without an increase in $^{222}Rn$ indicates that the water was stored at least 20 days (5 times half-life of $^{222}Rn$) in deeper zones while an increase in $^{222}Rn$ indicates a significant contribution of the soil zone to discharge. $^{222}Rn$ provides information on the location of storage because $^{222}Rn$ production is much larger in soil than in the limestone matrix and because $^{222}Rn$ decay with a half-life of 3.8 days. $CO_2$ concentrations indicate the origin of water because the $CO_2$ level during baseflow varies relatively little on an annual basis while water that originates from zones closer to the soil is more strongly affected by the annual fluctuation of the $CO_2$ concentration. Hence, especially during the periods of high $CO_2$ production in the soil, the arrival of water from the epikarst through the saturated LPV can be identified by an immediate increase of the $CO_2$ concentration in the saturated zone during flood events. Due to a variable $CO_2$ production, the response of this tracer varies seasonally. In addition, the $CO_2$ levels may be lower in the saturated LPV than in the epikarst due to consumption of $CO_2$ for carbonate dissolution during prolonged storage and degassing of the $CO_2$.

The application of the method at the Milandre test site demonstrated that during baseflow, the underground river discharge corresponds essentially to seepage flow from the LPV reservoirs and $CO_2$ concentration reflects an average annual buffered value. During baseflow, the $CO_2$ concentration in the river is lower than in the saturated LVP due to a degassing of the $CO_2$ in the cave atmosphere. Indeed the $CO_2$ concentrations in the cave atmosphere are lower than in the water making degassing of the dissolved $CO_2$ from the water to the cave atmosphere possible.

During small flood events, contribution of water from the LPV was observed as indicated by a constant electrical conductivity and a small immediate $CO_2$ increase. The $CO_2$ values in the saturated LPV are likely higher compared to the river and the small $CO_2$ increase is due to a fast contribution of the saturated LPV to the discharge with less time for degassing than during baseflow.

During medium flood events, no contribution of freshly infiltrated rainwater to the discharge was observed as indicated by a constant electrical conductivity. A delayed soil water contribution to the discharge is observed as demonstrated by the $^{222}Rn$ increase. During the recession phase, water from the soil zone contributes to the discharge during many days as indicated by the high $^{222}Rn$ concentrations. Finally during large flood events, rain water contributes between 20 and 25% to discharge similarly as observed by Perrin (2003).

These data confirm that soil and epikarst sub-systems act as important zone of groundwater storage (Chapman, 1992; Caballero et al. 1996). This conclusion is consistent with a study on oxygen isotopes carried out at the same site (Perrin et al., 2003), which also demonstrated a highly buffered reaction in the underground river and in springs compared to rainfall. Howerer the $^{222}Rn$ measurements allow to define if the soil water contribute or not to the discharge during flood events. Moreover, the contribution of the saturated LPV to the baseflow dicharge and small flood event is also expected by the $CO_2$ measurments. $^{222}Rn$ and $CO_2$ have also the advantage that they can be continuously measured. Thus more hydrological events can be investigated at less cost. While the study demonstrate the potential of $^{222}Rn$ and $CO_2$ as natural tracer, additional studies are necessary to characterize in more detail the input function of $^{222}Rn$ and how it varies as function of precipitation intensity.

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## 2.2. $^{222}$Rn under artificial recharge conditions

Three artificial sprinkling experiments were conducted at the Grand-Bochat test site in order to study the transport of the $^{222}$Rn enriched soil groundwater in the karst system at a local scale and identify the origin of water in the cave under artificial recharge conditions. The Grand-Bochat cave is well connected to the epikarst and soil zones and the influence of the unsaturated LVP to the discharge is low in contrast to the Milandre site. The sprinkling experiments were also conducted in order to compare the $^{222}$Rn variations (no $CO_2$ measurements) measured under natural conditions at Milandre test site during flood events with $^{222}$Rn variations measured under artificial recharge conditions. The sprinkling experiments were not conduct at the Milandre site due to the very long response time between sprinkling and discharge increase in the cave (more than 10 hours) and the difficulties to be in the cave at the right moment for sampling the discharge increase (see chapter 2.5). The tracing experiments were realized on 11$^{st}$ September 2002 (Savoy, 2002), 10$^{th}$ September 2003 and 31$^{st}$ August 2004.

### 2.2.1 Study area

As described in chapter 1, the Grand-Bochat cave (Swiss Jura, NE) is a small karst system with 15 meters unsaturated zone. The site is located in a forest and is covered by less than 0.4 m brown soils. The epikarst water is drained by a fissured media connected to the cave where observations were made. Measuring devices were installed on a single outlet. Flow in the unsaturated LPV is essentially controlled by the dip of the bedding planes (12°N) (Fig. 2.10a and b and 2.11).

*Figure 2.10a. Grand Bochat cave, sampling location with measurement devices.*

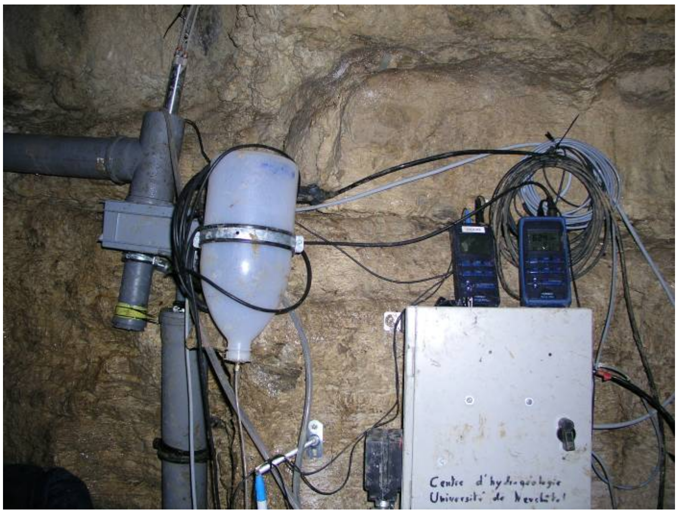


*Figure 2.10b. Grand Bochat cave, detail of the sampling location with measurement devices.*

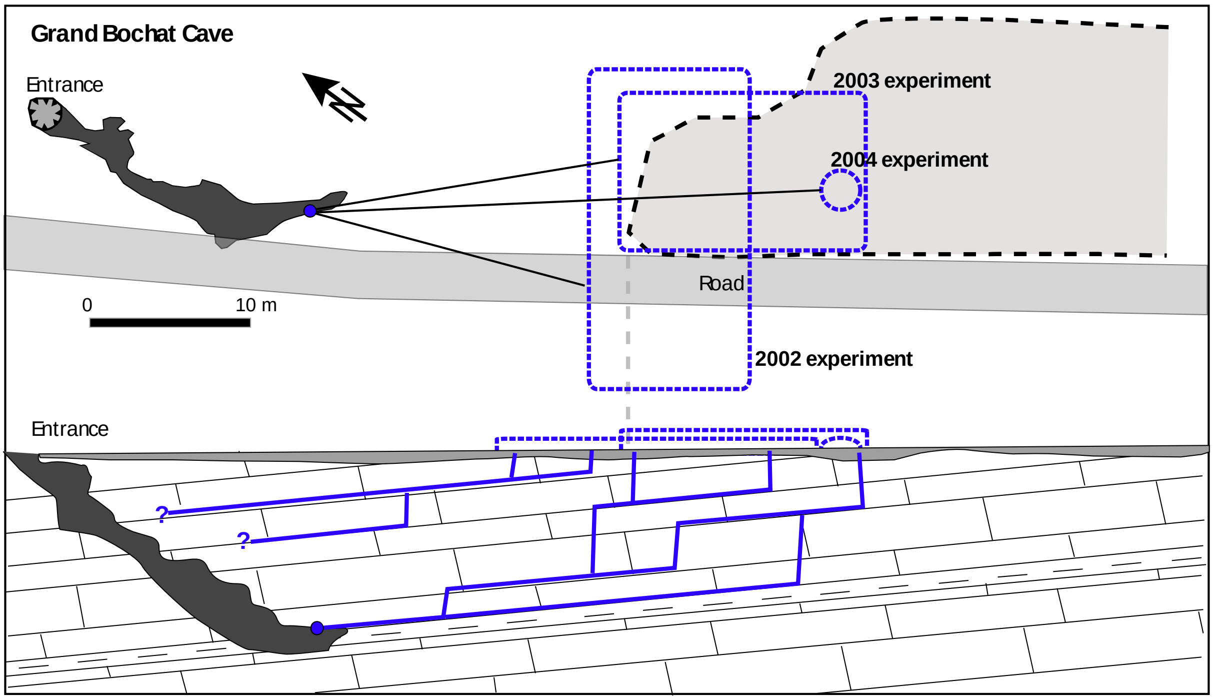


*Figure 2.11. Grand Bochat cave (plan and cross section).Catchement area (grey) and sprinkled area during 2002, 2003 and 2004 experiments (blue dashed lines).*

### 2.2.2 Experimental settings and Data acquisition

In order to create artificial rain conditions, an automatic rainmaking device was constructed with sprinkler traditionally used for garden watering (Fig. 2.12) to provide a uniform water flux to the soil surface. The sprinklers were installed 50 cm above the soil surface and the water distribution along the spray was assumed as uniform. Water tanks (4 $m^3$) supplied the water to the sprinkler device with a pump (for a detailed description of the sprinkling system, see chapter 3). For these experiments, natural rainwater with low conductivity (~40 µS/cm) was used for sprinkling except for the first test (spring water). The experiments were carried out during base flow periods to avoid perturbation due to rainfall events. A relative constant discharge intensity was applied onto the soil surface and controlled by adjusting the flow rate with a valve and a flow meter. The experiments were conducted with different flow rates and different irrigation surfaces. Water sampling took place at 15 meters depth in the cave on a conduit at the cave roof. Discharge was measured in a perforated PVC tube equipped with pressure probe. The discharge is obtained from the water level in the tube after the determination of the water level-discharge relationship from gauging experiments. The conductivity was measured and recorded every 5 minutes using portable field conductivity meter (WTW) with internal data logger. 40 ml samples for analyses of $^{222}Rn$ were manually collected in glass vials (PTFE/silicone septum cap). In the laboratory, as for samples collected under natural conditions (see paragraph 2.1.3) $^{222}Rn$ was extracted with an organic solvent/scintillator cocktail (Maxilight, Hidex Oy, Finland) and after 3 h, $^{222}Rn$ and $^{222}Rn$ decay products were determined by liquid scintillation counting (LSC).

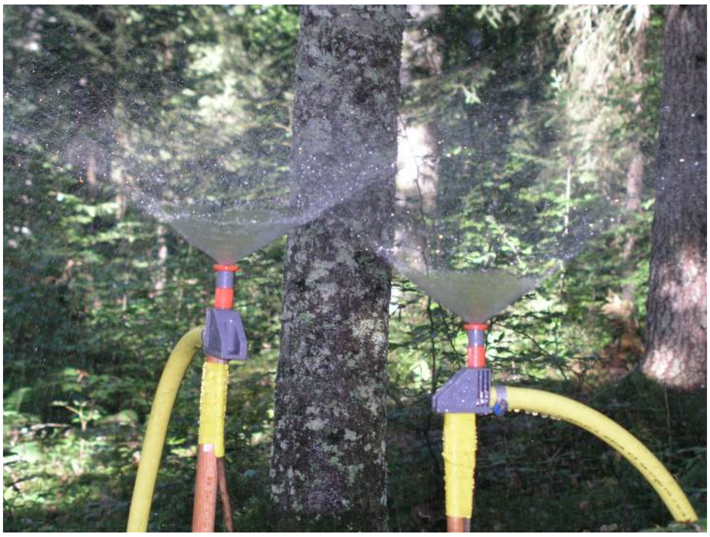

*Figure 2.12. Rainmaking device constructed with sprinkler traditionally used for garden watering.*

## 2.2.3 Results and discussion

### *Experiment 1*

On September 11th and 12th 2002, a 40 l/min mean flow rate was applied onto the soil surface during 6 hours separated by 3 short interruptions. The watering surface was 200 $m^2$ and the injection rate corresponds to a 12 mm/h rainfall event. The $^{222}Rn$ concentration in the injected water was below 0.8 Bq/l. The first flow increase in the cave was observed 33 min after the beginning of the sprinkling (Fig. 2.13). Due to a dysfunction of the pressure probe, the discharge data during the experiment are missing but punctual manual gauging was carried out. The discharge was 10 l/min and corresponded to a quarter of the sprinkling rate. The $^{222}Rn$ concentration before the discharge increase was 8.5 Bq/l for the percolating water and the initial electrical conductivity 584 µS/cm. The three small electrical conductivity increases are linked to the interruption of the irrigation. With the increasing discharge in the cave, an increase of the $^{222}Rn$ was observed for the first two samples. Seven minutes after the increase of the discharge, a maximum $^{222}Rn$ value of 12.2 Bq/l was measured and then $^{222}Rn$ started to decrease in parallel with the electrical conductivity (injection water ~40 µS/cm). The $^{222}Rn$ reached a value of 9 Bq/l and stayed around during the rest of the experiments.

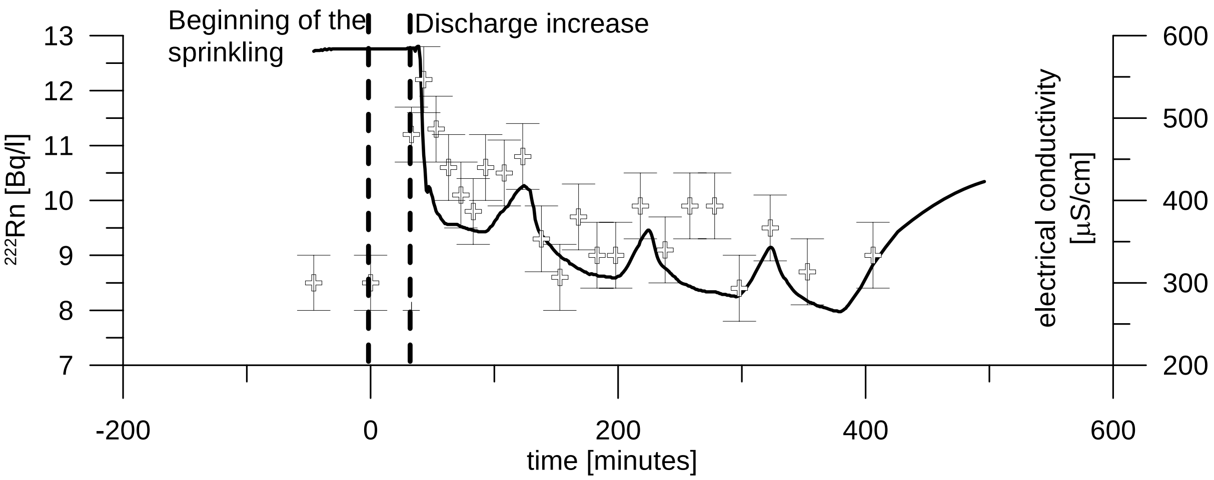


*Figure 2.13. Experiment 1, September 2002. White crosses represent $^{222}Rn$ concentration and black line the electrical conductivity. Discharge is missing but the initial discharge increase in the cave and the beginning of the sprinkling are represented by the vertical black dashed lines. The three local successive electrical conductivity increases are linked to the interruption of the irrigation on the soil surface*

### *Experiment 2*

On September 3rd 2003, a mean flow rate of 18 l/min was applied onto the soil surface (100 $m^2$) during 166 min, corresponding to a rain intensity of 10.8 mm/h. The first flow increase in the cave was observed 24 min after the beginning of the sprinkling and the electrical conductivity decrease 9 min after flow increase (Fig. 2.14). The mean recovery discharge was 8.5 l/min and corresponds to 48% of the injected discharge. With the increasing discharge in the cave, an increase of the $^{222}Rn$ was observed for the three first samples. $^{222}Rn$ concentration for the percolating water before the experiment was 5.8 Bq/l and 9 minutes after the initial discharge increase, a maximum $^{222}Rn$ concentration of 9 Bq/l was measured corresponding to the beginning of the electrical conductivity decrease. The $^{222}Rn$ value decreased in parallel with the electrical conductivity. The sprinkling was stopped 166 min after the beginning of the experiment and a $^{222}Rn$ and electrical conductivity increase were observed.

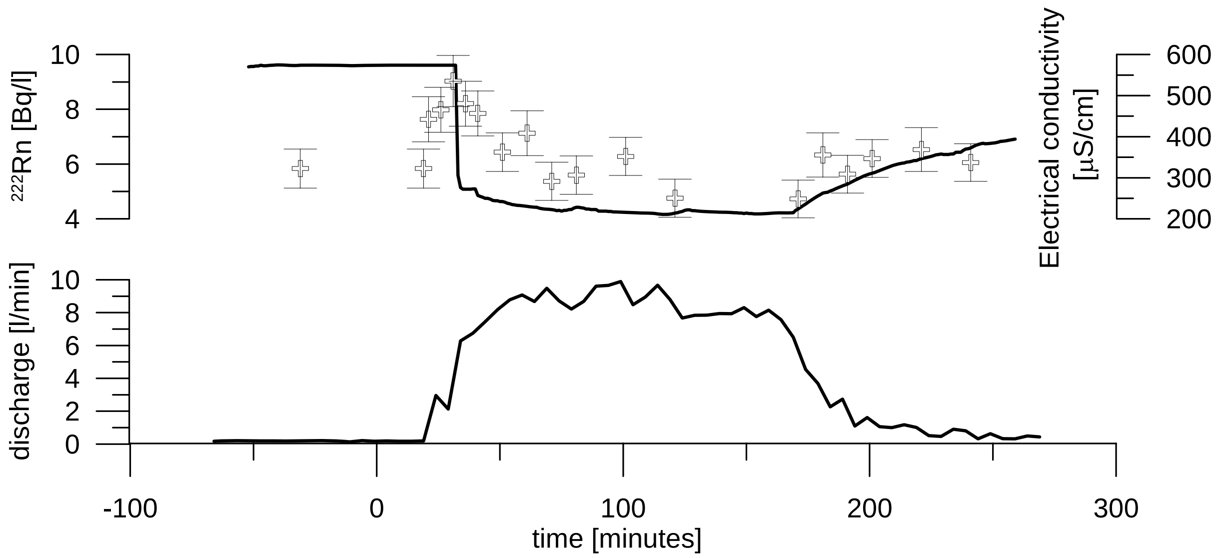


*Figure 2.14. Experiment 2, September 2003. White cross represent $^{222}$Rn concentration and black lines the electrical conductivity and the discharge. The initial discharge increase is represented by the vertical black dashed line.*

## *Experiment 3*

On August 31$^{st}$ 2004, a constant flow rate of 4 l/min was applied onto the soil surface (15 m$^2$) during 660 min corresponding to a rain intensity of 16 mm/h. The first flow increase in the cave was observed 40 minutes after the beginning of the experiment and the electrical conductivity decreased 10 min after the flow increase (Fig. 2.15). The mean discharge was 3.5 l/min and corresponds to 88% of the injected discharge. The initial electrical conductivity of the percolating water was 599 µS/cm and 120 minutes later a value of 360 µS/cm was measured. 190 minutes after the discharge increase a strong increase of the electrical conductivity is observed due to a pulse injection of salt for a parallel experiment (data not discussed). In parallel with the initial discharge increase, a $^{222}$Rn increase was observed. The $^{222}$Rn concentration of base flow water was 6.9 Bq/l and a maximal value of 9.3 Bq/l was reach 44 minutes after the flow increase. Elevated $^{222}$Rn concentrations were observed during 50 minutes before a decrease was observed.

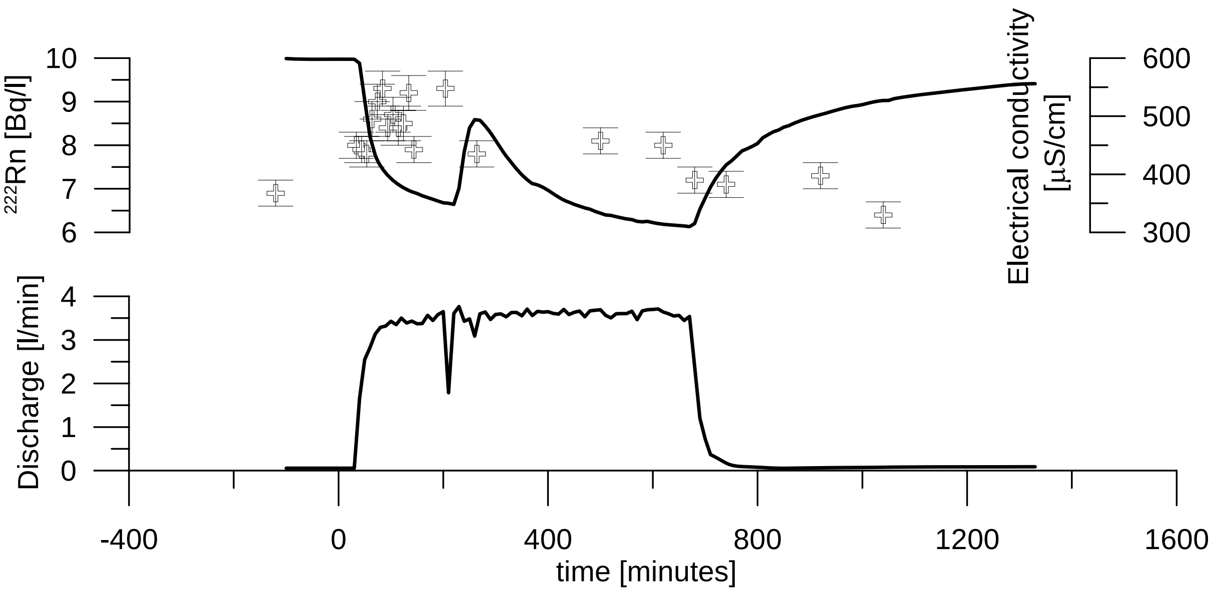


*Figure 2.15. Experiment 3, August 2004. White cross represent $^{222}$Rn concentration and black lines the electrical conductivity and the discharge. The initial discharge increase is represented by the vertical black dashed line.*

The variability of the water recovery can be explained by water losses to other groundwater catchments during the experiments depending on the location of sprinkled areas as indicated by multiple tracer tests carried out before the sprinkling experiments (Fig. 2.11). For the third experiment, the totality of the sprinkled zone was located in the catchment area of the sampling location and 88 % of the injection discharge was recovered. The ratio of the recovered discharge (%) during experiment 2 and 3 versus the sprinkled surface (%) located in the catchment area of the sampling location are proportional to the recovered discharge during experiment 1. For the second experiment, 48 % of the injection discharge was recovered and 50% of the sprinkled area was located within the catchment area (effective discharge recovery: 48/50=96%). For the first experiment, approximately 25% of the injected water was recovered and 30 % of the sprinkled area was located within the catchment area of the sampling location (effective discharge recovery: 25/30=83%).

For the artificial experiment at the Grand-Bochat test site, a similar conceptual model of water mixing and contribution to the discharge than for $^{222}$Rn measurements under natural conditions on Milandre cave can be applied (see chapter 2.1.5). At baseflow condition, before starting the sprinkling, an elevated $^{222}$Rn concentration was observed. This value is due to the influence of the water from the soil zone with not totally decayed $^{222}$Rn. Several studies (Puech et Bourret 1998, Madec 1999, Perrin 2003) have demonstrated that the storage in

Grand-Bochat site was essentially located in the epikarst and soil zones in contrary to the Milandre cave where storage during base flow was located in the saturated LPV and epikarst (see chapter 2.1.4). For Milandre test site, under natural conditions, the sustained presence of $^{222}$Rn during baseflow despite its short half-life indicates that some production of $^{222}$Rn occurs in the limestone as well. In the case of Grand-Bochat, the contribution of the LPV to the discharge can be neglected.

Under imposed artificial rain conditions, in a first step, an increase of the discharge is observed in parallel with an increase of the $^{222}$Rn concentration. This increase of the $^{222}$Rn concentration corresponds to an increase of the soil water contribution to the discharge. In a second step, a delayed electrical conductivity and $^{222}$Rn decrease is generally observed corresponding to the arrival of artificial rainwater with low electrical conductivity and $^{222}$Rn concentration. The delay between the increase of the $^{222}$Rn and the decrease of the electrical conductivity corresponds to a piston effect. Soil water is pushed into the epikarst reservoir, and the increasing hydraulic stress on the epikarst causes an increase of the discharge in the conduits.The result of the 2002, 2003 and 2004 experiments indicate a piston duration of respectively 7, 9 and 10 minutes. Perrin (2003) found similar results for the piston duration (12 minutes) under natural rainfall event at Grand-Bochat test site. Elevated infiltration rates will decrease the duration of the piston effect. The discharge during the piston effect corresponds to a mixing of water from the epikarst zone and soil zone containing more elevated $^{222}$Rn compared to the water measured during base flow only attributed to the epikarst zone. For the third experiment, in contrary to the 2002 and 2003 experiments, the $^{222}$Rn values stayed elevated during a long period. The elevated $^{222}$Rn values measured during more than 50 minutes can be due to a contribution to discharge of the diffuse part of the infiltrated water in the soil zone. Due to the elevated recharge, matrix water of the soil zone becomes completely mobile and can mix very effectively with fresh water. Perrin et al. (2003) explained this effect by an exceeding of the soil water storage capacity during recharge events. The hydraulic conductivity increases drastically and a part of the fresh water bypasses the soil reservoir through macropores.

### 2.2.4 Conclusion

The experiments carried out at Grand-Bochat test site confirm that the upper part of the unsaturated karst system (the soil and epikarst zone in this case) can store an elevated quantity of water. A similar conceptual model to this developed for the Milandre cave can be used, with water storage in the soil zone and contribution of this subsystem to the discharge during rainfall events. In the case of Grand Bochat cave, the contribution of the LPV to the discharge is neglected.

In conclusion $^{222}$Rn is a good natural tracer for soil water in karst systems. For karst system with thin unsaturated zone as Grand-Bochat cave, an elevated $^{222}$Rn concentration during base flow will generally indicate a contribution of soil water to the discharge. For karst systems with tick unsaturated zone, $^{222}$Rn during base flow is generally attributed to limestone production. In case of flood events a $^{222}$Rn increase will indicate a contribution of soil water to the discharge.

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## 2.3. Total organic carbon under natural recharge conditions[1]


**Abstract**

Total organic carbon (TOC), a natural tracer originating from the soil, was measured continuously in parallel with common hydrochemical parameters and two natural tracers also produced in the soil zone, radon ($^{222}Rn$) and carbon dioxide ($CO_2$). The measurement of these parameters makes it possible to get a detailed picture of the origin and flow paths of water and provide information about the dynamics of solute transfer across the unsaturated zone. In a karst aquifer characterized by diffuse infiltration and thick soil cover, a delayed increase of the TOC and electrical conductivity with respect to discharge was observed with constant 222Rn values. The constant $^{222}Rn$ level suggests that the water was stored at least 20 days (5 times half-life of $^{222}Rn$) in deeper zones and that no water from the soil zone contribute to the discharge. The constant TOC and electrical conductivity levels during the discharge peak indicate that the water contributing to the discharge corresponded to the water of the base flow. The delayed TOC and electrical conductivity increase observed several days after the small flood events is likely due to an indirect contribution of epikarst water via the low permeability volumes. For stronger rainfalls, a decrease of the delay in TOC increase occurs, due to a significant contribution of the epikarst zone to the discharge via preferential flow paths. The increase of the $^{222}Rn$ is likely due to a contribution of water from the soil zone to the discharge and the decrease of the electrical conductivity indicates that freshly infiltrated water contributes to the discharge. A second TOC increase is generally observed once the discharge had reached base flow values again, due to the delayed contribution of the epikarst through the LPV.

These results indicate that the soil and epikarst zones have a highly buffer and storage capacity. The study demonstrates the potential of TOC as natural tracer. TOC has the


[1] **TOC as natural tracer for flow separation in the unsaturated zone of karst systems**

Ludovic Savoy; Daniel Hunkeler

*Paper prepared for Journal of Hydrology*

advantage that it can be continuously measured and more hydrological events can be investigated at less cost.

**Keywords**

Karst hydrology, soil, epikarst, TOC, unsaturated zone.

### 2.3.1 Introduction

Recent karst studies, based on natural tracers, have revealed the importance of the upper part of the unsaturated zone (soil and epikarst) as buffer and water storage location in karst aquifers (Perrin, 2003; Vesper and White, 2004). During flood events, freshly infiltrated water is mainly stored in the soil and epikarst zone, although a large discharge peak is observed in the cave and a delayed contribution of the soil water to the discharge is observed in the saturated zone. These studies allow to define the location of the groundwater storage in the upper parts of the unsaturated zone, but they can not explain the distribution of flows between the drainage conduits and the fractured limestones. This study focuses on the use of total organic carbon (TOC), coupled with electrical conductivity, as natural tracer to gain insight into the groundwater storage and transport in the unsaturated zone of the karst system.

Flow in karst systems is characterized by a duality (Smart and Friederich, 1986; Ford and Williams, 1989; White, 1988) of the infiltration conditions (diffuse and concentrated recharge); storage condition (saturated zone and unsaturated zone) and flow condition (diffuse and concentrated flows). This specificity leads to a high variability of the flow and transit time with variable geochemical signature. In karst systems, groundwater flow is generally separated between concentrated quick flow in the drainage conduits and seepage flows in the fractured limestone and the matrix considered as low permeability volumes (LPV) (White, 2001; Worthington et al., 2004; White, 2005). According to Worthington et al. (2000) and White (2005), the main portion of the storage takes place in the fractured limestones, although the main portion of the flow takes place in the drainage galleries. In dense and low permeability limestones, the matrix flow is negligible (White, 2005) and discharge consists of a mixture of flow through the drainage conduits and the LPV of the saturated zone. Several studies have demonstrated the high spatial and temporal variability of hydraulic response of water inlets of the unsaturated zone (Smart and Friederich, 1986; Destombes et al., 1997; Delannoy et al., 1999; Sanz and Lopez, 2000; Perrette et al., 2001; Perrin, 2003). Perrin (2003) identified 3 types of flows depending of the hydraulic response, drainage conduit flows for nervous hydraulic responses, seepage flows through the LPV of the unsaturated zone for the dampened responses and intermediate flows for a combination between the two types of flow. The repartition of water during infiltration between drainage conduits and LPV of the saturated zone was calculated by Jeannin (1996) for the Milandre test site. Several methods (boreholes observations, base flow evolution, water balance) allowed to estimate that around 50% of the infiltrated water during a rain event participates to the recharge of the LPV of the saturated zone (Jeannin, 1996). Based and discharge measurement in the saturated zone and Jeannin (1996) and Charmoille (2005) have demonstrated the importance of the LPV in the Jura karst systems for the prolonged storage of groundwater and their possible participation to the discharge.

Natural tracers that originate from the soil zone and diminish in deeper parts of the unsaturated zone are particularly useful in order to provide information about the dynamics of solute transfer across the unsaturated zone. These substances are potentially sensitive indicators for water storage in different subsystems (soil, epikarst, low permeability volumes) of the unsaturated zone and may indicate the travel time of the recharge water due to their decay below the soil zone. Several authors (Emblanch, 1997; Bakalowicz, 2003; Batiot, 2002; Pronk, 2006) demonstrated that the total organic carbon (TOC), produced in the soil zone and degraded and transformed by microbial activity in the unsaturated zone, is a good indicater of the transit time of water and flow conditions in the karst system. The TOC content in soil water can reach 300 mg/l, whereas it rarely exceeds 100 mg/l in river water and 1 mg/l in groundwater (Thurman, 1985). The TOC in groundwater is a mixture of diverse organic compounds often arbitrarily subdivided according to their size. The division is based on filtration through a 0.45µm filter. The dissolved organic carbon (DOC) regroups molecules smaller than 0.45µm such as humic and fluvic acids and short-chained hydrocarbons. The particulate organic carbon (POC) regroups macromolecules and particles with a size more than 0.45 µm. Generally in natural water, POC is found in smaller abundance than TOC, accounting typically for approximately 10% of the total organic carbon (Thurman, 1985; Aiken, 2002; Batiot, 2002).

The aim of this study was to evaluate if continuous TOC measurements provide information on the dynamics of solute transfer from the soil zone across the unsaturated zone. The study was carried out at the Milandre test site during year 2004. The concentration of the TOC was indirectly measured in the underground river using fluorescence with a field fluorometer generally used for continuous measurement of artificial dye tracers. For comparison and to aid in data interpretation, a number of different parameters (discharge, electrical conductivity, $^{222}$Rn and $CO_2$) were measured as well.

## 2.3.2 Material and methods

### *Field site description*

See chapter 1.4.1 and 2.1.2

### *Sampling and Data acquisition*

Between September 2003 and September of 2004, the most upstream part of the Milandrine underground river (Fig. 2.1) was equipped with a continuous recording station measuring TOC concentrations in parallel with discharge, electrical conductivity, water temperature and two complementary natural tracers, radon ($^{222}Rn$) and carbon dioxide (CO2) (see annex 1 for complete chronicles). Continuous water level and electrical conductivity measurements were carried out with pressure and electrical conductivity probes. The discharge was calculated from the water level using the water level-discharge relationship obtained from punctual gauging experiments. For continuous $^{222}Rn$ and $CO_2$ measurements in water, a closed circuit of air-filled semipermeable (polypropylene) tubing was immerged directly into the water of the river (Surbeck, 1996). The detailed results of the $^{222}Rn$ and $CO_2$ measurement are presented in chapter 2.1.

For the TOC measurement a field fluorometer (GGUN-FL30, University of Neuchâtel, Switzerland), initially developed in order to carry continuous measurement of artificial dyes, was used (Schnegg and Bossy, 2001; Schnegg and Costa, 2003; Flynn et al., 2005). The fluorometer is equipped with four excitation and detection systems, allowing the simultaneous measurement of three tracers and an independent turbidity measurement. Each excitation-detection system is composed of an excitation LED (light emitting diode) set on one axis, whereas a photodetector is mounted on another axis at 90 degrees and measuring the intensity of emitted fluorescence by the excited tracer. The measured fluorescence by the photodetector is filtered by an optical filter. The light sources and filters are selected according to the absorption-emission spectra of artificial dye presenting different optical properties. The turbidity signal is determined by measurement of the scattered light from the excitation source. The wavelength for turbidity measurement is located in the red range apart from the wavelength from tracer fluorescence in order that the emitted light can not generated fluorescence if a tracer is present in the water (Schnegg, 2003). For the TOC measurement, a light source for the tinopal artificial tracer detection, a 370 nm UV LED, was used for the

TOC excitation. The fluorescence was measured between 440 nm and 540 nm using a photodetector.

Several studies have investigated the fluorescence dissolved organic matter in rivers and marine waters (e.g. Senesi et al., 1991; Coble et al., 1993; Mobed et al., 1996; Baker and Genty, 1999; Baker, 2001; Batiot, 2002). These authors have studied fluorescence of organic carbon by spectophotometry using excitation/emission matrix (EEM). These studies have revealed the presence of fluorescence intensity peaks for an excitation between 220 and 400 nm and a measured emission beetwen 300 to 500. As describe in Batiot (2002), a maximal fluorescence intensity of TOC in karstic springs for an excitation-emission value of 346 / 439 nm is observed.

The 370 nm UV lamp used for TOC excitation in this study is relatively near this value. Batch samples were taken in Milandre cave in 100 ml glass vials under variable discharge conditions along the year (PTFE/silicone septum cap) and analysed by 680°C catalyst-aided combustion and non-dispersive infrared detection method. The results of the batch samples were used to calibrate the fluorescence intensity measured by the field fluorometer (Fig 2.16).

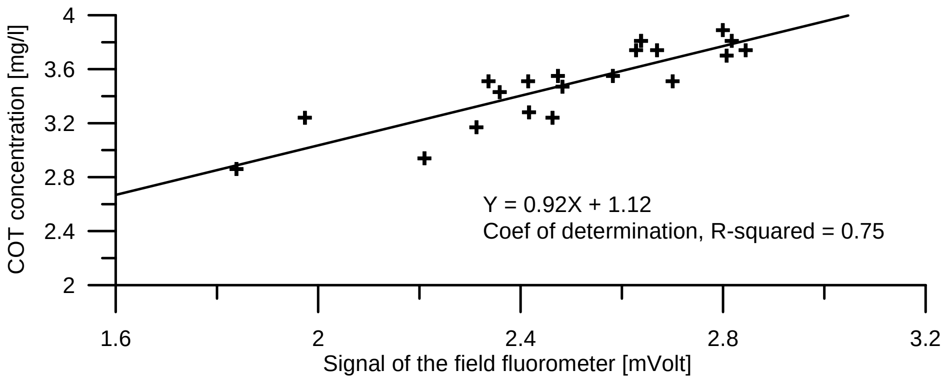


*Figure 2.16. Calibration of the field fluorometer for TOC measurement*

### 2.3.3 Results

The results were separated in different groups depending on discharge: 1) annual baseflow conditions 2) small flood events with discharge less than 150 l/s; 3) Larger flood events with discharge up to 150 l/s.

#### *Annual TOC evolution*

Figure 2.17 illustrates continuous TOC measurements over one full year. The TOC concentration varied between 1 mg/l and 7.6 mg/l. The highest concentration peaks were observed between October and November 2003 and between May and September 2004 (end of measurement) although discharge peaks were as high or higher during other periods of the year. When taking into account only the TOC concentrations during base flow, again a seasonal trend is observed with higher TOC values during summer and the lowest values in February and March as illustrated by a regression line through the baseflow values (Fig. 2.17). The higher TOC peak amplitude and baseflow concentrations in summer are likely due to a higher TOC production in the soil zone. According to chapter 2.1.4, a similar seasonal trend during baseflow was observed for the $CO_2$ concentration in the river (Fig 2.17). Regarding the TOC fluxes in the underground river, a constant level is observed throughout the year, although minimal TOC concentrations are observed in the river during winter (Fig. 2.16). This effect is due to the elevated base flow observed in the river during winter when $CO_2$ and TOC concentrations are lower than during summer. As for the $CO_2$ concentrations during base flow conditions (see paragraph 2.1.4) the information contained in the TOC concentration signal is completely masked by the discharge. The TOC fluxe allowed to observe that when the TOC concentrations are elevated and baseflow discharge low, during the end of summer, the mass of transported TOC is similar than the value observed during winter when more elevated baseflow discharges and smaller TOC concentrations are measured. However when the contribution of the different subsystem to the discharge is researched, a model using concentrations appears to be more adapted.

In the following, TOC concentration variations are presented and discussed in more detail for shorter time periods according to the intensity of flood events.

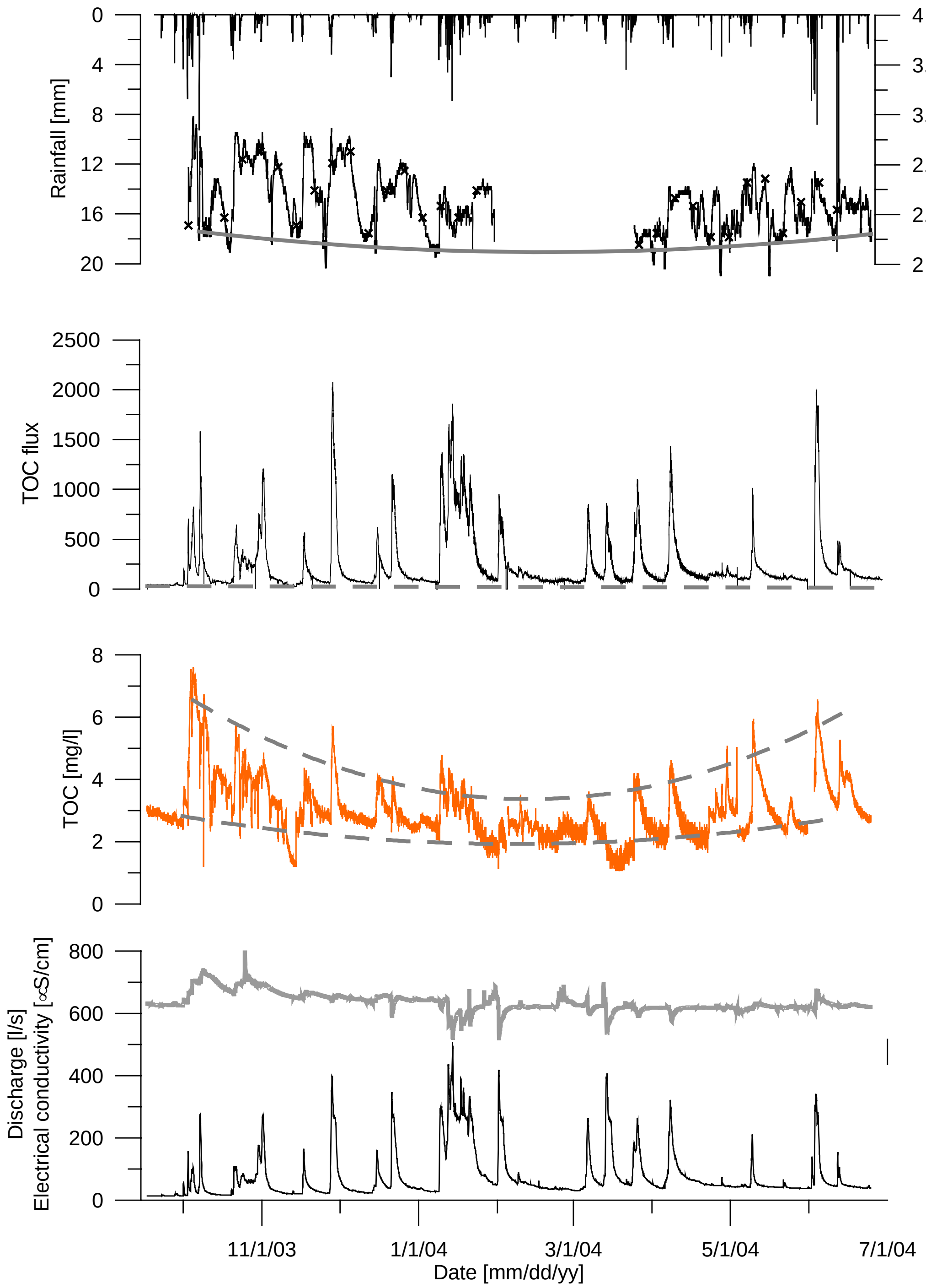


*Figure 2.17. Comparison of the annual TOC concentration and flux in the Milandrine river with discharge, electrical conductivity and $CO_2$ concentration. The curved grey lines are regression lines through the $CO_2$ and TOC baseflow values and TOC peak maximal values.*

## *Small flood events*

During July 2004 a prolonged base flow period occured during more than 30 days (55.9 mm rainfall in 30 days). During this period, a succession of ten small flood events was observed during 20 days between 5th July and 25th July (Fig. 2.18). The maximum discharge event reached 140 l/s, three events reached a maximum discharge comprised between 65 and 75 l/s and the 6 other event reached less than 55 l/s maximum discharge. The base flow discharge was 35 l/s. For all these events a delayed and smoothened increase of the

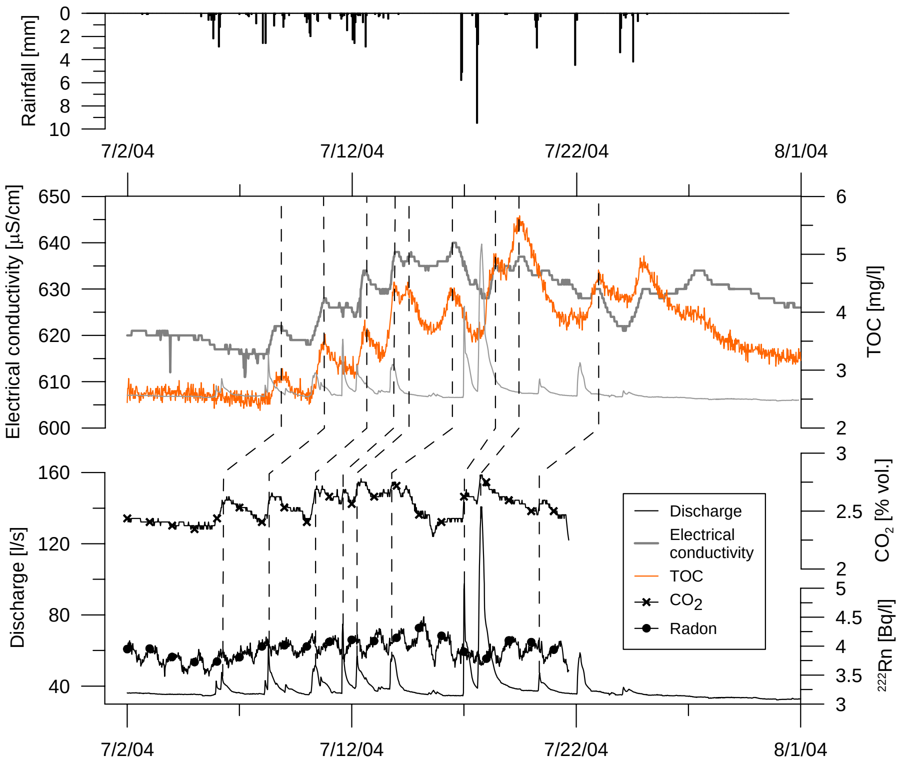


*Figure 2.18. Comparison of the TOC concentration in the Milandrine river with discharge, electrical conductivity and $CO_2$ concentration. The dashed black lines linked the discharge and $CO_2$ peaks to the corresponding TOC and electrical conductivity peaks.*

TOC and the electrical conductivity is observed. For each successive TOC increase a higher concentration is reached. The delay of the TOC increase varies between 1.5 day and 3 days depending on the flood intensity. The higher the discharge, the shorter is the delay (Fig. 2.18). With the discharge increase a simultaneous $CO_2$ increase was observed without variations of the $^{222}$Rn concentrations ($^{222}$Rn data not represented).

Several other small flood events showed similar results as illustrated for two flood events with 75 l/s (Fig. 2.19 a, April event) and 68 l/s (Fig. 2.19 a, Mai event) discharge, and for two very small flood events with 10 l/s discharge (Fig. 2.19 a, June events). During all of these events, little variations in TOC, electrical conductivity and $^{222}$Rn were observed during the flood peak, except for a small TOC reaction in the April event (Fig. 2.19a) and an immediate small increase of $CO_2$ for all events. An increase of the TOC concentration together with the electrical conductivity only occurred once the discharge had reached baseflow values again. Similarly as discussed above, the delay in TOC response increased with decreasing flood intensity with 36 hours delay for the April event (75 l/s), 42 hours for the Mai event (68 l/s), and 69/64 hours for the June events (10 l/s).

### *Larger flood events*

The June 11$^{th}$ 2004 flood event (Fig. 2.20) can be separated in two successive floods of decreasing magnitude. The first discharge peak started 2 hours after 22.4 mm rainfall during 5 hours and reached 154 l/s. The second discharge peak started 14 hours later after 15.8 mm rainfall during 4 hours and reached 105 l/s. During the initial discharge increase of this double event, little variations in TOC, electrical conductivity and $^{222}Rn$ are observed, except for an immediate small increase of $CO_2$. A double increase of the TOC concentration is observed during the later phase of the flood peak. The first TOC increase from 3.2 mg to 5.2 mg/l was observed 16 hours after the first discharge increase and a second TOC increase 27 hours after the second discharge increase. In parallel to the first discharge increase a very small $CO_2$ increase by 0.25 % vol. from 2.35 to 2.6 % vol. was observed while no significant $^{222}Rn$ variations occurred similarly to the small flood events described above. $^{222}Rn$ and $CO_2$ data are missing for the second peak, but no significant increase is expected during the peak discharge considering the relative constant concentrations during the recession period. Similarly as for the small flood event, the first double TOC increase is followed by a delayed increase of TOC concentration once the discharge had reached baseflow values again, more than 70 hours after the beginning of the flood event.

The December 2003 flood event showed (Fig. 2.21) a discharge increase by 310 l/s, 5 hours after the start of the rainfall (22mm/day). A rapid TOC increase from 2.8 to 4.1 mg/l was observed, in parallel with a $^{222}Rn$ increase and an electrical conductivity decrease, 6 hours after the beginning of the discharge increase. The maximum TOC (4.1 mg/l) concentration was observed 9 hours after the maximum discharge peak and the maximum $^{222}Rn$ (4.94 Bq/l) 17 hours later. As for the June flood event describe above, a second TOC increase once the discharge had reached baseflow values again. During this flood event, the first TOC increase is accompanied by a $^{222}Rn$ increase and electrical conductivity decrease.

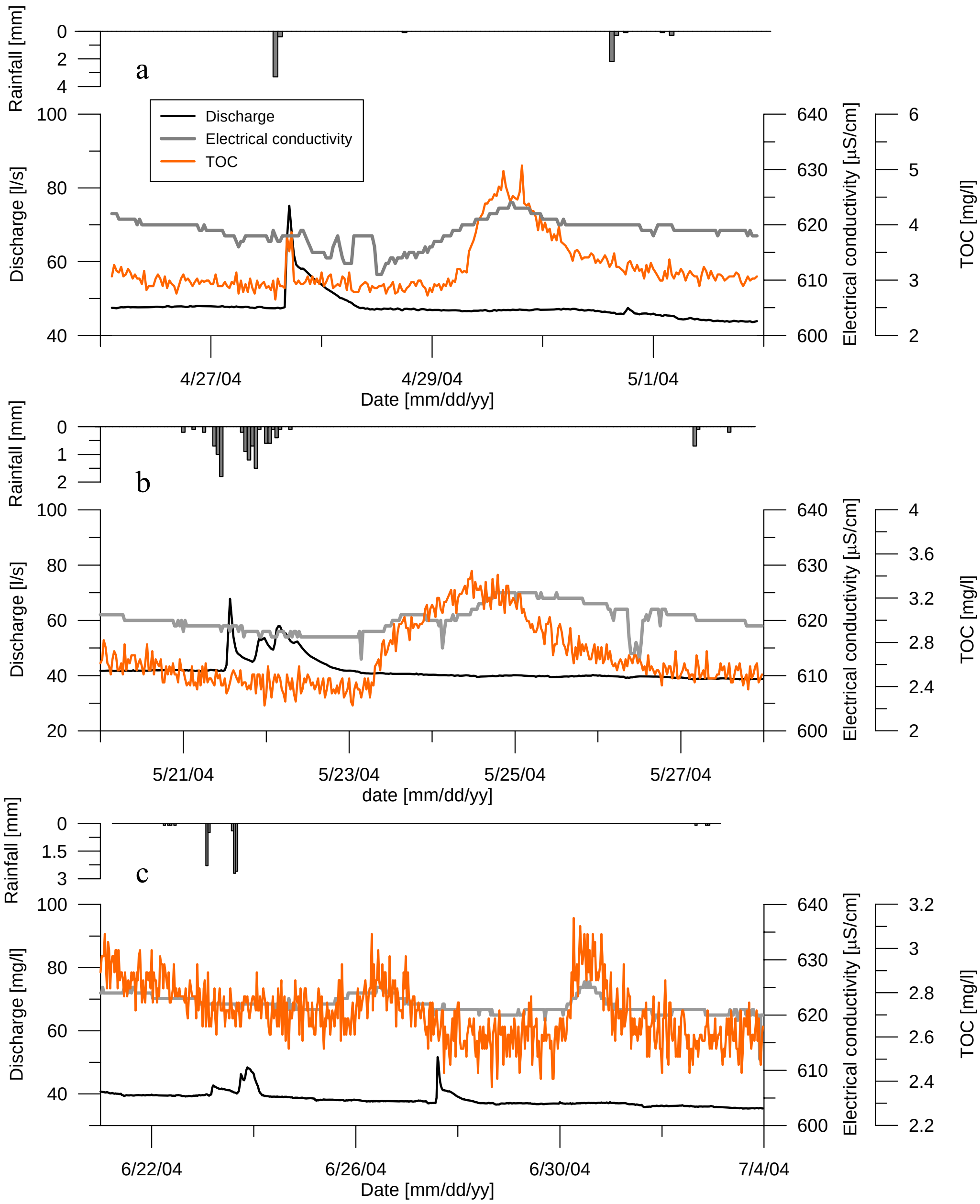


*Figure 2.19. a) April ,b) May and c) June small flood events. TOC concentration in the Milandrine river, discharge, electrical conductivity and $CO_2$ concentration.*

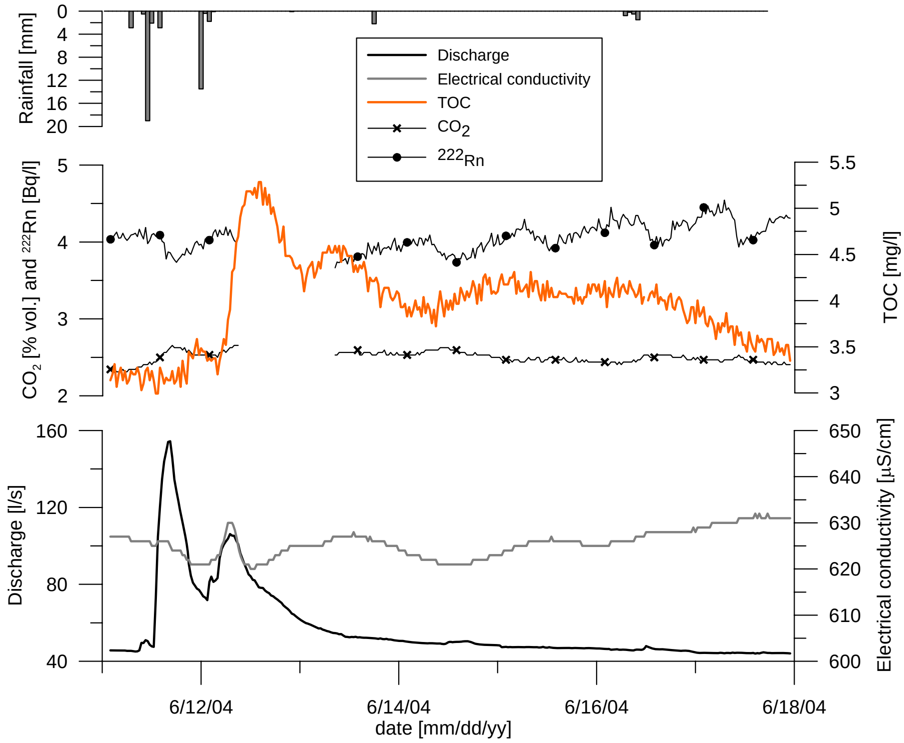


*Figure 2.20. June 2004 flood event. Comparison of the TOC concentration in the Milandrine river with discharge, electrical conductivity and $CO_2$ concentration*

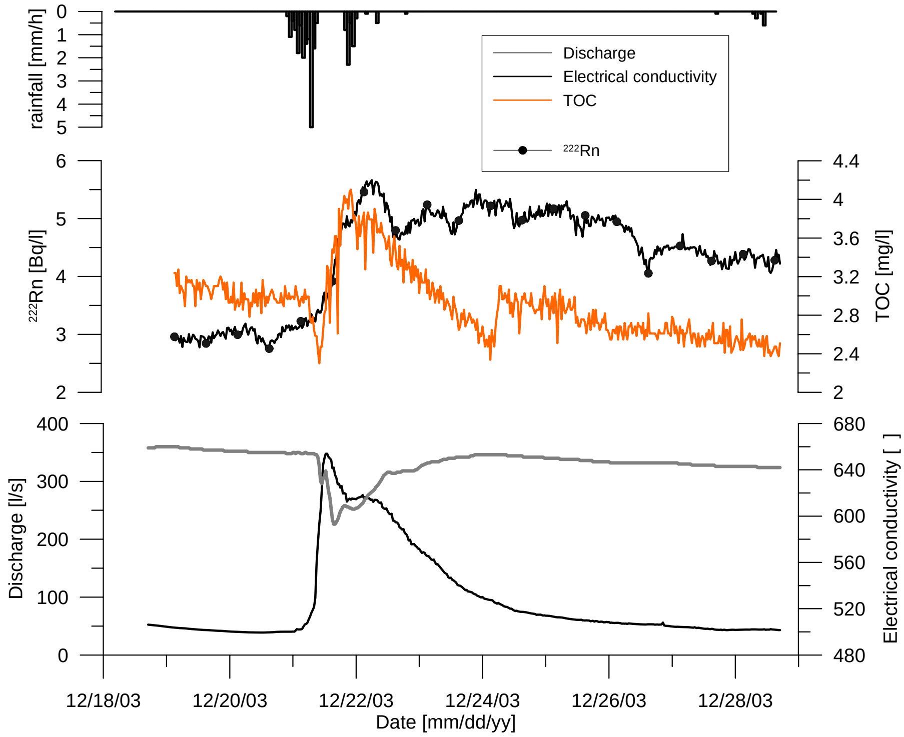


*Figure 2.21. December 2003 flood event. Comparison of the TOC concentration in the Milandrine river with discharge, electrical conductivity and $CO_2$ concentration*

The October 26th 2004 flood event showed a rapid TOC increase associated with a $^{222}$Rn increase and an electrical conductivity decrease (Fig. 2.22) summary as the December 2003 event. The October 2004 event can be separated in two successive floods of increasing magnitude each linked to a rainfall event. A first TOC increase (from 1.9 mg/l to 2.7 mg/l) was observed 8 hours after the first discharge increase associated with an immediate slight $CO_2$ increase (3.1 to 3.7 % vol.). No $^{222}$Rn increase was observed. During the second discharge peak, a second TOC increase (2.2 mg/l to 3.2 mg/l) was observed in parallel with a $^{222}$Rn increase (from 3.3 to 10.1 Bq/l), 9 hours after the beginning of the second discharge increase. The electrical conductivity decreased from 667 to 490 µS/cm during the flood event.

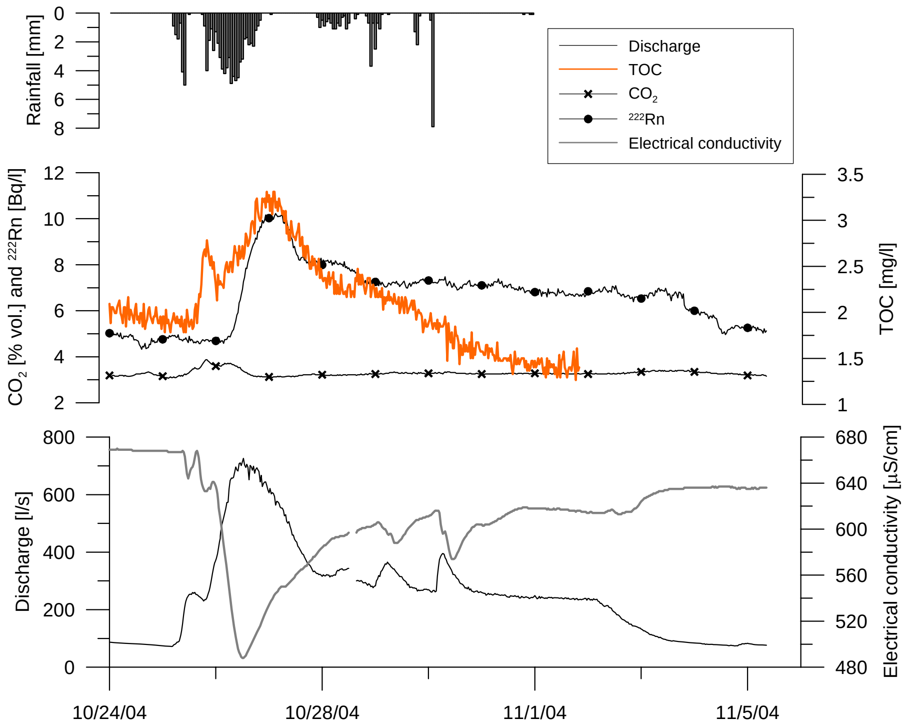


*Figure 2.22. October 2004 flood event. Comparison of the TOC concentration in the Milandrine river with discharge, electrical conductivity and $CO_2$ concentration*

### 2.3.4 Discussion

As shown above, independent of the strength of the flood event, the $CO_2$ concentration increases simultaneously with the increase of the flood peak while the TOC only reacts with a delay. The water contributing to the small flood events is the same to the water contributing to the base flow. The water was stored in the LPV of the saturated zone for a sufficiently long time not only for $^{222}Rn$ to decay but also for TOC to be degraded or retained. The flood peak likely consists of water that is displaced from the LPV by piston effect (Fig 2.23a). The immediate contribution of the saturated LPV to the discharge, leads to a slight $CO_2$ increase as explained in paragraph 2.1.4 (small flood events). During baseflow, the $CO_2$ concentration in the river is lower than in the saturated LVP due to a degassing of the $CO_2$ in the cave atmosphere. During flood events, less time is available for degassing than during baseflow and a small $CO_2$ increase is observed (see chapter 2.1). The delay between the beginning of the flood and the TOC increase can be explained by a slow TOC transfer from the epikarst through the LPV of the saturated and unsaturated zones (Fig 2.23c) and corresponds to a delayed contribution of the epikarst water to the discharge. According to several authors (Chapman, 1992; Caballero et al., 1996; Perrin, 2003; Vesper and White, 2004), a plausible location for the storage is the epikarst zone. Moreover, according to chapter 2.2, a low $^{222}Rn$ production is expected in the epikarst zone and no $^{222}Rn$ are measured with the delayed TOC and electrical conductivity increase.The slight increase in electrical conductivity during the TOC peaks suggests that the water receives a slightly higher mineralization in the epikarst.

For the larger flood events, an increase of the TOC is already observed during the flood peak, suggesting that a more significant volume of water containing TOC contributes to the discharge. The TOC shows a double response with a second delayed increase once the discharge had reached baseflow values again. The first TOC increase is likely due to water not only transited slowly across the LPV of the unsaturated and saturated zones but also through conduits possibly due to an overflow of the epikarst zone. Such an overflow could explain the first strong TOC response while the dampened response may be due to a slow transfer through the LPV similarly to the small flood event. The relatively small offset and similar peak shape of discharge and the first TOC increase suggest that the water from the epikarst and soil zones rapidly transited across the unsaturated zone likely along conduits (Fig. 2.23b). Indeed, $^{222}Rn$ increase observed in parallel with the TOC increase, indicate a contribution of water from the soil zone.

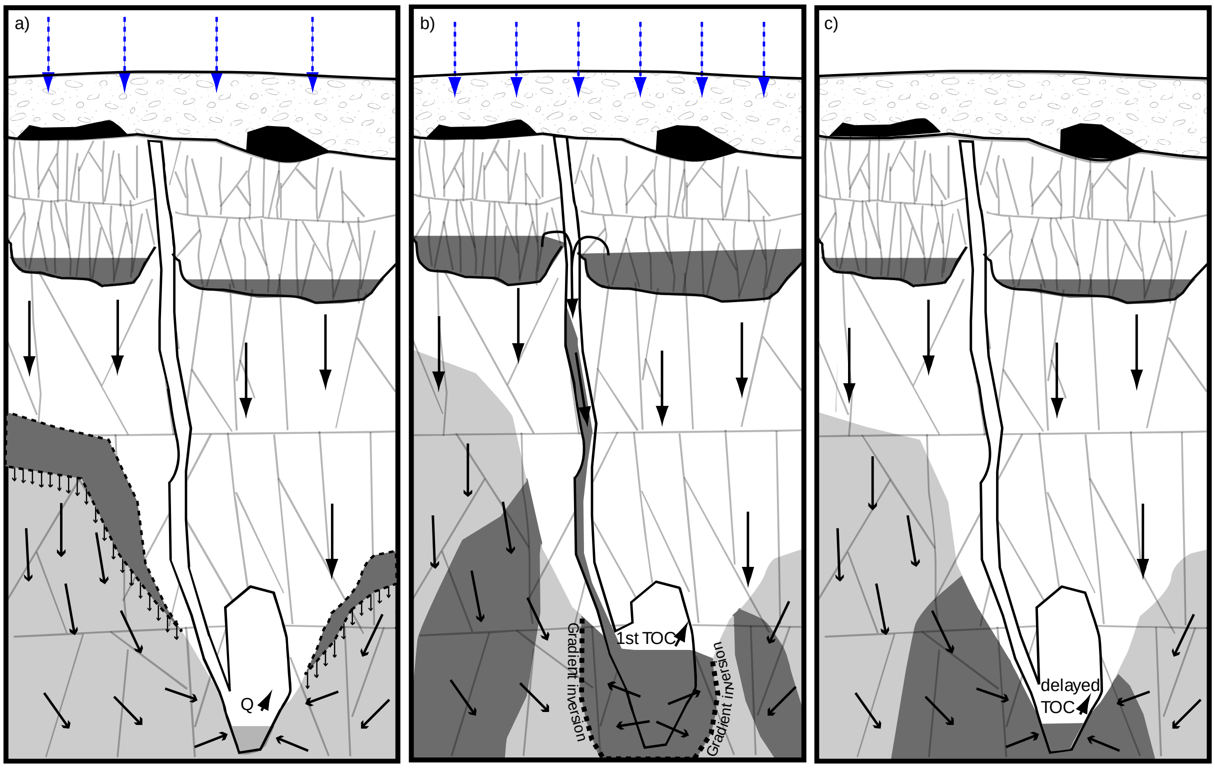


*Figure 2.23. Conceptual model of groundwater storage and solute transfer across the unsaturated zone of the karst system.a) Piston flow, b) Inversion of the hydraulic gradient, c) Second delayed TOC peak.*

The longer delay between the initial discharge increase and the second dampened TOC peaks compared to the small flood events may be due high flow conditions in the conduits leading to an inversion of the hydraulic gradient in the drainage conduits (Fig. 2.23b). The water level in the karst conduit network becomes higher than the one of the saturated LPV near the conduits and prevents the contribution of water form the saturated LPV to the discharge. This effect was described by Jeannin (1996) for the Milandre test site, based on water level and discharge measurements in boreholes and conduits. This inversion of the hydraulic gradient will stop the contribution of saturated LPV to spring discharge (Kiraly, 2002). Similarly, Charmoille (2005) studied the inversion of the hydraulic gradient during tracing experiment and flood events at Fourbanne spring (Doubs, France) and conclude that a pressure increase in the conduit can stop the contribution of the saturated LPV to the discharge. Finally, Trcek et al. (2006) observed in a Slovenian karst spring that during flood events, more than 50% of the discharge consists of epikarst water resulting in an inversion of the hydraulic gradient. Thus the delay before the second TOC increase during larger flood events corresponds to the transit time of water through the LPV as for small flood events and in addition the duration of the hydraulic gradient inversion in the conduits. For the larger flood events, the $^{222}Rn$ increase associated with the TOC increase (December 2003 and October 2004) suggests that, unlike for the smaller events, water that has recently transited through the soil contributed to discharge as well. For the first large flood event (Fig. 2.20) , the soil water contribution was likely smaller as indicated by a constant $^{222}Rn$ concentration compared to the second and third events (Fig. 2.21 and 2.22) where the $^{222}Rn$ increased. The decrease of the electrical conductivity for the second and third events may be attributed to the contribution of fresh rainwater to the discharge. As discussed in chapter 2.1.4, the contribution of fresh rainwater to the discharge can be evaluate between 20 and 25% (see October 2004 flood event, Fig. 2.8b and Fig. 2.22).

The separation between flood flow and intermediate base flow value during discharge recession was done using a graphical method (Shaw, 1994) already used by Perrin (2003) at the Milandre test site. The beginning of the flood corresponds to the inflexion point of the discharge curve (not represented on figure 2.24). The end of the flood corresponds to the point from which on the discharge follows a straight line when plotted on a semi logarithmic plot. This method was applied to the June 2004 and December 2003 flood events where a second delayed TOC increase was observed (see paragraph 2.3.3). The intermediate base flow – flood flow separation for the June 2004 and December 2003 flood events shows that the second TOC increase occurs immediately after the base flow – flood flow separation time (Fig. 2.24). The determined base flow point do not represents a real base flow period but an intermediated state of the system between flood and base flow. In the case of the December 2003 flood event, the observed elevated $^{222}Rn$ concentrations when discharge as reached intermediate base flow values again (inversion of the hydraulic gradient) can likely be attributed to the contribution of soil water to the discharge. Soil water can contribute to the discharge in parallel to the saturated LPV during recession phase.

These results strengthen the hypothesis of the hydraulic gradient inversion in the conduits during large flood events with interruption of the contribution of the saturated LPV to the discharge.

The Figure 2.25 summarises the delay of TOC, $^{222}Rn$ and $CO_2$ increase plotted versus the maximum discharge for most of the flood events measured during the whole period of observation. For the small flood events (less than 150 l/s) the delay in TOC response decreased with increasing flood intensity. The delay is probably due to a slow TOC transfer from the epikarst through the LPV as describe above. For the larger flood events, the delay shows little variations between 200 and 750 l/s and may be attributed to quick flow through conduits probably due to an overflow of the epikarst. A solute transfer velocity across the unsaturated and saturated LPVs (55 meters) can be calculated, comprised between 19 m/day and 55 m/day. During base flow period without recharge these values are smaller.

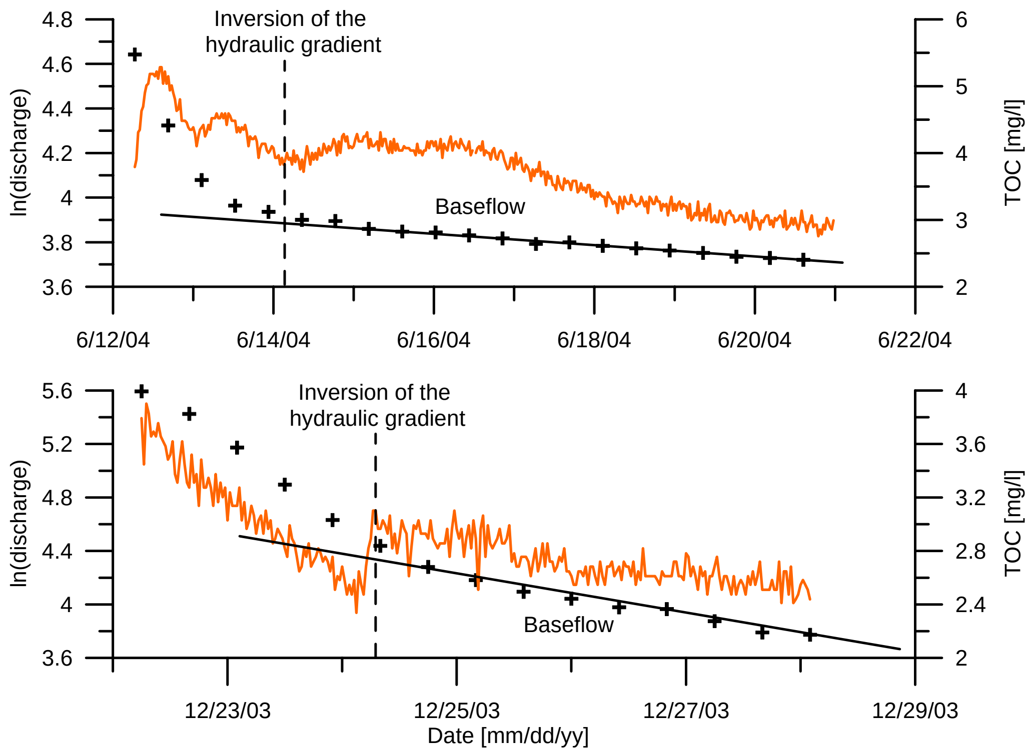


*Figure 2.24. Graphical separation of the base flow- quick flow for June 2004 and December 2003 flood events. The inflexion point correspond to the base flow with contribution of the saturated LPV to the discharge and second TOC delayed increase.*

These observations confirm that water transits across the unsaturated zone by different flow paths, rapid transfer along conduits and delayed transfer along LPV and that the relative importance of the two varies depending on flow conditions. Interestingly, water that has recently transited through the soil zone only contributes to discharge during large flood events as indicated by $^{222}$Rn measurements. The study suggests that TOC measurement together with $^{222}$Rn are good tracers to separate water that has been in storage in deeper zones of the system (low TOC, low $^{222}$Rn), in intermediate zone, likely the epikarst (elevated TOC, low $^{222}$Rn) and in the soil zone (elevated TOC, elevated $^{222}$Rn). In addition, the delay with respect to discharge and the peak shape of TOC provides information about the mode of transit of water from the epikarst zone. Such information cannot be obtained from $^{222}Rn$ because it is already decayed to limestone background values in this zone.

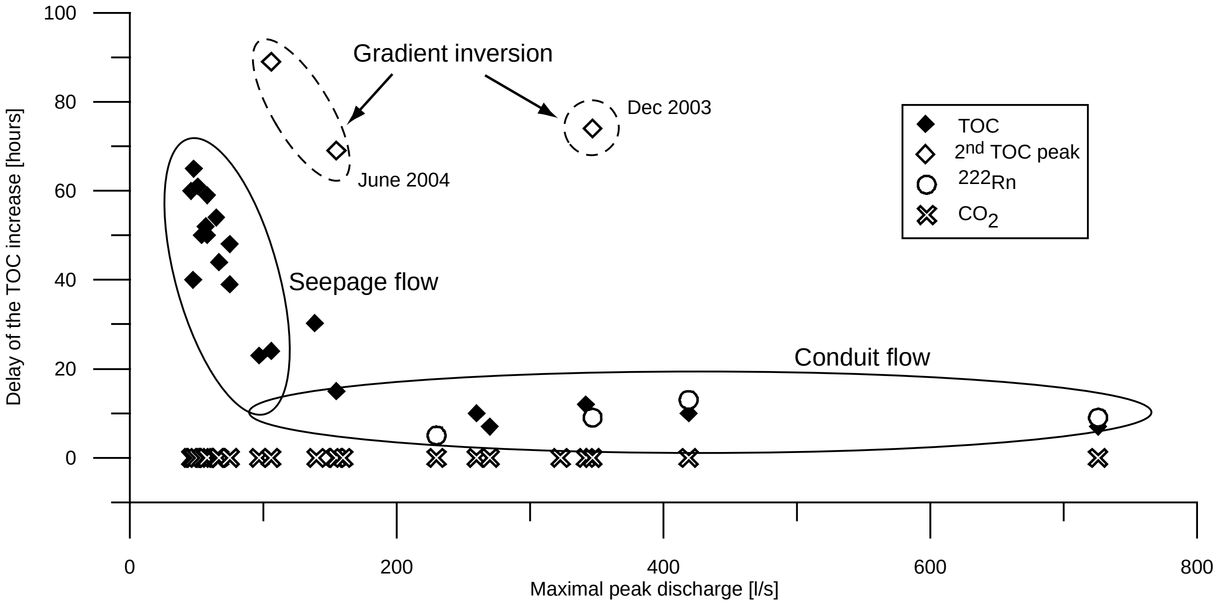


*Figure 2.25. TOC, $CO_2$ and $^{222}Rn$ increase delay versus discharge. Two hydraulic comportments are highlighted: seepage flow and conduits flow during flood event of more than 150 l/s.*

### 2.3.5 Comparison with existing conceptual models

The results of the TOC, $^{222}Rn$ and $CO_2$ measurements confirme some aspects of the conceptual model proposed by Perrin (2003) and summarized in paragraph 1.1.2 and add new elements concerning the storage and the flows. A part of the storage is located in the soil and epikarst zones as proposed by Perrin (2003), but the role of the saturated LPV as to be considered, as they are necessary for explaining the observed hydraulic responses and TOC - $CO_2$ observations during base flow and small flood events. For a flood event, the following steps can be observed:

In steady-state conditions, the base flow discharge is sustained by the saturated LPV, and the saturated LPV are recharged through the unsaturated zone by the diffuse infiltration as already observed by Charmoille (2005). Discharge and chemistry are stable in the saturated zone. In the Perrin's model, the base flow discharge is sustained by the epikarst reservoir.

During phase 1, rainfall has started and soil water is pushed into the epikarst reservoir. The hydraulic stress on the epikarst causes a discharge increase in the unsaturated LPV but the recharge is not enough sufficient to create an overflow of the epikarst reservoir. The water pushed in the unsaturated LPV causes a hydraulic stress on the saturated LPV and a discharge increase is observed in the saturated zone as postulated by Mudry (1987) and Charmoille (2005). The water is the same as during base flow period. In Perrin's model, during base flow and small flood events, the system is fed by epikarst water mainly but this hypothesis does not allow to explain the immediate $CO_2$ increase and the delayed TOC increase.

During phase 2, rainfall continues, more soil water is pushed into the epikarst reservoir. An overflow of the epikarst reservoir through preferential flow paths is observed and a mixing of soil water and epikarst water reaches directly the saturated zone. The system is fed by epikarst and soil waters as for Perrin's model. In addition to Perrin's model, the measurement of $^{222}Rn$ allows the detection of the soil water contribution.

During phase 3, rainfall continues. Some fresh water bypasses the soil reservoir and reaches the saturated zone. The system is fed by a mixing of fresh water, soil water, and epikarst water as for Perrin's model. The contribution of fresh rainwater to the discharge is similar for the two models (20 and 30%).

During phases 2 and 3, the saturated LPV contribute to the discharge at the beginning of the flood and are then stopped by an inversion of the hydraulic gradient caused by the rapid contribution of soil and epikarst water through preferential flow paths

During phase 4, rainfall has stopped, the soil releases water into the epikarst reservoir. The system is mainly fed by epikarst and saturated LPV water, but soil water can still be present (sustained $^{222}Rn$ concentrations, see paragraph 2.1.4).

The flow velocities through the unsaturated zone during base flow or flood events conditions (in Milandre test site) are different than those proposed by Perrin (2003):

- During base flow, flow velocity in the unsaturated and saturated LPV is below 19 m/day. In Perrin's model (2003), the velocity in the unsaturated (from the epikarst to the main drainage conduit) is on the order of 100 m/h. This value is plausible for flow in the more permeable parts of the system (conduits) but do not take in account the less permeable parts (LPV) where slower velocities are expected.

- During small flood events, the estimated flow velocity in the unsaturated and saturated LPV is comprised between 19 m/day and 55 m/day when water transit through the LPV and higher than 10 m/h when water transit though preferential flow paths (higher flood events). In Perrin's model, during flood events, the velocity in the unsaturated zone is estimated in the order of 50 m/h for the small flood events as for the large flood events. An elevated velocity through the most permeable parts of the unsaturated zone has to be involved in order to explain the rapid contribution of the epikarst zone to the discharge. For the small flood events, where no overflow of the epikarst are expected, an immediate contribution of the saturated LPV (piston flow) without increase of the TOC concentration can also explain the discharge increase as already observed by Charmoille (2005). In this case, slower velocity from the epikarst through the unsaturated LPV can be expected.

### 2.3.6 Conclusions

The continuous TOC measurements along with common hydrochemical parameters makes it possible to get a more detailed picture of the flow path of water during baseflow and flood events. Moreover the study suggests that TOC together with $^{222}Rn$ are good tracers to separate water that has been in storage in the LPV of the system (low TOC, low $^{222}Rn$), in intermediate zone, likely the epikarst (elevated TOC, low $^{222}Rn$) and in the soil zone (elevated TOC, elevated $^{222}Rn$). In addition, while classical parameters such as electrical conductivity are usually used to distinguish between rain and pre-event water arriving at a sampling point, TOC make is possible to characteristics the flow paths of the pre and post-event water in more detail. The delay with respect to discharge and peak shape of TOC provides information about the mode of transit of water from the epikarst zone. Such information cannot be obtained from $^{222}Rn$ because it is already decayed to limestone background values in this zone.

During flood events, an increase in discharge without variations of the other parameters (conductivity, TOC, $^{222}Rn$), indicates that the water is identical to baseflow water. During baseflow, the underground river discharge corresponds essentially to seepage flow from the LPV. An increase in TOC indicates a contribution of the epikarst and soil reservoirs to the discharge and provides information on the flow paths of water. After small rainfall events, the TOC rich water of the epikarst zone percolates through the LPV and leads to a delayed and smoothed increase of the TOC signal in the saturated zone. During higher recharge conditions an overflow of the epikarst and soil reservoirs is observed with TOC increase and contribution to the discharge.

**Acknowledgements**

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## 2.4 TOC measurements at additional test site

### 2.4.1 TOC measurements at Vers-Chez-le-Brandt

The continuous measurement of the TOC in parallel with discharge and electrical conductivity was conduct at the Vers-Chez-le-Brandt test site in October 2005. As for Grand Bochat test site, the monitoring at Vers-Chez-le-Brandt was performed in order to have additional measurements at a local scale and to identify the origin of water in the cave under recharge conditions. The Vers-Chez-le-Brandt cave is well connected to the epikarst and soil zones and the influence of the unsaturated LVP to the discharge is low in contrast to the Milandre site (Gratte-Roche gallery). No $^{222}$Rn and $CO_2$ samples were collected during this period. Vers-chez-le-Brandt groundwater is sampled and monitored on a vadose perennial flow issued from a conduit at the cave roof. The sampling location is located 30 m below the ground surface; the discharge at low stage is 0.4 l/min, corresponding to a basin of 100 m2.

The sampling location at Vers-Chez-le-Brandt corresponds to a drainage conduit directly connected to the epikarst without significant contribution of the saturated LPV to the discharge. Perrin (2003) observed during flood events that stable oxygen isotopes in percolation water followed the same pattern as in the rainfall with a small dampening of the signal, indicating the contribution of two waters to the discharge: base flow water stored in the unsaturated zone (most probably in an epikarst reservoir), and fresh infiltrated water having the chemical composition of rainfall.

During years 2004 and 2005, similar irrigation experiments were conduct at Vers-Chez-le-Brandt and Milandre site (see chapter 3 and paragraph 2.5). Conservative tracers were injected in the unsaturated zone under artificial steady state conditions. At Milandre test site, in the Gratte-Roche gallery located at the base of the unsaturated zone, very low recovery rates were measured (<1%), and persistent tracer concentration observed at the sampling points (see paragraph 2.5) during several weeks. The tracer experiments (see paragraph 2.5) suggested that the buffer capacity in the unsaturated zone is mainly linked to the presence of an elevated thickness of LPV and the storage to the epikarst. Preferential flow path in the limestones through vertical drainage conduits seems to be unimportant and seepage flow in the LPV with mixing of epikarst and soil water an important process. In contrary, irrigation experiments at Vers-Chez-le-Brant test site indicated a rapid discharge response and elevated recovery rate (>50%) of the injected tracers (see chapter 3). This account that storage in this site is not located in the unsaturated LPV but more probably in the soil and epikarst, drained by preferential flow paths/conduits through the unsaturated zone.

### 2.4.2. Results and discussion

Two flood events where observed at Vers-Chez-le-Brandt test site during the period of measurement. On October 19th 2005 an increased in discharge by 8 l/min was observed after 15.3 mm rainfall throughout a half day (Fig. 2.26). The discharge increase was observed 1 hour after the maximal rainfall intensity. A TOC increase by 7.2 mg/l from 3.7 to 10.9 mg/l was observed 140 minutes after the beginning of the discharge increase. An electrical conductivity decrease by 110 µS/cm from 438 µS/cm to 328 µS/cm was observed in parallel with the TOC increase. Three days later, on October 22nd, a second smaller discharge increase by 1.2 l/min was observed after 4.5 mm rainfall. A very small electrical conductivity increase from 408 µS/cm to 415 µS/cm was observed one hour before the discharge increase. A delayed TOC increase was observed 250 minutes after the discharge and electrical conductivity increases. The beginning of the TOC increase corresponded to the maximal electrical conductivity.

During the beginning of the first flood, a small piston effect occurs probably mobilizing water from the less permeable parts of the epikarst as TOC concentrations do not increase. The decreasing of the electrical conductivity in parallel with the TOC increase is due to the direct contribution of a mixture between TOC rich soil water and rainwater by preferential flow paths along the vertical drainage conduits. In this case, a part of the fresh water and soil water bypasses the epikarst reservoir. The TOC reaction of this event can be compared to the large flood event observed at Milandre cave (see paragraph 2.1.4) where rainwater and epikarst water contribute to the discharge, but in the case of Vers-Chez-le-Brandt, the piston flow observed at the beginning of the flood is not attributed to the saturated LPV as for Milandrine river but to the water stored in the epikarst as for the experiments realised at Grand Bochat cave (see paragraph 2.2.3). Indeed, as described above, no contribution of the LPV to the discharge are observed at Vers-Chez-le-Brandt sampling location. During the second discharge event, the beginning of the discharge increase is attributed to a piston effect through the epikarst as for the first event. The delayed increase of the TOC corresponds to a delayed contribution of the percolating water from the soil reservoir through the epikarst. The rainfall is not sufficiently elevated to exceed the soil storage capacity and a direct contribution of rainwater to the discharge is unlikely as indicated by the slight increase of the electrical conductivity.

### 2.4.3 Conclusion

As describe above, the sampling location at Vers-Chez-le-Brandt test corresponds to a drainage conduit without important contribution of the LPV to the discharge. Similar hydrological reactions than for Milandre test site were observed with less delayed contribution of the epikarst reservoir due to absence of seepage flow through the unsaturated and saturated LPV. During large recharge events, a direct contribution of soil water and fresh rainwater to the discharge is observed. During small recharge events a delayed contribution of the soil reservoir containing a small amount of fresh rainwater is observed after percolation through the epikarst reservoir.

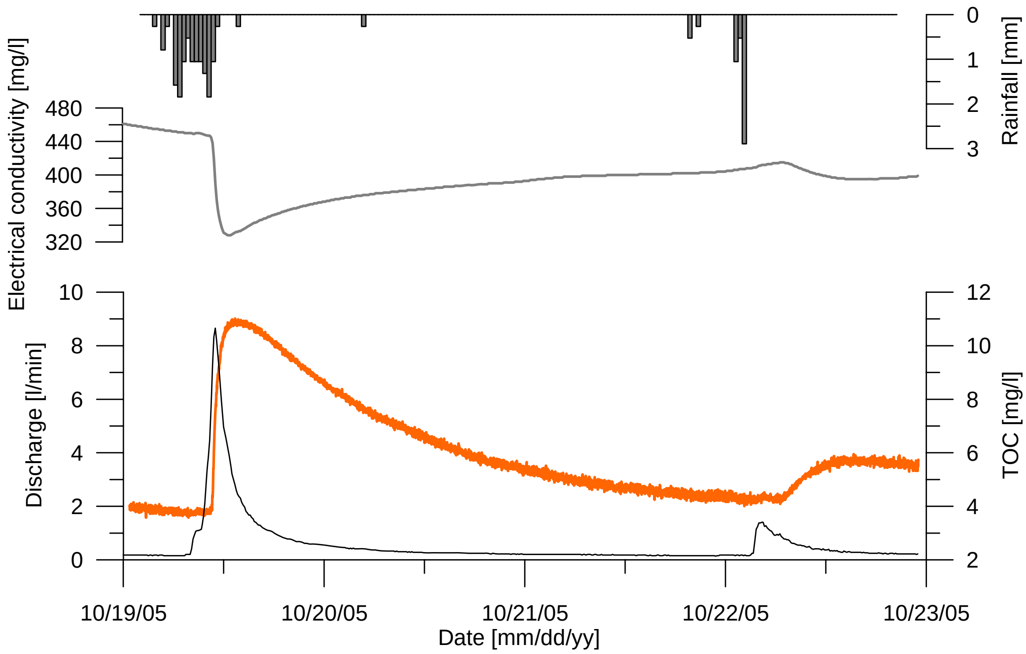


*Figure 2.26. October large and small flood events at Vers-Chez-le-Brandt test site.*

The measurements carried out at Vers-Chez le Brandt test site as the artificial experiment done at Grand-Bochat confirm that the upper part of the unsaturated karst system (the soil and epikarst zones) can store and mix a considerable amount of water. Moreover the measurements strengthen the hypothesis that under strong recharge conditions, preferential flow paths through the different subsystems of the unsaturated zone can be activated due to an exceeding of the storage capacity.

## 2.5. Artificial tracing experiments and discharge measurements in the unsaturated zone[1]

### 2.5.1 Introduction

Recent karst studies, based on natural tracers, have revealed the importance of the soil and epikarst zone as buffer and storage subsystems in karst aquifers during rainfall events although discharge varies substantially in the saturated zone (Chapman, 1992; Caballero et al., 1996; Perrin, 2003; Vesper and White, 2004) (see chapter 1.1.1.b)

Several studies have demonstrated a high spatial and temporal variability of the hydraulic response of water tributaries in the unsaturated zone (Smart and Friederich, 1986; Destombes et al., 1997; Delannoy et al., 1999; Sanz and Lopez, 2000; Perrette et al., 2001; Perrin, 2003) and two types of hydraulic responses were identified; conduit flow leading to "nervous" hydraulic responses and seepage flow through the fractured LPV of the unsaturated zone leading to a dampened responses In karst aquifers the hydraulic responses at the spring correspond generally to a combination of the two types of flow (see chapter 1.1.1.c).

The present study focuses on the Milandre cave case-study, where a freeway is being constructed on top of the cave, which is one of the most highly decorated in Switzerland. The protection of the cave was included since the beginning of the planification of the road construction. Another important reason to protect the cave is that the catchement area is drained by underground streams feeding a drinking water supply. In order to evaluate the possible impacts linked to the construction of the Swiss national road N16 on the Milandre cave and karst groundwater, three discharge measurement systems were installed in the Gratte-Roche gallery, in the axe of the planned road, and collecting the water percolating

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Modified from [1] **Characterisation of flow in karst unsaturated zone. The case-study of A16 freeway and its impact on Milandre cave.**

Savoy Ludovic, Pochon Alain, Jeannin Pierre-Yves

*Paper prepared for Environmental Geology* from the roof of the gallery. The aim of the measurement is to characterize flow before and after the road construction. In order to minimize the impact (drying up of a significant part of the cave) an infiltration system under the road was designed. Tracing experiments were conducted in order to verify the correct position of this system for feeding the cave. These data and experiments represented a highly valuable opportunity to better understand the flow dynamic of the unsaturated zone of karst systems during high flow conditions and base flow conditions.

### 2.5.2 Study area

Milandre cave is situated in the Swiss Jura, 8 km N-W of Porrentruy. It is an 11 km cave of national importance (registered in the national list of geotopes) because of its high speleothems density. The Milandre karstic network is developed in the Rauracian (Oxfordian) limestone overlying the impermeable Oxfordian marls. The thickness of the unsaturated zone ranges from 40 to 80 meters (Jeannin, 1996). The system discharge area consists of the perennial Saivu spring, (20 to 200 l/s) and the Bame temporary overflow (0-1500 l/s). A part of the water discharged at Saivu spring is artificially injected in an alluvial aquifer exploited as drinking water supply by the Boncourt village. Two parts of the karstic network, the Milandrine river and the Gratte-Roche gallery (GR), are directly threatened by the construction of the Swiss national road N16 (Fig. 2.27) that will join the city of Bienne (Bern, Switzerland) with Belfort (France). The Milandrine river is one of the three more developed tributaries of the cave and corresponds to the best known upstream part of the cave. The discharge varies between 20 to 700 l/s. The Gratte-Roche (GR) gallery is located in the Milandrine catchment above the saturated zone. The GR gallery is connected to the Milandrine river by a small tributary (perennial discharge around 1 l/s).

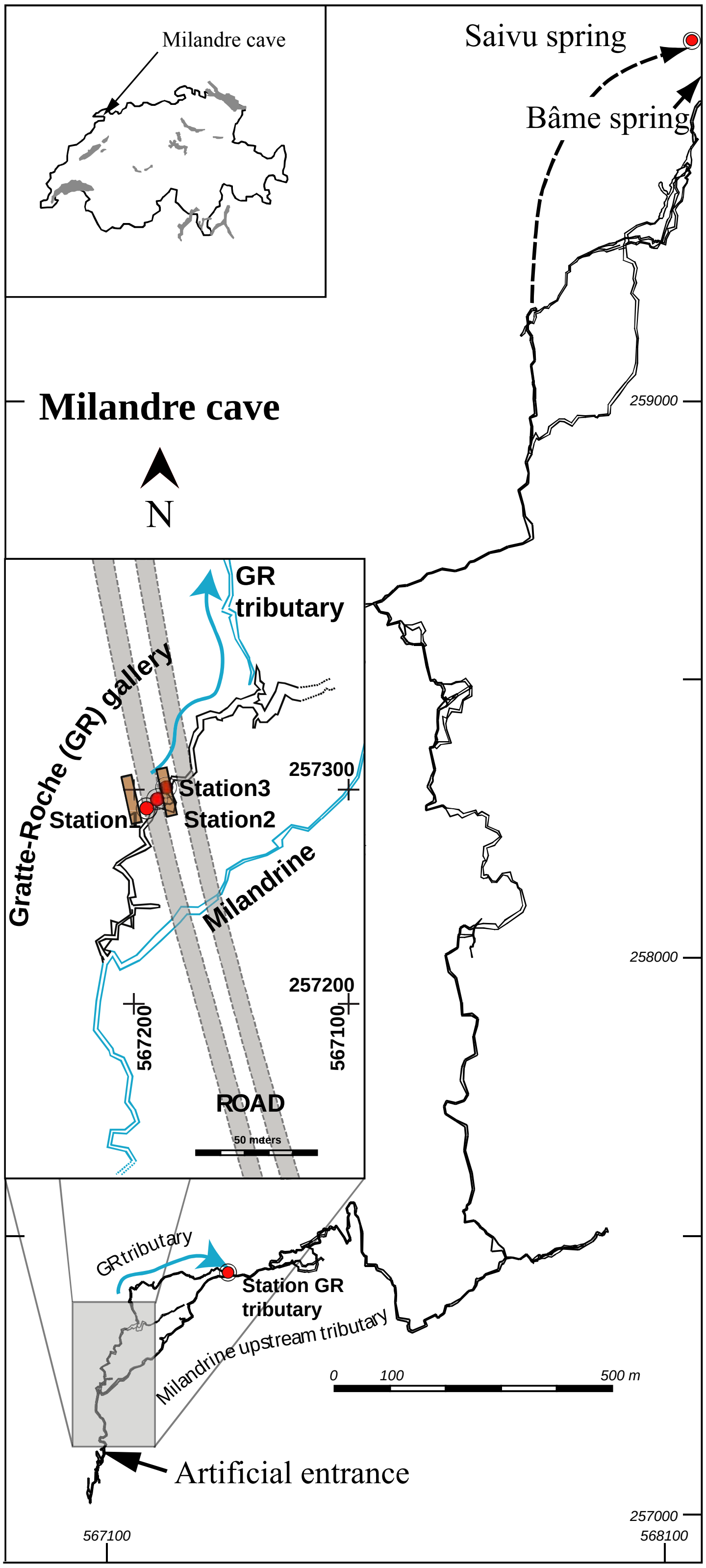


*Figure 2.27: Location of the Milandre cave, the three discharge measurement stations, the GR tributary, the Saivu spring and the two irrigated areas. Arrows correspond to flow direction*

### 2.5.3 Methodology

#### *Continuous discharge monitoring*

Since November 2002, drip water discharge in the GR gallery (Fig. 2.27) is measured with three tipping-bucket pluviometers linked to dataloggers. The measurement stations are located 45 meters under the ground, directly under the planed highway. Three locations in the cave were chosen. Station 1 (S1) presents concentrated continuous discharge (Fig. 2.28), Station 2 (S2) collects punctual drip water of the fractured cave roof (Fig. 2.29) and Station 3 (S3) collects continuous drip water of one isolated speleothem. Each rain collector is equipped with a self-emptying tipping-bucket of 8 or 20 ml (volume depending on discharge rate) which sends an electrical pulse to the datalogger (DT5, dataTaker) at every toppling. The data are downloaded on a laptop every month. During the whole observation period, continuous discharge measurements were recorded at the Milandrine river which allowed comparing the hydrologic reaction of the local catchment observed in the GR gallery to the overall reaction of the system.

*Figure 2.28: Discharge measurement station S1.*

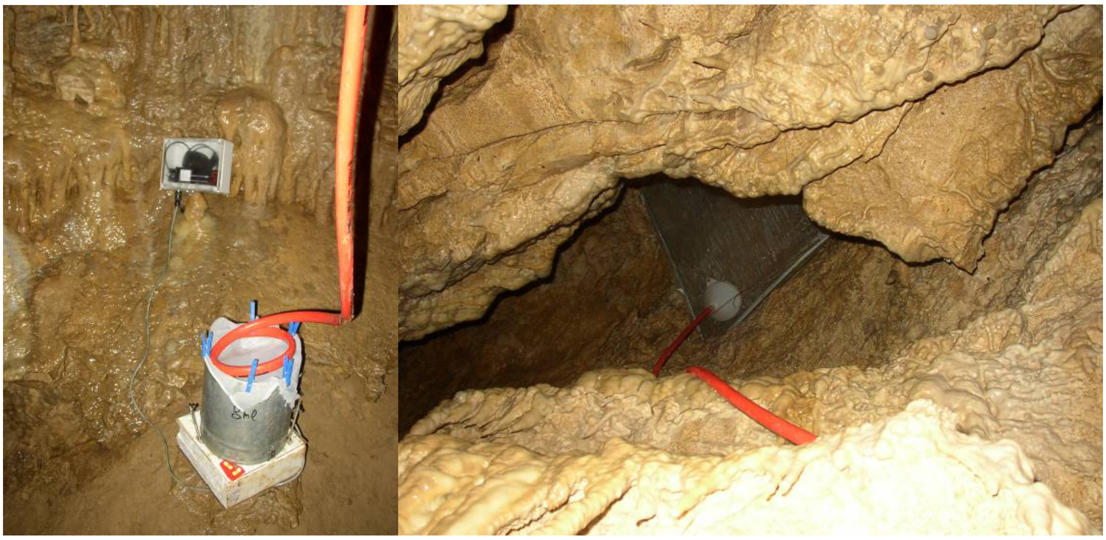

*Figure 2.29: Discharge measurement station S2.*

### *Tracing experiment*

Two tracing experiments were performed (April and June 2004,) directly above the monitoring stations. The injection area was divided into two rectangles of 3 by 20 meters (60 $m^2$) along the axis of the planned road and the soil was removed (from April to June 2004) from one of the areas until the limestone bedrock was reached. The aim of these experiments was to demonstrate the connection between the area sprinkled at the surface and the monitored inlets in the GR gallery, to estimate the buffer effect of the unsaturated zone on infiltration recharge and to evaluate the impact of soil removal on vadose flow by comparing the propagation of tracer in presence and in absence of soil cover. The two areas were not on exactly the same location but it was assumed that infiltrated water flows through similar flowpaths in the unsaturated LPV hence results from the two areas can be compared. In the trench, the measured soil thickness varied between 2.0 and 3.5 meters. An automatic garden sprinkler system was installed on each area to provide a uniform water flux at the soil surface. Two 4 $m^3$ water tanks linked with two pumps supplied the water necessary for the irrigation. A constant sprinkling intensity was maintained, simultaneous on the both surfaces, by adjusting the flow rate with a valve and a flow counter. For the first experiment, a tracer (uranine or sulforhodamine B) was added to each of the surfaces in order to evaluate the connections between the surfaces and the three stations in the cave. Uranine, which is generally considered as a conservative tracer was sprinkled at the soil surface, while sulforhodamine B, which is potentially more influenced by sorption processes (Käss, 1998), was released in the trench, directly on the bedrock. The tracers were sprinkled on a 60 $m^2$ area with a constant concentration of 45 g/l, during 7 hours with a discharge corresponding to a rain intensity of 30 mm/h. The total mass of each tracer injected during the experiment was about 650 g. After seven hours, the injection of the tracers was stopped. The discharge intensity was then increased using clear water to reach 50 mm/h, and maintained during 2 hours in order to improve the infiltration of the tracers into the system.

For the second experiment, a pulse of uranine was injected in the trench under constant irrigation rate (intensity of 50 mm/h maintained during 6 hours).

In the cave, drip water was sampled at station 1 using a programmable autosampler (Isco) collecting 400 ml samples. The discharges were measured by the systems already described above. The fluorescent dye breakthrough curves at the 3 stations were recorded every minute using field fluorometers (Schnegg and Costa, 2003; Flynn et al., 2005). Fluorometers were also installed at the end of the Gratte-Roche tributary (Fig. 2.27), which collects all the water percolating in the monitored area, and at the Saivu spring. In order to enhance the infiltration, the tracing experiments were realized during high to medium water conditions in the GR gallery, corresponding to relatively high water content in the unsaturated zone.

### 2.5.4 Results

#### *Discharge measurement*

The discharge of the three measurement stations in the GR gallery and the Milandrine river are presented in Figure 2.30 for the period between July 2003 and December 2005 together with rainfall and potential evapotranspiration (PET) data. Rainfall and PET data (mm/day) were obtained from a METEOSWISS automated station located at Fahy, 6 km in the south-west of the measurement area. Data gaps for discharge measurements occured at several periods due to datalogger failures. The elevated and nervous discharge increases observed in the GR gallery between April and August 2004 do not reflect natural conditions but artificial infiltration of water linked to the tracing experiments. Throughout the measurement period, marked flood are observed in the Milandrine river linked to the rainfall events. In the GR gallery, the discharge of the three measurement stations presented different flow intensities (mean values: 43.7 dl/h for S1, 0.24 dl/h for S2 and 3.4 dl/h for S3) but the discharge variations were comparable. The station 1 (S1) presented the most elevated discharge varying between 18 and more than 150 dl/h. The maximum discharge values were too large to be measured by the recording station. Three base flow periods were observed (between August 2003 and January 2004; July and September 2004; July and November 2005). For the station 2 (S2), with discharge varying between 0.1 and 1 dl/h, and for the station 3 (S3) with discharge varying between 1.5 dl/h and 5.5 dl/h, the same periods of base flow than for S1 were observed. For the three stations, two high flows periods were observed during winter and spring. The high flow periods began after rainfall events more than 80 mm/week corresponding to flood event in the Milandrine with a discharge of more than 400 l/s. An exception was October 2003 when no hydraulic responses in the GR gallery and only flood less than 400 l/s in the Milandrine river occurred despite a rainfall of more than 110 mm/week.

Between April 18th and 25$^{th}$ 2005 a strong discharge increase was measured in the three stations (Fig. 2.31). The discharge increase was observed 48 hours after a 62 mm/day rainfall event (April 16$^{th}$). The PET was 0.13 mm. The total measured rainfall during this recharge event (10 days) was 115 mm and corresponded to 10 % of the annual rainfall (1033 mm, Gretillat, 1996). In the Milandrine river, a discharge increase by 630 l/s during 15 hours from 55 l/s to 685 l/s was observed. Considering the 50 meters distance from the surface to the GR measurement stations and the time interval between the beginning of the rainfall and the discharge increase, the estimated mean transit velocity of the pressure pulse through the unsaturated zone (soil, epikarst and LPV) is less than 1 m/hour. This value is not representative of the velocity of the pressure pulse only through the LPV but considered the whole thickness of the unsaturated (with soil and epikarst). In order to obtain a more representative velocity, the thickness of the unsaturated LPV should be known in more detail. The measured soil thickness was around 3 meters and the epikarst thickness is estimated between 1 and 5 meters (Perrin, 2003) leading to higher velocity between 1 and 1.5 meters.

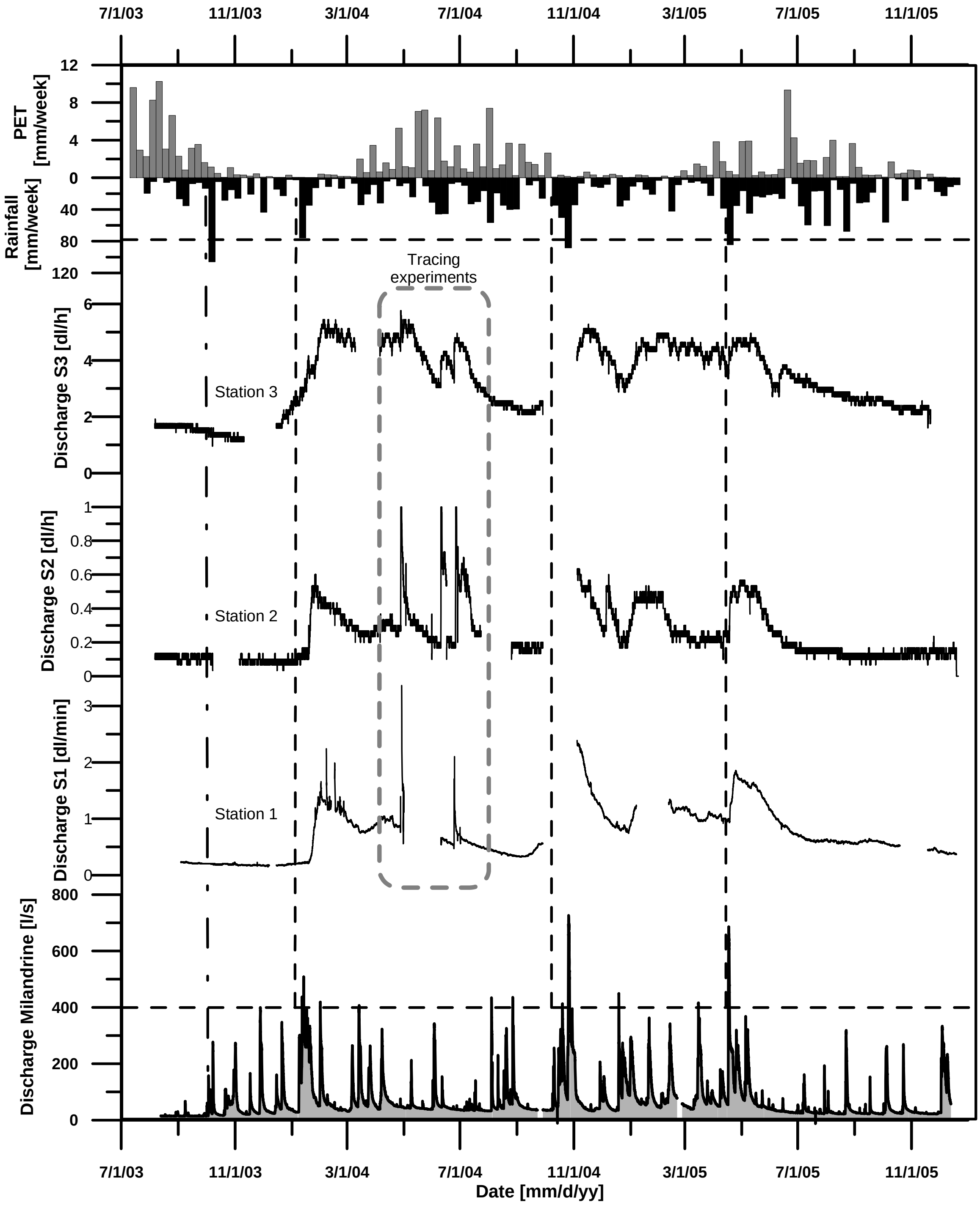


*Figure 2.30: Continuous measurements of the discharge for Milandrine river and stations 1, 2 and 3 with seasonal base flow and high flow conditions. Rainfall and PET at the Fahy METEOSWISS station. Vertical dashed lines correspond to discharge>400 l/s in the Milandrine (horizontal dashed line) and rainfall>80mm/week (horizontal dashed line).*

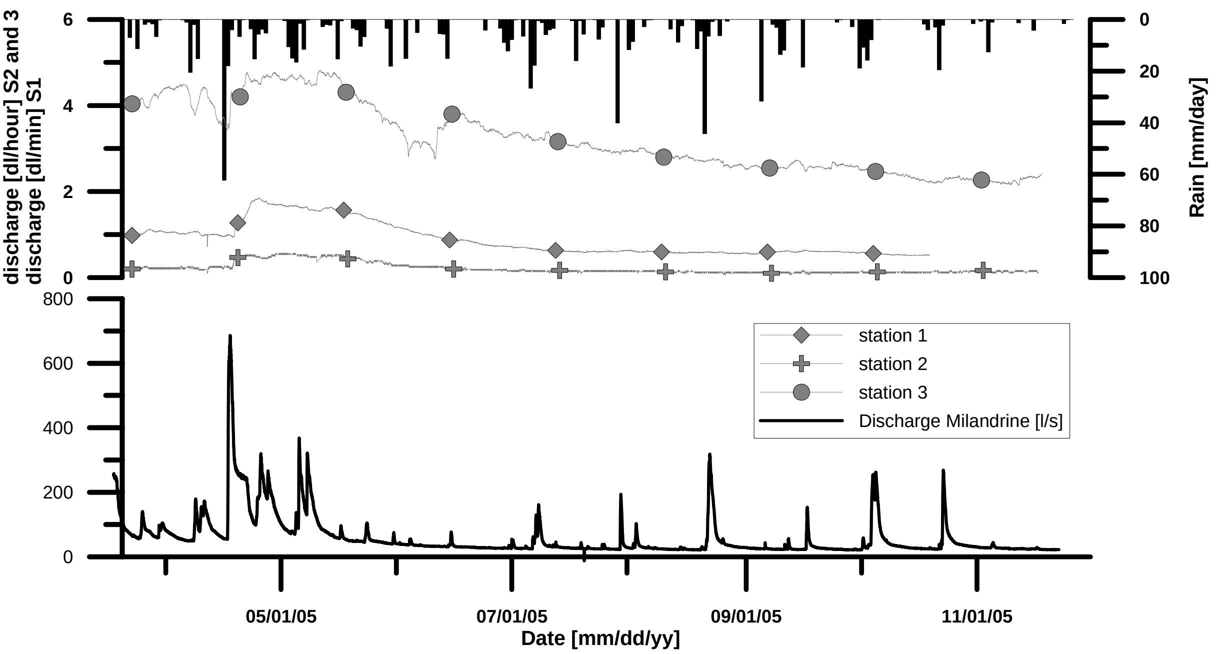


*Figure 2.31: Continuous measurements of the discharge for stations 1,2 and 3 (one symbol every 30 days). April 2005 discharge increase at S1, S2, S3 and Milandrine linked to 62 mm/day rainfall event. Rainfall from the Fahy METEOSWISS station.*

## *Tracing experiments*

The first tracing experiment was performed on April 27th 2004 after an extended period of rainfall without elevated PET leading to a significant discharge increase at the percolation zone between December 2003 and April 2004 (Fig. 2.30). Two different tracers were applied on the two areas along the planned road, above the Gratte-Roche gallery (Fig. 2.27).

In the cave, the first hydraulic reaction was observed between 12 to 14 hours after the beginning of the sprinkling at the three discharge measurement stations (Fig 2.32a). Uranine was detected at the station 1, 3.5 hours after the discharge increases (Fig 2.32b). No tracers were detected at the two other stations. The maximum uranine concentration at S1 was 3.5 μg/l. The concentrations measured by the field-fluorometer located at the end of the GR tributary was close to the detection limit but are still reliable due to a constant signal of the natural fluorescence background before this period (Fig 2.32c). The GR tributary is assumed to collect water from the entire percolation zone activated during the experiment. Therefore the mass recovery can be calculated based on the tracer concentration and the discharge measured in the GR tributary (0.8 l/s without significant variation of the discharge). 15 mg of uranine were recovered during the six days following the injection, which represent less than 0.003% of the injected mass. Although the two stations (S2 and S3) located directly under the trench showed a clear hydraulic reaction in response to the irrigation, no sulforhodamine B was detected. The sulforhodamine B was also not detected at the station 1 and at the end of the GR tributary.

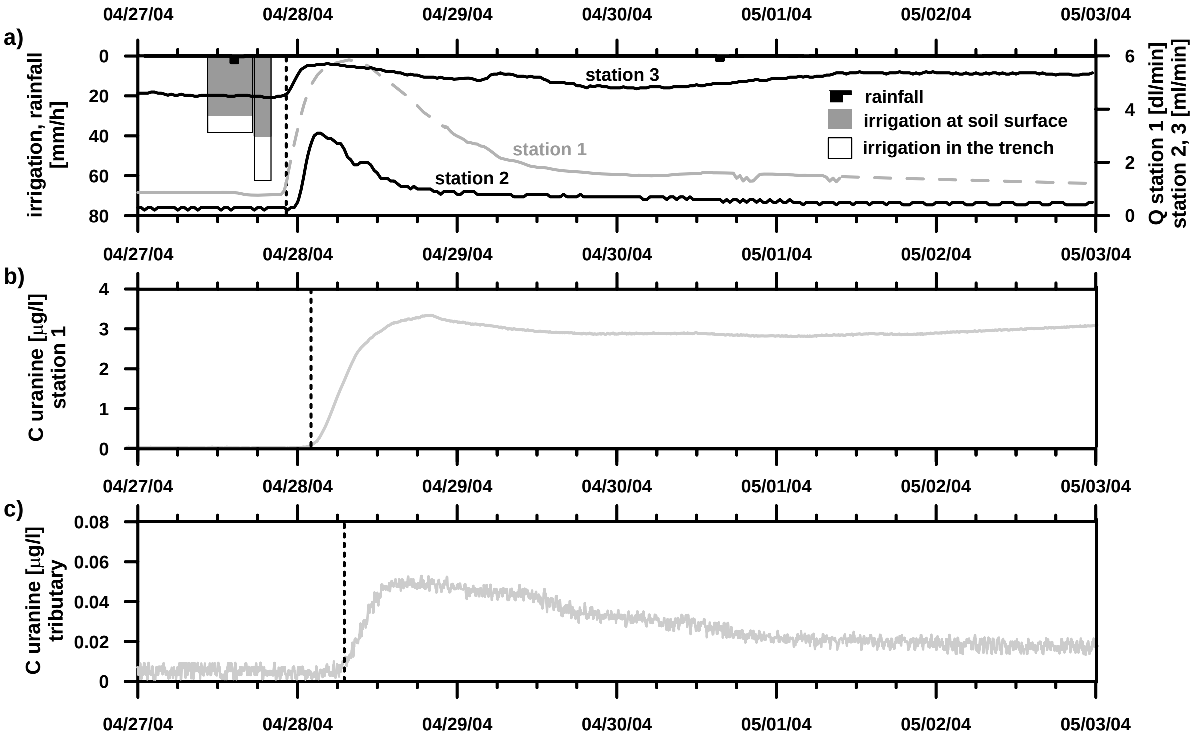


*Figure 2.32: Results of the first experiment performed in April 2004. a) irrigation intensity at surface and hydraulic reaction monitored in the cave b) measured uranine concentration at station 1 c) measured uranine concentration at the end of Gratte-Roche tributary. Dashed line corresponds to the beginning of the discharge reaction and uranine detection*

In order to evaluate why sulforhodamine B was not detected during the first tracing experiment and to test the reactivity of the system under higher infiltration rate, a second tracer test was performed on June 9th 2004, seven weeks after the first experiment. Since no uranine injected on the soil zone during the first experiment was detected at the stations 2 and 3, the same tracer was injected in the trench for the second experiment. The tracer was released as a pulse to simplify the injection procedure and to induce higher concentration in the conduits network. A higher tracer mass (2 kg as 10% solution) was used to increase the chance of finding the tracer.

A higher rate of irrigation was imposed compared to the first experiment with an intensity of 50 mm/h maintained during 6 hours. After 2 hours of sprinkling the tracer was injected and the irrigation was maintained during 4 additional hours. The higher irrigation rate compared to the first experiment resulted in an accumulation of several cubic meters of water at the end of the experiment in the trench. A discharge increase at S2 and S3 was observed 13 and 14 hours respectively, after the beginning of irrigation (Fig. 2.33a). Discharge measurement at S1 was out of order during the first hour following the injection but a significant hydraulic reaction at this outlet can be excluded (no variation of the discharge is observed at S1 once the measurement was functioning, while floods are observed at S2 and S3, Fig. 2.33a). Uranine was not detected by the field fluorometers at S2 and S3 during the discharge increase. Manual sampling and observations performed in the cave the day after the injection showed that most of the water percolating in response to the injection discharged along a five meters broad zone located between the stations S2 and S3. Manual sampling in this zone and laboratory measurements gave concentrations around 4 μg/l 16 hours after the beginning of the experiment and around 165 μg/l two weeks later (Fig. 2.34). Two days after the first detection of uranine between S2 and S3, an increase of uranine was measured at S3 and uranine persisted during more than 2 weeks (Fig. 2.34).

The breakthrough curve at the end of the GR tributary obtained by the field-fluorometer and the manual samples (Fig. 2.33b) shows a maximum concentration between 6 and 7 μg/l and a mass recovery around 0.05%. The uranine was also detected above the tracer detection threshold (0.02 μg/l) at Saivu spring (Fig. 2.33c), which is the main perennial outlet of the system (Fig 2.27).

When considering long term measurement of fluorescence in the GR gallery, uranine persisted for a prolonged period after the first experiment (Fig. 2.34b) at S1. The concentration decreased slowly and reached 2 μg/l 8 weeks after the first experiment. A second peak of uranine (3.5 μg/l) is observed 6 days after the first peak. No rainfall events are observed before and during this peak. Discharge measurements are missing for S1, however measurement at S3 (Fig. 2.34a) and S2 do not indicate any variation of the discharge. As for the first experiment, uranine persisted for several days at S3 after the second experiment.

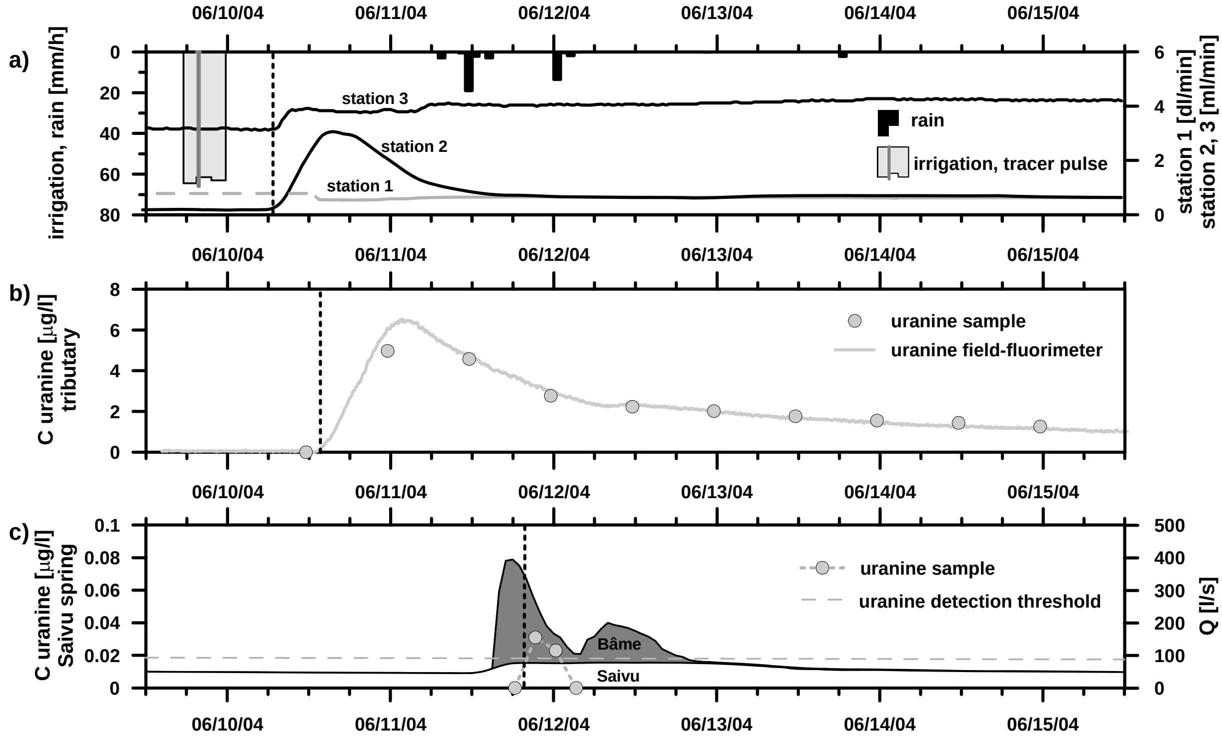


*Figure 2.33: Second experiment performed in June 2004. a) irrigation intensity at surface and hydraulic reaction monitored in the cave b) uranine concentration measured at the end of Gratte-Roche tributary c) discharge and uranine concentration at main springs. Dashed line corresponds to the beginning of the discharge reaction and uranine detection. The discharge curve is divided between Saivu spring discharge (white surface) and Bame spring discharge (grey surface).*

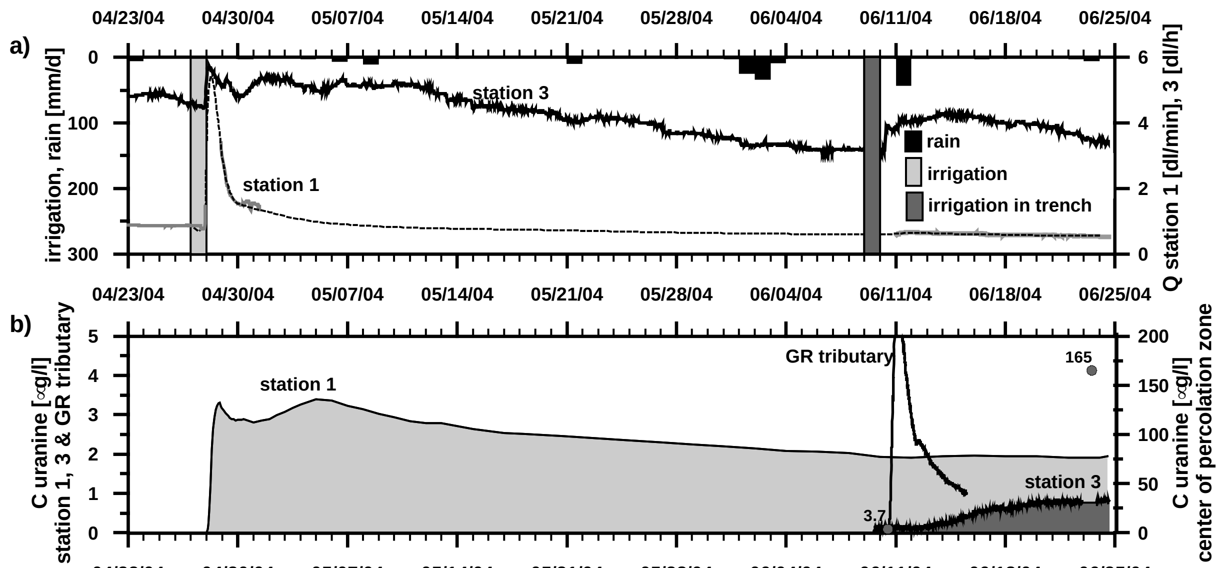


*Figure 2.34: Observations performed in Gratte-Roche percolation zone during the entire period of observation. a) rainfall, irrigation experiment and discharge measurement at stations 1 and 3, b) evolution of uranine concentration at station 1, 3 and GR tributary*

### 2.5.5 Interpretation and discussion

In the GR gallery, in short term, discharge variations are generally not correlated with rainfall events except in case of elevated rainfall events associated with low PET. In long term, the measurement in the GR gallery indicated a strong seasonal effect of the discharge with base flow during summer period in parallel with the increasing PET. According to several authors (Chapman, 1992; Caballero et al., 1996; Perrin, 2003; Vesper and White, 2004), the storage is likely located in the upper part of the unsaturated zone, in the soil and epikarst zones. In case of large recharge events, preferential flow paths in the LPV of the unsaturated zone are activated due to an exceeding of epikarst storage capacity and a discharge increase is observed in the cave. The base flow discharge sustained during long periods suggests substantial groundwater storage in the unsaturated zone. In case of high water deficit in the unsaturated zone, elevated recharge events more than 80mm/week are not sufficient to create a discharge increase in the GR gallery and flood event in the Milandrine river are less than 400l/s (Fig. 2.30). This effect is observed for the October 2003 recharge event after a period of elevated PET.

The two tracing experiments confirmed the connection between the area located along the planned road and the percolation zone located in the Gratte-roche gallery 45 meters above. The absence of a hydraulic reaction and tracer detection at S1 during the second experiment suggests that the catchment areas of S1 and S2-S3 are partially independents. Overflows between the two reservoirs are not excluded during high recharge conditions

Despite the high amount of water infiltrated during each experiment (> 250 mm), sufficient to create a discharge increase in the GR gallery, very low tracer recoveries (< 1%) were observed. For the first experiment, very low concentrations, compared to the injection solution (45000 μg/l), close to 3 μg/l, are observed at station 1 during several weeks after the experiment with a recovery rate of 0.003% (Fig. 2.33b). A higher mass would be calculated when considering a longer period however the total recovery would be anyway inferior to 0.1 % even after several months. The absence of sulforhodamine B is likely due to an elevated sorption in the epikarst and LPV of the unsaturated zone. For the second experiment, the tracer recovery at the end of the GR tributary (0.05%) (Fig. 2.33b) is clearly higher than the data obtained during the first experiment (0.003%), but remains very low. The concentrations observed at Saivu spring (0.024 μg/l) are also coherent with those measured at the end of the tributary (6.5 µg/l) considering a dilution factor of 300 (flow rates = 0.8 l/s in the GR tributary and 240 l/s for the karst system discharge area). These very low recovery rates are attributed to a strong dilution effect in the epikarst and probably to the recharge of the less permeable parts of the epikarst.

The two peaks of uranine observed at S1 after the first tracing experiments are likely due to a contribution of water through different flow paths. The first peak is likely due to a direct contribution of the epikarst reservoir through preferential flow paths in the more permeable parts of the LPV of the unsaturated zone and the second delayed peak of uranine 6 day later to a contribution of the epikarst reservoir through less permeable parts in the LPV of the unsaturated zone. For the first experiment, the delay between the discharge increase and the uranine detection at S1 (3.5 hours) is due to a piston effect linked to the expulsion of water stored in the LPV of the unsaturated zone. For the second experiment a similar dual response can be observed. A rapid arrival of uranine occurs at GR tributary likely via the percolation zone located beetwen S2 and S3, 2 hours after the discharge increase. This zone is activated by overflow of the epikarst and flow via preferential flow paths. Later uranine also arrives at S3 likely through unsaturated LPV.

A similar behaviour is observed in the Milandrine river (see chapter 2.3). During flood events, a first increase of the TOC concentration is observed, due to a rapid contribution of the epikarst water to the discharge through the conduits. A second delayed increase of the TOC is observed due to the delayed contribution of the epikarst water through the LPV.

Based on results a conceptual hydraulic model of the unsaturated zone above the GR gallery can be developed. The major part of the water is stored in the soil and epikarst zone. The epikarst contributes to the discharge by seepage flow through the LPV of the unsaturated zone. A considerable amount of slowly percolating water is flowing through the LPV of the unsaturated zone (Fig 2.35 a, b). A strong seasonal effect of the discharge with base flow during summer period (Fig 2.35a) and high flow during winter (Fig 2.35b) is observed. The volume of the groundwater storage allows sustaining base flow during long period. Discharge is generally not correlated with rainfall events except in case of elevated rainfall events associated with low PET. In this case preferential flow paths in the LPV of the unsaturated zone are activated due to an exceeding of epikarst storage capacity (Fig 2.35c). In case of high water deficit in the system, elevated recharge events more than 80mm/week are not sufficient to create a discharge increase in the GR gallery and flood event in the Milandrine river are less than 400l/s.

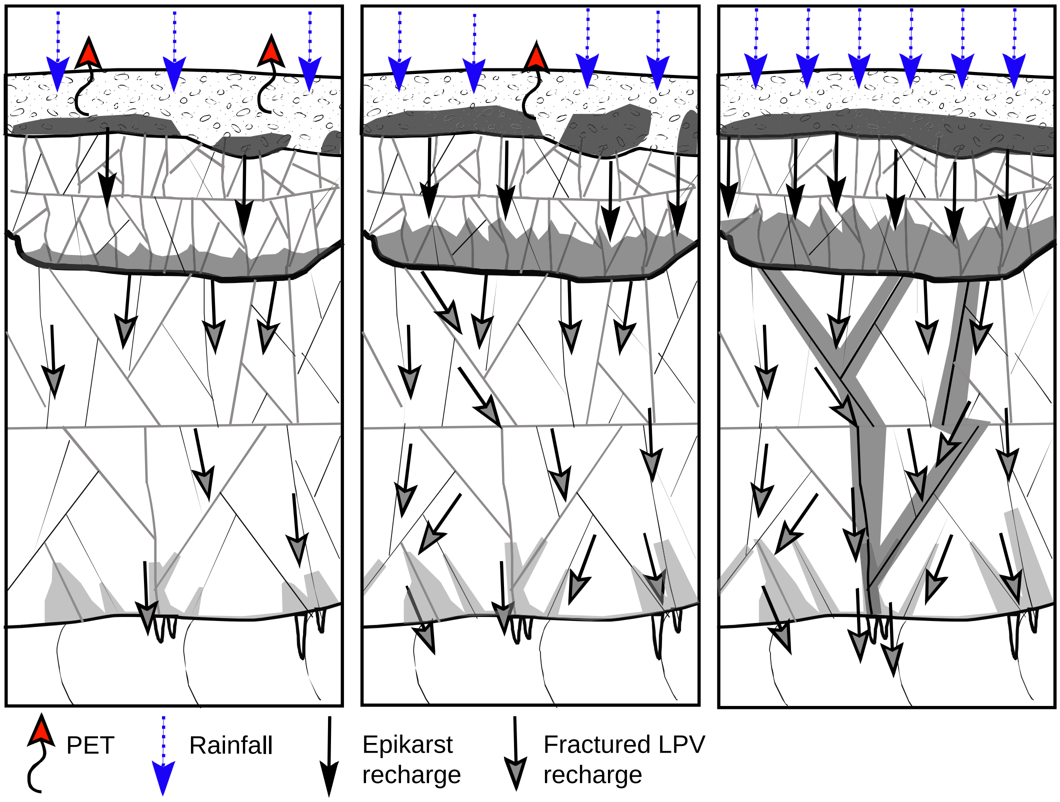


*Figure2. 35: Conceptual hydraulic model of the unsaturated zone above the GR gallery. A) base flow conditions with low recharge and elevated PET; b) High flow conditions with elevated recharge and low PET; c) Large recharge event with preferential flow paths through the LPV of the unsaturated zone.*

### 2.5.6 Conclusion

The long term discharge measurements at percolation zones located at the base of the unsaturated zone of karst systems coupled with specific tracing experiments makes it possible to get a detailed picture of the critical importance of water storage, preferential flow paths and buffer capacity in the epikarst and the LPV of the unsaturated zone.

More specifically, the data obtained during this study allowed to develop a conceptual model of flow and transport in the unsaturated zone. The elevated buffer effect of the epikarst and the LPV of the unsaturated zone is illustrated by a dampened hydraulic response, and low tracer recovery. In this case, the contribution of the soil zone to the buffer capacity of the system is clearly lower compared to the epikarst and the LPV of the unsaturated zone. Indeed the epikarst and specially the LPV in this part of the catchment area present an extent over more than 40 m thickness and therefore have a higher buffer capacity than the soil zone (2 to 3 meters thick). The discharge in the percolation zone is dependent on the rainfall and the PET. It is characterized by a seasonal cycle with discharge increase during winter corresponding to higher rainfall and low PET. In some exceptional cases when the recharge capacity is exceeded a direct reaction of the discharge in response to heavy rainfall is observed.

In addition, these experiments make it possible to characterize the flow paths of water during base flow conditions and high flow conditions, as for the measurement of TOC which make it possible to distinguish the flow paths of the pre event-water and the post event-water. During high flow conditions, the water flows through the unsaturated LPV and preferential flow paths are activated. Rapid and slow tracer responses are observed at the base of the unsaturated zone. During base flow, the discharge corresponds essentially to seepage flow from the LPV.

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# Chapter 3

# Use of reactive tracers to study biodegradation

# 3 Use of reactive tracers to study biodegradation

This part of the study focussed on the biodegradation of artificial compounds in the unsaturated zone of karst systems. As explain in the chapter 2, to study the biodegradation of contaminants in the karst system, a detailed knowledge of the hydraulic behaviour of the unsaturated zone under variable hydrogeological conditions is needed. Once the location of the storage, the transit time and the flow paths of solutes through the unsaturated zone of the karst system is understood, the biodegradation of specific compounds can be studied.

In order to estimate the biodegradation rate of specific compounds, continuous monitoring of artificial biodegradable tracers was carried out at several test sites during sprinkling experiments. A method was developed in order to inject the artificial reactive tracers as conventional conservative tracers. While natural tracers have been used for decades to study transport of solutes related to limestone dissolution (e.g. Bakalowicz, 1979; Mudry, 1987; Plagnes, 1997; Batiot, 2002; Maloszewski et al., 2002; Mudry et al., 2002; White, 2002; Celle-Jeanton et al., 2003) or more recently dissolved organic carbon (e.g. Bakalowicz, 1979; Plagnes, 1997; Emblanch et al., 2003; Batiot, 2002; Pronk et al., 2006) the use of reactive tracers is a relatively new research area in karst systems. Reactive tracers are compounds that are injected into the subsurface to study transformation processes. Transformation processes can be assessed based on the production of a characteristic stable intermediate. If the reactive tracers are transformed to non-unique products, they are injected in combination with non-reactive compounds. Reactive tracers have been used to gain insight into the general biological activity in porous aquifers or to evaluate if specific contaminants are degraded. Examples for these use of reactive tracers is the injection sodium benzoate, ethanol, hexanol and pentanol to study the spatial variability of in-situ microbial activity in a sandy aquifer (Sandrin et al., 2003), injection in soil of de-icing chemicals containing propyleneglycol, also known as 1,2-propanediol, and potassium acetate to study the degradation of these specific compounds (French et al., 2001).

The occurrence of biological activity in the unsaturated zone of karst systems was evaluated using reactive tracers at two test sites: Vers-Chez-le-Brandt and Gänsbrunnen gallery. The main test substances that have been used were (i) lithium acetate (LiAc) and (ii) ethylacetate (EAc) that have a low toxicity to ensure approval from regulatory agencies for injection into the subsurface. The test substances are transformed to characteristic products, which provide information about microbial activity in karst infiltration systems along the flow paths of the water. These reactive tracers (LiAc or EAc) were injected in the soil surface of the two test sites during summer 2004 and 2005. In parallel, conservative tracers (Bromide, uranine) were injected. The degradable compounds and the conservative tracers ($Br^-$ or Uranine) were dissolved in water and pulse-injected during artificial steady state conditions. The steady state condition was obtained by watering with constant flow in the karst surface. Constant flow rate was maintained during the flow establishment, pulse tracer injection and breakthrough curves recession. Breakthrough curves of conservative tracers and reactive were observed in underground outlets. Two methods allowed to estimate the first order biodegradation rate of the injected compounds. The first method is based on the comparison between the concentrations of the reactive compounds versus conservative tracers. The results of these tracing experiments have been summarized in one paper presented below: Estimation of degradation potential in unsaturated zone of karst system using artificial reactive tracers. The second method tested is based on the measurement to the shift of the carbon isotopic composition during biodegradation.

## *3.1. Comparison of reactive tracers with conservative tracers*[1]

### 3.1.1. Introduction

As discussed in the introduction chapter, most of the studies dealing with reactive processes in karst aquifers have focused on mineral dissolution (e.g. Plummer and Wigley, 1976; White, 1977; Buhman and Dreybrodt, 1985a and b; Dreybrodt, 1988; Palmer, 1991; Groves and Howard, 1994a and b; Dreybrodt, 1996; Siemers and Dreybrodt, 1998; Zaihua and Dreybrodt, 1998; Dreybrodt and Eisenlohr, 2000) and there are few studies that deal with chemical and microbial transformation of organic compounds and contaminants in karst aquifers. The studies performed so far have mainly focused on transformation of nitrogen compounds (e.g. Kastrinos and White, 1986; Dubreucq, 1987; Montandon et al., 1995; Panno et al., 2001; Perrin, 2003).

As describe in chapter 1, little is known about the potential for degradation of organic compounds in karst aquifers and the biological activity in karst aquifers in general. Some information on the occurrence of biological transformation processes in karst aquifers can be derived from pesticide studies (Kozel and Garazi, 2001). The detection of pesticide degradation products in some spring waters (Börger and Poll, 1998) and the absence of pesticides in groundwater of poorly karstified areas (Goody et al., 2001) suggest that transformation and/or retention occur during transport across soil, epikarst and unsaturated zone. Furthermore, mass balance calculations for a catchment area of a karst spring indicated that more than 99% of the applied pesticides are retained or degraded (Börger and Poll, 1998). However, little is known about where and to what extent transformation processes occur. Microcosm studies with material from the unsaturated zone of a chalk aquifer in England, demonstrated that microorganisms capable of pesticide transformation are present in the unsaturated zone (Johnson et al., 2000). Generally, it is assumed that pesticides may sorb or

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[1] **Estimation of degradation potential in unsaturated zone of karst system using artificial reactive tracers** Ludovic Savoy[1], Daniel Hunkeler[1]

be transformed in the LPV with a relatively low permeability and long water transit time while they migrate fast through conduits (Kozel and Garazi, 2001). However, this assumption has not been experimentally verified.

The aim of this study was to develop and test a method in order to assess and, if possible, quantify the biological activity in the unsaturated zone of karst aquifers. The elevated heterogeneity of the karst aquifers and the complexity of water sampling and monitoring in the unsaturated zone implies high difficulties to get solid samples for laboratory studies and to install representative in situ microcosm experiments. A method based on the use of reactive tracers (e.g. Barker et al., 1987; French et al., 2001; Sandrin et al., 2003) was used for this study. This method has the advantage that the reactive tracers can be injected in the system like for conventional tracing experiment (e.g. catchment limits determination).

In this study, the developed method in order to evaluate the biodegradation was based on the comparison of relative concentration between reactive and conservative tracers. Between years 2004 and 2005, during separated field experiments, at two site (Vers-Chez-le-Brandt and Gansbrunne cave) reactive and conservative compounds were injected on the soil surface. Breakthrough curves of conservative tracers and test substances were observed on vadose flows issued from fissures at the cave/gallery roof. The reactive test substance used for this study was lithium acetate (LiAc). This reactive tracer was dissolved in water and pulse-injected with conservative tracers (bromide or uranine) during artificial steady state conditions. The steady state conditions were obtained using an automatic rainmaking device specially designed for these experiments. The steady state condition was maintained during the flow establishment, pulse tracer injection and breakthrough curves tailing. Based on the transit time and the concentrations of the reactive compounds relative to the conservative tracers, first order degradation rates (K) were estimated, and the minimal degradation rates that can be detected with this approach was evaluated.

## 3.1.2. Material and methods

### *Field site description*

The Vers-chez-le-Brandt cave (Fig. 3.1) is located in the unsaturated zone of the Areuse karst system (Swiss Jura). The total thickness of the unsaturated zone is about 300 m and the catchment size is on the order of 130 $km^2$. The mean discharge of the Areuse spring is 4.5 $m^3/s$. At the local scale, the epikarst is covered by less than 1m soil and the unsaturated zone is 30 meters thick. Vers-chez-le-Brandt groundwater is sampled and monitored on a vadose perennial flow issued from a fissure at the cave roof. It is located 30 m below the ground surface; the discharge at low stage is 0.4 l/min, corresponding to a basin of 100 $m^2$ (Perrin, 2003).

The artificial Gänsbrunnen galleries, which are owned by the Canton of Solothurn, was selected as comparative test site in order to evaluate the importance of the soil zone and transit time for biodegradation. The site has a thin soil layer, less than 10 cm. The galleries are dug in fractured limestones and the hydraulic boundary conditions are well-defined due to the location of the galleries on the top of a hill. At the experimental zone, the galleries are located 10 m below the ground. As for Vers-chez-le-Brandt site, the groundwater is sampled and monitored on a vadose flow issued from a fissure at the gallery roof.

### *Experimental settings*

Preliminaries tracing experiments were carried out on the two test sites in order to delimit the water catchment area of the sampling location in the cave and to minimize water loss to other local groundwater catchment during irrigation experiments. An automatic rainmaking device was designed (Fig. 3.1 and 3.2) in order to provide a uniform water flux to the soil surface. The rainmaking device was constructed with a sprinkler traditionally used for garden watering.

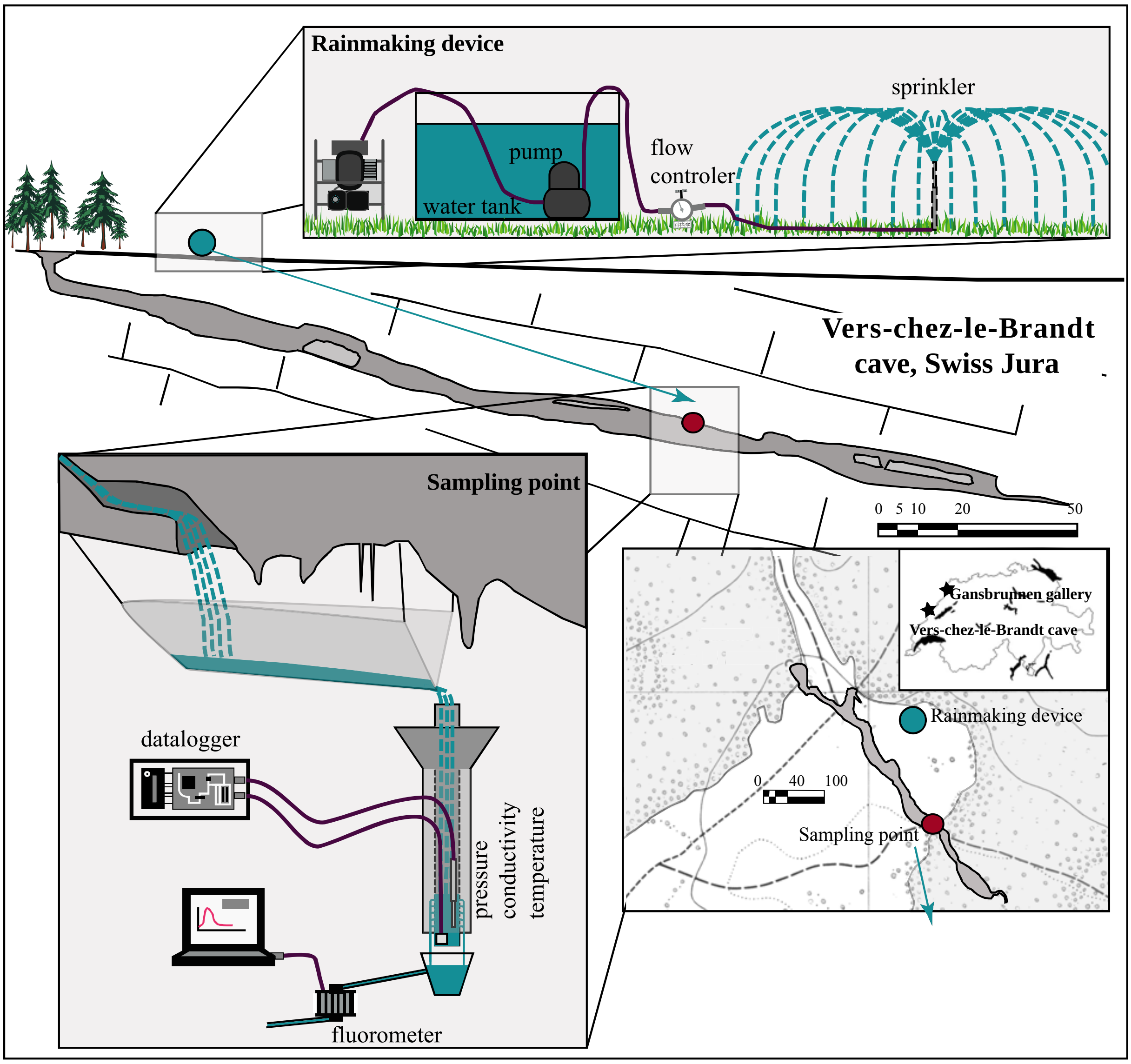


*Figure 3.1: Location of the Vers-Chez-le-Brandt and Gänsbrunnen test sites. Cross section of the Vers-Chez-le-Brandt cave with position of the sampling location. Schematic representation of the rainmaking device and sampling system.*

The sprinkler was installed 50 cm above the soil surface. The water distribution along the spray was uniform. A pump immerged in a 7m$^3$ water tank (separated in two tanks) supplied the water to the rainmaking device. The discharge rate was set at a defined value using a valve and verified using a flow meter. Experiments with different flow rates were conducted. The artificial rain intensity was measured by dividing the watering surface by the irrigation discharge rate. The experiments were carried out during a dry period in order to avoid perturbation due to rainfall events. The rainmaking device thus created a relative constant flux to the watering surface and imposing artificial steady-state conditions on the vadose flow in the cave. During all experiments, constant flow rates were maintained to the surface in order to avoid changes in the discharge rate in the karst system.

*Figure 3.2: Picture of the rainmaking device (left) and sampling location (right)*

Before the tracer application, water was sprinkled at the desired flow rate until steady state conditions were obtained in the cave. The tracer solution consisted of a mixture of conservative (fluorescent and anions) tracers and a reactive tracer. Tracer application took place without changing the discharge rate.

At both sites sampling and tracer monitoring took place in the cave on a vadose flow issued from a fissure at the cave roof (Fig. 3.1 and 3.2). Groundwater was collected in a perforated PVC tube equipped with pressure probe. The discharge is obtained from the water level in the tube after determination of the water level-discharge relationship from gauging experiments. The electrical conductivity was measured and recorded every 5 minutes using a portable field electrical conductivity meter (WTW 340i) with internal data logger. The fluorescent dye breakthrough curves were obtained using a flow-through fluorometer (GGUN-FL30, University of Neuchâtel, Switzerland) connected to the water tube. The fluorescence signal was recorded every minute. A computer, connected to the fluorometer, allowed to follow the fluorescent breakthrough curve and to adjust the sampling spacing rate. 500ml samples for analyses of reactive tracers were manually collected in glass bottle and immediately basified to pH 11 with NaOH (1 M) to stop degradation and hermetically closed. The samples were kept refrigerated for later analysis. For comparison, and in order to evaluate the potential degradation of the reactive tracer in the water out of the karst system, control samples were taken and stored without basification and refrigeration. The sampling for conservative tracers was automated by using a programmable autosampler (Teledyne Isco, USA) that collected 400 mL samples.

### *Tracers selection*

Two conservative tracers, one anion ($Br^-$) and one fluorescent dye (uranine), and one reactive tracer, Acetate$^-$ ($Ac^-$) were selected for the experiments. $Br^-$ is considered as conservative groundwater tracers (Davis et al., 1985). The background concentration of $Br^-$ was null. The uranine is the most conservative fluorescent dye tracer and is easily detectable (Käess, 98). It was applied during the first experiment (Table 1). The $Ac^-$ was injected as reactive tracer for the three experiments. Lithium acetate, hydrated ($LiC_2H_3O_2.2H_2O$ (Table. 1) was dissolved in water before the experiment. The reactive test substance that was used for this study has a low toxicity to ensure approval from regulatory agencies for injection into the subsurface. This reactive tracer was chosen for his high degradation potential.

### *Chemical analysis*

$Br^-$ and $Ac^-$ concentrations were determined by ion chromatography (DX-120, Dionex, USA) with a detection limit of 0.1 mg/L. Before the analyses, the samples were filtered through 0.45 um filters. Uranine was measured in the field with a fluorometer (GGUN-FL30, University of Neuchâtel, Switzerland) and additional measurements of uranine were conducted in the laboratory with a spectrofluorometer (LS50B, PERKIN ELMER, USA) to calibrate the field fluorometer.

### *Calculation of first order rate coefficient*

In order to calculate the first order degradation rate (k), the karst system was simplified as a reservoir overflow under constant steady state recharge conditions. The first order degradation rates were calculated during the recession period of the breakthrough curves. The method used for the determination of the first order degradation rate (k) is developed on the basis of the theory of Continuously Stirred Tank Reactor (CSTR) as an analogue for the groundwater storage in the epikarst zone (Field, 2002).
The dynamics of the CSTR can be described by a mass balance equation (Silebi and Schiesser, 1992):

$$\frac{d(M)}{dt} = -QC - VkC \tag{1}$$

the solution to equation (1) is

$$\frac{C}{Co} = e^{-\left(\frac{Q}{V}+k\right)t} \tag{2}$$

with $(\frac{Q}{V} + k)$ corresponding to the slope of the breakthrough curves of the reactive tracer plotted on a natural logarithmic scale in function of time and $(\frac{Q}{V})$ to the slope of the conservative tracer.

The first order degradation rate (k) corresponds to the difference between the two slopes:

$$k = Sr - Sc \tag{3}$$

With *Sr*, the slope of the reactive tracer and *Sc* the slope of the conservative tracer
Moreover the volume of the reservoir can be calculated with:

$$V = \frac{Q}{\ln Cc(t)} \tag{4}$$

The statistical significance of differences among the slopes was calculated according to Student's *t* test. The statistic test was computed as the difference between the two slopes (b1 and b2) divided by the standard error of the difference between the slopes (Sb1-b2) on (N-4) degrees of freedom.

$$t_{obs} = \frac{b_1 - b_2}{S_{b_1 - b_2}} \tag{3}$$

The standard error of the slope is given by the Excel regression module and the standard error of the difference between the slopes is given by:

$$S_{b_1 - b_2} = \sqrt{S_{b_1}{}^2 - S_{b_2}{}^2} \tag{4}$$

The calculated $t_{obs}$ values are compared to a t table at (n1 + n2 -4) degrees of freedom (d.f) for 95% (P > 0.05) confidence interval (bilateral distribution).

### *Experiments*

Three tracing experiments were conducted between years 2004 and 2005 with different flow rates. The Table 1 summarizes the different experiments with injected tracer quantities and flow rates. The watering surfaces were 20 and 28 $m^2$ for the two experiments at Vers-chez-le-Brandt site with flow rates corresponding to a rain intensity of 19 mm/h and 15 mm/h respectively**.** At Gänsbrunnen test site, the watering surface was 6 $m^2$ corresponding to an elevated rain intensity of 54 mm/h.

*Table 3.1: Description of the three experiments with injected mass of reactive and conservative tracer, discharge and corresponding rain intensity and finally the experiments duration. The experiments 1 and 2 were realized at Vers-Chez-le-Brandt and the third one at Gänsbrunnen.*

| | Date | Uranine [mg] | Br- [g] | Ac- [g] | Inj. flow [l/min] | Rain [mm/h] | Infiltration [hours] |
|---|---|---|---|---|---|---|---|
| **Exp. 1** | 16.09.2004 | 329 | - | 177.5 | 6.3 | 19.0 | 14.2 |
| **Exp. 2** | 27.09.2005 | - | 386 | 518.5 | 7.0 | 15.0 | 13.2 |
| **Exp. 3** | 11.11.2004 | - | 40 | 40 | 5.4 | 54.0 | 7.7 |

## 3.1.3. Results and discussions

### *Vers-Chez-le-Brandt, experiment 1*

On September 16th and 17th 2004, a mean constant flow rate of 6.3 l/min was applied on the soil surface during 855 minutes. A flow increase in the cave was observed 55 min after the beginning of the irrigation and a constant flow rate was reached 100 minutes later (Fig. 3.3).The mean discharge in the cave during semi-permanent flow was 5 l/min and 70 % of the injected water was recovered. The irrigation was stopped after 855 minutes and a total of 5435 litres of water was applied. Due to a dysfunction of the pressure probe, the tailing of the discharge curve after the experiment was estimate from manual gauging. The difference between the volume of the injected water and the volume of the recovered water is due to groundwater storage in the soil and epikarst. Indeed the experiment followed a sunny period of 20 days with small rainfalls (11 mm) and high soil water deficit. The evapotranspiration during the experiment is negligible compared to the sprinkled water intensity (19 mm/h). The injection of a 50 litres solution containing reactive ($LiAc2H_2O$) and conservative (uranine) tracers was realized 205 min after the beginning of the irrigation during semi-permanent flow (Fig. 3.3). The solution contained an initial concentration of 3.55 g $Ac^-$/l in the form of $LiAc2H_2O$ and $6.6*10^{-3}$ g uranine/l.

The first arrival of uranine in the cave was observed 65 minutes after the injection of the tracer solution, and the maximum concentration 55 minutes later (Fig. 3.3). The samples were collected at 10 min intervals immediately after tracer injection. After 3 hours, the sampling interval decreased to 1 hour intervals.

The irrigation was stopped 660 min after the injection of the tracers and the sampling was carried out during 12 hours until base flow discharge was similar to conditions measured before the experiment. The last sample was collected 1400 min after the injection of the tracers.

$Ac^-$ decreased more rapidly than uranine during the tailing of the breakthrough curves. This decrease indicated an $Ac^-$ degradation during transport in the unsaturated zone of the aquifer. $Ac^-$ was below the detection limit at the end of the experiment. The cumulative recovery is 55.5% for $Ac^-$ and at least 74.5 % for Uranine (Fig. 3.3). Due to the presence of uranine at the end of the experiment (uranine = 16.4 ppb) the observed cumulative recovery for uranine is under evaluated. The first order degradation rate calculated based on the slopes of Ac- (-10.49 $day^{-1}$) and uranine (-3.39 $day^{-1}$) was 7.1 $day^{-1}$ (Fig. 3.3). The calculated volume of the reservoir is 2.676 m3 (49% of the injected water) corresponding to a stock of 133.8 mm of water. The Student test gives a $t_{obs}$ value of 7.13, significantly different than the value on a Student *t* table (2.056, 95% of confidence interval and 26 degree of freedom) and the difference between the slopes is thus highly significant.

### *Vers-Chez-le-Brandt, experiment 2*

On September 27th 2005, a repetition of the 2004 experiment was conduct with a mean constant flow rate of 7 l/min applied on the soil surface during 793 minutes. The aim of the experiment was to use an other conservative tracer than for the first experiment. A flow increase in the cave was observed 68 min after the beginning of the irrigation and a constant flow rate was established 172 minutes later. The mean discharge in the cave during semi-permant flow was 5.3 (l/min) and 62.6 % of the injected flow was recovered. The irrigation was stopped 793 minutes after the beginning of the experiment with an injection of 5450 litres of water. The injection of conservative and reactive tracers was carried out 200 min after the beginning of the irrigation during semi-permanent flow (Fig. 3.4). The 54 litre test solution contained an initial concentration of 7.1 g $Br^{-}$/l in the form of NaBr and 9.6 g$Ac^{-}$/l in the form of $LiAc2H_2O$.

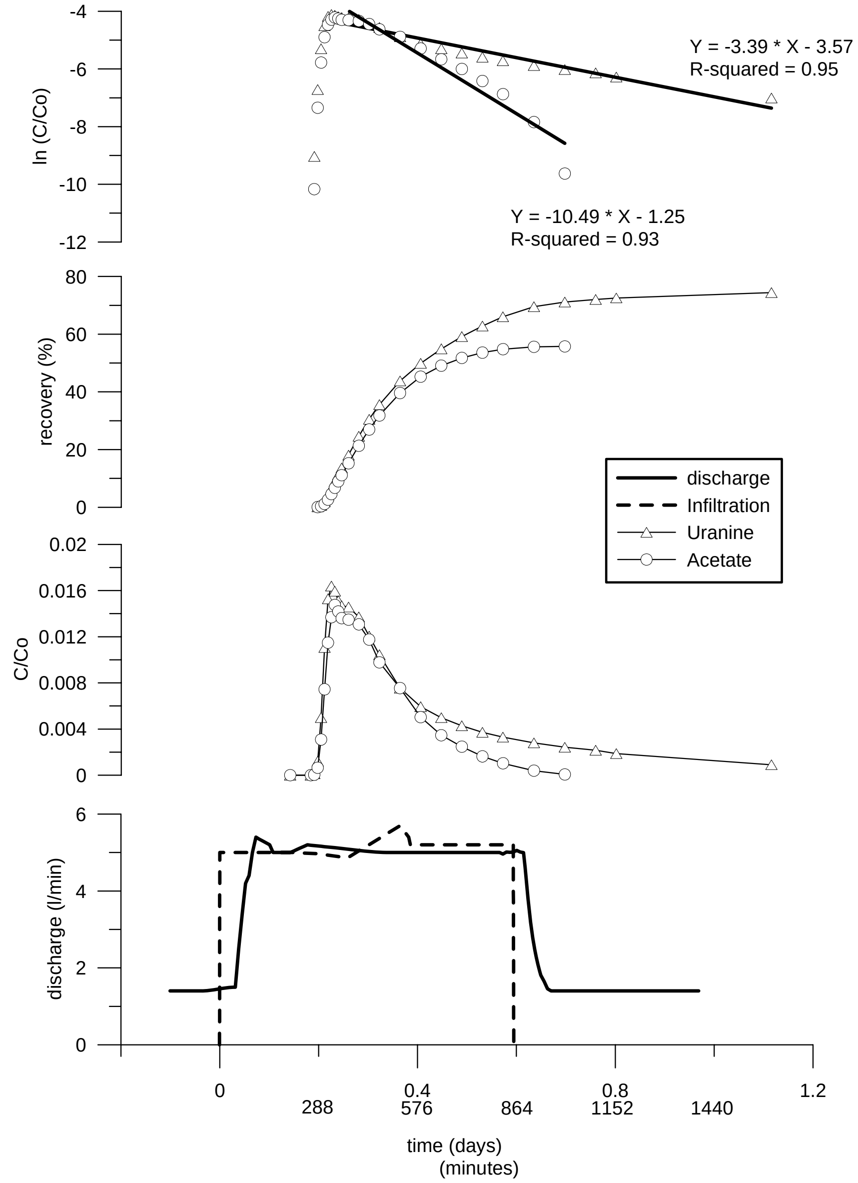


*Figure 3.3: Results of the September 2004 experiment. Infiltrated discharge and recovered discharge in the cave. Normalized concentration of the recovered tracers. Recovery (%). Ln curves of the reactive and conservative tracers with k obtained with lnCr(t) – lnCc(t)*

The first arrival of test solution in the cave was indirectly observed by the electrical conductivity increase 80 minutes after the tracer injection, and the maximum concentration 49 minutes later (Fig. 3.4). Samples were collected at 10-min intervals immediately after the tracers pulse. 150 minutes after injection the sampling interval was increased to 30 minutes intervals. With the same interval, samples were taken and stored without basification at room temperature in order to evaluate the degradation of the reactive tracer in the water. The irrigation was stopped 600 min after the injection of the test solution and the sampling was carried out during 2 hours more. The last sample was collected 706 min after the tracers injection.

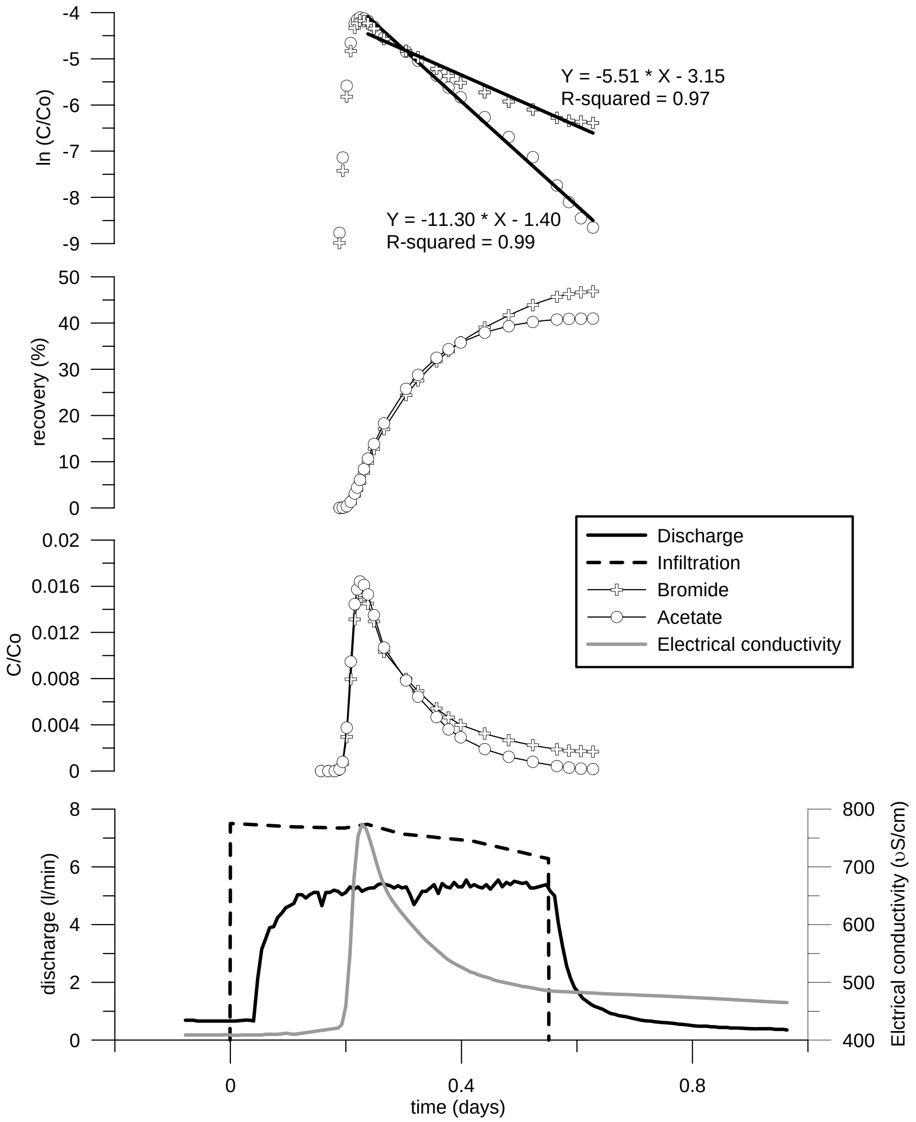


*Figure 3.4: Results of the September 2005 experiment. Infiltrated discharge and recovered discharge in the cave. Normalized concentration of the recovered tracers. Recovery (%). Ln curves of the reactive and conservative tracers with k obtained with lnCr(t) – lnCc(t)*

The timing of this experiment was shorter than for the first experiment due to a more elevated infiltration rate. Indeed the $Ac^-$ was detected in the cave 18 minutes after the tracer injection, shorter than the 45 minutes measured during the first experiment. As for the first experiment, a more rapid decrease of the Ac- is observed during the tailing of the breakthrough curve compared to the conservative tracer. The tracer analyses in the laboratory indicate that no $Ac^-$ was present in the system at the end of the experiment. At the end of the experiment $Br^-$ was no totally recovered and we observe elevated values ($Br^-$ = 12.0 mg/l) compare to the initial values measured before the experiment (less to 0.05 mg/l). The cumulative recovery is 41% for $Ac^-$ and minimum 47 % for $Br^-$ (Fig. 3.4). The calculated first order degradation rate based on the slope of $Ac^-$ (-11.30 $day^{-1}$) and bromide (-5.51 $day^{-1}$) was 5.79 $day^{-1}$ (Fig. 3.4). The calculated volume of the reservoir is 1.803 m3 (32.5% of the injected water) corresponding to a stock of 63.7 mm of water. The Student test gives a $t_{obs}$ value of 15.85 significantly different than the value on a Student *t* table (2.056) and the difference between the slopes is then thus highly significant.

The calculated first order degradation rate (k) is in the same range as the one obtained at the same site during the first experiment and suggests that the biological activity is fairly constant.

No significant difference in Ac- concentration was observed between unpreserved samples at room temperature and basified refrigerated samples. This indicates that biodegradation occurs rather due to an interaction of the substance with the soil and epikarst subsystem than during storage in the sampling vials.

### *Gänsbrunnen, experiment 3*

Similar experiment was carried out at Gänsbrunnen test site in order to evaluate the importance of the soil zone and transit time for biodegradation. A constant infiltration rate of 54 mm/h was applied during 460 min corresponding to 2480 litres of water. The injection of conservative and reactive tracers was started 100 min after the beginning of the irrigation during semi-permanent flow. A 3 litre test solution containing an initial concentration of 13.3 g $Br^-$/l in the form of NaBr and 13.3 g$Ac^-$/l in the form of $LiAc2H_2O$ was injected.

The irrigation was stopped 360 min after injection of the test solution. Sampling was carried out at one percolating fissures on the roof of the gallery. The first arrival of test solution in the cave was observed 8 minutes after the tracer injection, and the maximum concentration 16 minutes later. Samples were collected at 10-min intervals (Fig. 3.5). The first order degradation rate calculated based on the slope of $Ac^-$ (-26.87 $day^{-1}$) and the slope of bromide (-19.70 $day^{-1}$) corresponds to 7.2 $day^{-1}$ (Fig. 3.5).

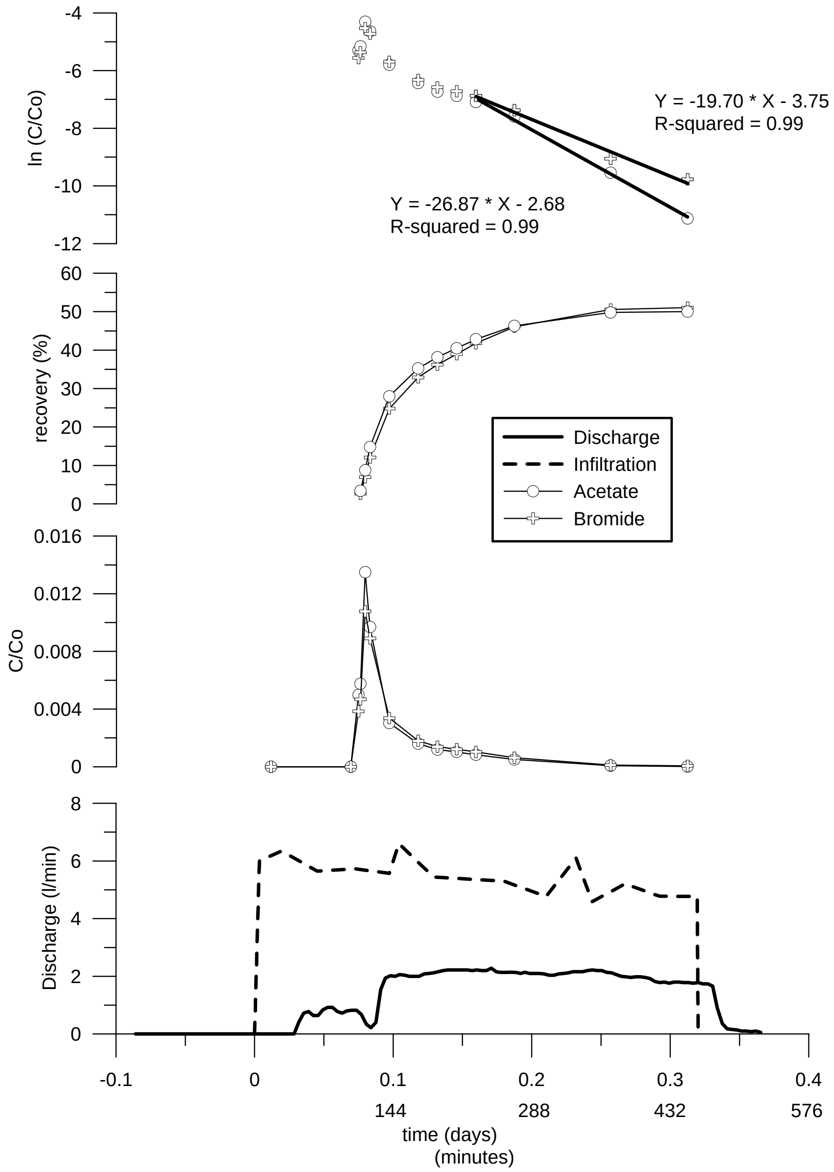


*Figure 3.5: Results of the Gänsbrunnen experiment. Infiltrated discharge and recovered discharge in the cave. Normalized concentration of the recovered tracers. Recovery (%). Ln curves of the reactive and conservative tracers with k obtained with lnCr(t) – lnCc(t)*

The cumulative recovery is 50.0 % for $Ac^-$ and 51.0 % for $Br^-$. The calculated volume of the reservoir is 0.410 $m^3$ (16.5 % of the injected water) corresponding to a stock of 68.3 mm of water. The Student test gives a $t_{obs}$ value of 2.7, near the corresponding value on the Student *t* table (2.306). The elevated transit time of the tracers allowed to calculate a degradation only for the last four sample of the breakthrough curves. At Gänsbrunnen test site, for more elevated infiltration rates, calculation of the first order degradation rate k will be imprecise.

As demonstrated by the three experiments, the biodegradation rate is similar for different test sites but the amount of biodegradation is higher if the storage is longer. The relative good match of the linear regression and the reasonable storage/mixing volumes that were obtained confirm that the conceptual model is appropriate. The mathematical model was chosen according to a specific conceptual model that was developed in previous section of the thesis. The karst system is simplified as a reservoir overflow under constant steady state recharge conditions based on the theory of Continuously Stirred Tank Reactor (CSTR). Several authors (e.g. Yonge, 1985; Chapman, 1992; Caballero et al., 1996, Perrin et al. 2003; Aquilina et al., 2005) have describe the epikarst sub-system as an important groundwater sub-systems for storage in the karst system. The proposed model considers the epikarst as the location of the storage during the experiments. The steady state irrigation conditions create an overflow of the reservoir in the vertical drainage conduits. The tracers solution injected on the soil surface migrates through the unsaturated soil and epikarst and reach the saturated epikarst reservoir. The epikarst reservoir presents a relative uniform tracer concentration and overflows in the vertical drainage conduits. Steady state conditions allow an exponential decrease of the concentration in the reservoir and biodegradation of the reactive tracer. At the end of the experiment, reactive tracer is totally degraded or pushed out of the epikarst.

Using the critical t value of 2.056 for the two experiments at Vers-Chez-le-Brandt (26 degree of freedom and 95% of confidence interval) and the standard errors of the difference between the slopes calculated for the two experiments (exp 1, S= 0.78; exp 2, S=0.37), a minimal difference between the two slopes can be determined. The obtained value is comprised between 0.75 $day^{-1}$ and 1.61 $day^{-1}$and corresponds to the minimal biodegradation rate (k) that could be calculated during these experiments.

The method used for calculation of first order degradation rate (k) allows to estimate the volume of water stored in the epikarst. This volume corresponds to a stock of water varying between 60 and 135 mm for Vers-Chez-le-Brandt cave and 60 mm at Gänsbrunnen gallery. Jeannin and Grasso (1995) have obtained a similar result (140 mm) for a test site located in the Swiss Jura (Milandre cave).

For comparison with other studies, French et al. (2001), for example, estimated a degradation rate for acetate corresponding to 0.02 day$^{-1}$. The biodegradation experiments where realized in porous aquifer during snowmelting in order to expected biodegradation of de-icing chemicals. The value estimated by French et al. (2001) is smaller than the values obtained from the tracing experiments at Vers-Chez-le-Brandt and Gänsbrunnen but as explained by French et al. (2001) the experiments were realized during snow melt conditions with a possible bypass of the potentially most active degradation zone near the surface due to the low temperatures. Sandrin et al. (2003) used sodium benzoate, ethanol, hexanol and pentanol to study the spatial variability of in-situ microbial activity in a sandy aquifer and obtainded first-order biodegradation rate between 0.1 and 0.6 day$^{-1}$ downgradient of the injection wells, and between 0.7 and 2.6 day$^{-1}$ near the injection wells.

### 3.1.4. Conclusion

The proposed method makes it possible to get a more detailed picture of the biodegradation of contaminants in the unsaturated zone of karst aquifers. The comparison between relative concentrations of reactive tracers with conservative tracers is used to determine the first order biodegradation rate of the whole system. The biodegradation rate can be similar for different test sites but the amount of biodegradation will be higher if the storage is longer. The application of different infiltration rate at the Vers-Chez-le-Brandt and Gänsbrunnen test sites demonstrated that during steady state flow, the amount of biodegradation will decrease with an increase of the recharge. Indeed an elevated infiltration rate decreases the duration of storage in the system and then the possibility to the contaminant to be degraded, while a lower infiltration rates increase the available time for biodegradation in the system. The biodegradation occurs due to an interaction of the substance with the soil and epikarst subsystem. These data confirm that biodegradation of contaminants in a karst system is possible but depends on the transit time.

The minimal biodegradation rate (k) that could be calculated during these experiments (value between 0.75 $day^{-1}$ and 1.61 $day^{-1}$) remain high and method the has to be modified in order to measured smaller biodegradation rates. For example, the irrigation could be stopped after the injection of the reactive tracers resulting to an increase of the transit time of the tracer in the unsaturated zone.

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## 3.2. Appendix to chapter 3.1

The first order degradation rate obtained for the reactive tracer experiments (between 5.5 and 7.5 day$^{-1}$) was used in order to simulate the degradation of a reactive tracer under prolonged storage and transit time in the unsaturated zone. The degradation rate calculated during the tracing experiments was tested using a first order degradation equation: $C = Co * e^{-kt}$ with Co corresponding to the concentration of the tracer measured at a time (t) after the tracer injection. The aim of this calculation is to determine if a reactive tracer, injected during the Milandre tracing experiment (Chapter 2.6.4 Fig 2.33), in parallel to the conservative tracers, could have been detected in the GR tributary or S3.

The breakthrough curves obtained for the artificial experiment at Milandre test site were used in order to simulate the restitution of the reactive tracer. For this experiment, on June 9$^{th}$ 2004, an irrigation rate with an intensity of 50 mm/h and maintained during 6 hours was imposed at the soil surface. After 2 hours of sprinkling, the tracer was injected. The tracer was released as a pulse (2 kg as 10% solution). The breakthrough curve at the end of the GR tributary (Fig. 2.27) shows a maximum concentration between 6 and 7 µg/l and a mass recovery around 0.05% (Fig. 2.33b). Two days after the first detection of uranine at the end of the GR tributary, an increase of uranine was measured at S3 and measured during more than 2 weeks (Fig. 2.34). The rapid detection of the uranine at the end of the GR tributary corresponds to an overflow of the epikarst through preferential flow paths and the delayed uranine increase at S3 corresponding to the delayed contribution of the epikarst water through the unsaturated LPV.

The result of the calculation shows that with a first order degradation rate (k) between 5 and 7.5 day$^{-1}$, corresponding to those obtained for the tracing experiments at Vers-Chez-le-Brandt and Gänsbrunnen, the reactive tracer would have been totally degraded before arriving at the end of the GR tributary or S3 (Fig. 3.6). Other breakthrough curves with arbitrary smaller first order degradation rates have been calculated (0.1; 0.05 and 0.005 day$^{-1}$) and indicated that in order to test the biological activity in the unsaturated zone of karst systems with thick soil cover, epikarst and LPV, without well developed conduit network, reactive tracers with small first order degradation rate should be used. The figure 3.6 shows that with elevated degradation rate (k>0.1 day$^{-1}$) the reactive tracer will almost be degraded before the detection in the cave. Even for moderate degradation rate (e.g. 0.05 day$^{-1}$) the substance will be highly degraded before arriving in the cave.

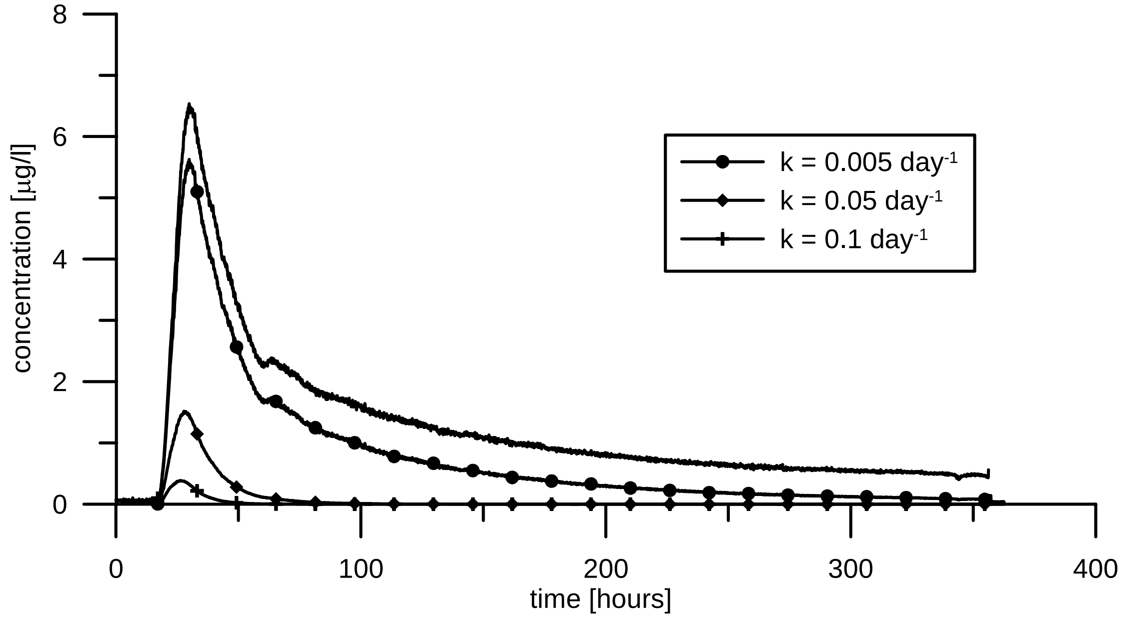


*Figure 3.6: Results of the calculated breakthrough curves with arbitrary first order degradation rates. The curve without symbols corresponds to the conservative tracer.*

## *3.3. Shift in the isotopic $^{13}C$ composition.*

Biodegradation of organic compounds is also often accompanied by shifts in the isotopic carbon composition of the compounds. It has been shown that these shifts can serve as a sensitive method to demonstrate biodegradation and the method was tested at several field sites (Hunkeler et al., 2002a; Hunkeler et al., 2002b; Hunkeler et al., 2001; Richnow et al., 2003a and 2003b; Griebler et al., 2004; Steinbach et al., 2004). In this study, the $^{13}C/^{12}C$ isotope fractionation of reactive tracer was used as an indicator of biodegradation in the unsaturated zone of karst system. In order to limit the influence of the high variability of the hydraulic conditions in the karst system (elevated variation of discharge, water storage in different sub-systems, flow paths heterogeneity) a specific method of tracer injection based on artificially imposed steady state conditions was developed. This method was successfully used for injection of reactive tracer, $Ac^-$ in the form of $LiAc2H_2O$, in parallel with conservative tracers, uranine or Br- as described above. The aim of the experiment was to evaluate if biodegradation in the unsaturated zone can be detected based on changes of the carbon isotope ratio of injected organic compounds.

### 3.3.1. Material and methodology

As describe above, for this experiment, a different approach to the one described in paragraph 3.2.1.b has been used in order to characterize first order biodegradation rates of EAc. The $^{13}C/^{12}C$ isotope fractionation of reactive tracer was used as an indicator of biodegradation. The test solution contained 5 litres of ethyl acetate (EAc) partially dissolved in 54 litres of water containing an initial concentration of 5.4 g $Br^-$/l. The initial $\delta^{13}C$ of the EAc was -31‰. During a field experiment with injection of 3 litres of EAc under similar hydraulic conditions no EAc was detected in the cave. The $Br^-$ was used as conservative tracer. The sampling spacing for EAc was chosen based on the evolution of the electrical conductivity according to the breakthrough curve of $Br^-$.

### *Tracer selection*

Ethylacetate is transformed into Acetate + Ethanol and soluble in water (8 g/100 mL). Ethyl acetate would not be expected to adsorb to sediment or particulate matter. If released on land, ethyl acetate will partially evaporate and partially leach into the ground. Biodegradation will probably occur both in soil and groundwater, however, experimental data are lacking (source: Australia's national database of pollutant emissions http://www.npi.gov.au/database/substance-info/profiles/38.htm, and MSDS).

### *Isotopic analysis*

The carbon isotope ratio of Ethylacetate was extracted using a Tekkmar Purge and Trap system and analysed using a TRACE™ gas chromatography coupled to an ThermoFinnigan™ Delta Plus XP isotope-ratio mass spectrometer via a ThermoFinnigan™ GC combustion III interface.

Carbon isotope ratios are reported in the δ-notation relative to VPDB:

$\delta^{13}C = ((R/R_{std})-1)*1000$ ‰

### *Calculation of first order rate coefficient*

By coupling the simplified Rayleigh equation:

$$\delta^{13}C = \delta^{13}Co + \varepsilon*\ln(f) \qquad (3)$$

with a first order degradation equation

$$f= e^{-k*t} \qquad (4)$$

an equation is obtained that links the change in $\delta^{13}C$ to the degradation rate

$$\delta^{13}C = \delta^{13}Co - \varepsilon*k*t \qquad (5)$$

where $\delta^{13}$Co is the initial $^{13}C/^{12}C$ ratio, k is the first order biodegradation rate and $\varepsilon$ the isotope enrichment factor determined by Bouchard et al. (in prep) equal to 7.7 ‰ ± 0.3‰. If the reactive tracer decays at a first-order degradation rate we expect a straight line for a plot of $\Delta^{13}$C versus time. The negative slope of the line corresponds to the first-order degradation rate k multiplied by the isotope enrichment factor ($\varepsilon$).

### 3.3.2. Results and discussion

On October 17th 2005, a constant flow rate of 4.1 l/min was applied on the soil surface during 1080 minutes at Vers-chez-le-Brandt site. The flow increase in the cave was observed 70 min after the beginning of the sprinkling and constant flow rate after 210 minutes (Fig. 3.7). The mean discharge in the cave during semi permanent flow was 4.1 l/min. 96.6% of the injected water was recovered. The flow increase was observed 70 min after the beginning of the sprinkling and the establishment of the constant flow rate in the cave 140 minutes later.

The test solution was injected 270 min after the beginning of the sprinkling during semi-permanent flow (Fig. 3.7) with a constant flow rate of ~4 l/min. The first arrival of the solution in the cave was indirectly observed by an electrical conductivity increase 72 minutes after the test solution injection, and the maximum concentration 60 minutes later (Fig. 3.7). Samples were collected at 10-min intervals just after the tracers pulse. The sprinkling was stopped 810 min after the test solution injection.

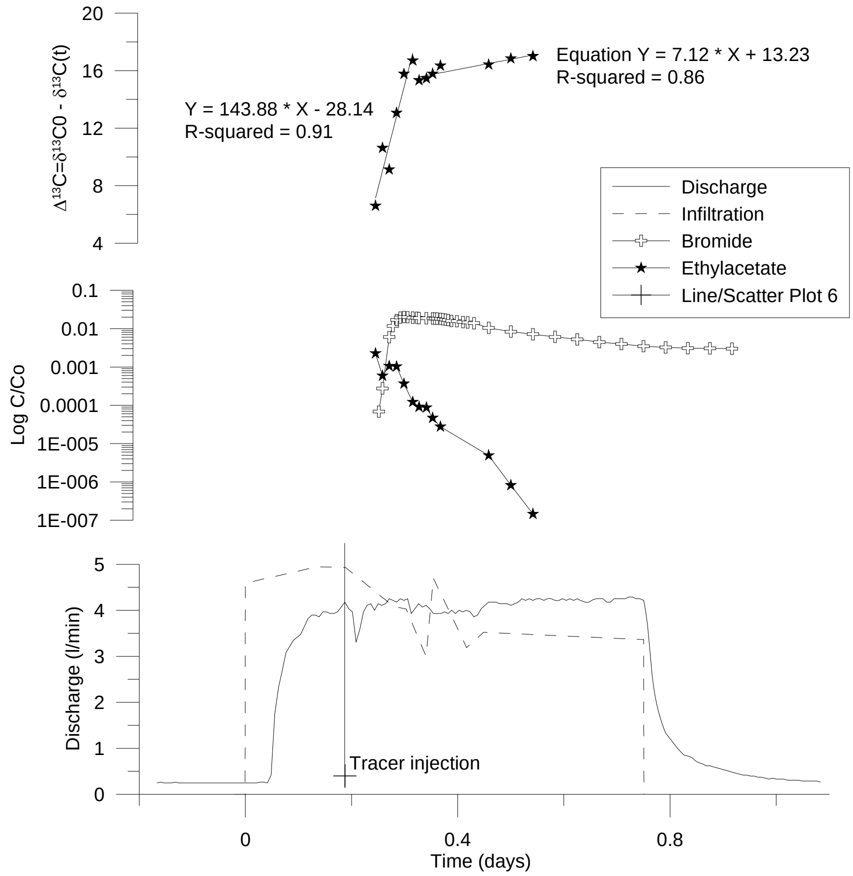


*Figure 3.7: Results of the October 2005 experiment. Infiltrated discharge and recovered discharge in the cave. Log normalized concentration of the recovered tracers. $\Delta^{13}C$ with k corresponding to the slope of the curves divided by the isotope enrichment factor.*

The isotopic analyses indicate a strong increase of the $\delta^{13}C$ for the 6 first samples and a smaller increases for the last other samples. The results are represented in the figure 3.7, where $\delta^{13}C$ - $\delta^{13}Co$ (-31‰) is plotted versus the time. Two linear trends are observed and for each of them a first order degradation rate constant was estimated. Values of 18.7 day$^{-1}$ for a first period of 101 minutes and 0.9 day$^{-1}$ for a following period of 309 minutes were obtained.

Considering the CSTR theory explained in paragraph 3.1.2, the observation of two different degradation rates in the same reservoir can not be explained without the hypothesis that a decrease of the microbial activity occurs in the system during the experiment.

Another explanation for this phenomenon can be the presence of two successive reservoirs presenting different microbial activity. In the case of this experiment, the first elevated degradation rate could be attributed to a first short storage in the soil reservoir with an elevated degradation rate of the reactive tracer and a second storage in the epikarst reservoir with lower degradation rate. However these two curves are not observed for the experiments discussed in paragraph 3.1.2.

### 3.3.3. Conclusion

The measurement of the shifts in the isotopic $^{13}C$ composition of the compounds makes it possible to determinate first order degradation in the karst system. As for the first used method, elevated first order degradation rates have been calculated in the unsaturated zone. This method is a promising tool to evaluate the biodegradation in unsaturated karst systems. More detailed investigations could certainly provide information about the biodegradation occurring in the different subsystems of the unsaturated zone.

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# Chapter 4

## Conclusion

# 4 CONCLUSION

The main conclusions of the BIOKARST project are summarized in the following chapter. The new methods developed during this study are discussed in the paragraph 4.1. The conceptual model of storage and transport of solutes across the unsaturated and saturated zones of the karst system under variable hydraulic conditions is described in the paragraph 4.2 and compared with existing conceptual models. The conceptual model is generalised to other karst systems or specific sampling points located at the base of the unsaturated zone (e.g. karst system without soil zone, seepage flow through the unsaturated LPV, conduits connected to the epikarst) in the paragraph 4.3. The degradation for dissolved contaminants in the karst systems is integrated in the hydraulic conceptual model and strategies are proposed to evaluate the degree of contamination and the biodegradation potential in karst systems (paragraph 4.4). Finally, the practical application of the developed tracer methods for contamination and vulnerability assessments are discussed and further works are proposed (paragraph 4.5 and 4.6).

## *4.1. Development of new methods*

In this study, a particular emphasis was given to the development of new methods to study the unsaturated zone of the karst aquifers. The concept of the BIOKARST project was based on a new natural tracing approach which involves the analysis of substances that originate from the soil zone and then decrease below the soil zone (epikarst, unsaturated LPV). The measurment of these substances make it possible to draw conclusions on how long the water containing solutes has resided in the karst infiltration system below the soil zone. Continuous measurement methods were preferred since only very dense sampling intervals provide sufficient information to understand the dynamics of karst systems.

In the study, it was demonstrated that $^{222}Rn$, $CO_2$ and TOC can be measured in situ in karst conduits for periods of several months. For continuous $^{222}Rn$ measurement in water, the instrument consisted of a closed circuit of air-filled semipermeable (polypropylene) tubing connected to a Lucas-cell and coupled to a photomultiplier (detection limit is at 0.1 Bq/l, the standard deviation is 3% at 1 Bq/l for an integration time of 30 min). The $CO_2$ concentration was determined in the same closed air circuit by IR absorption. The standard deviation is 0.05

% vol. at 1% vol. (Surbeck, 1996). The TOC measurement was based on the fluorescence properties of the TOC. A field fluorometer (Schnegg, 2003) was used, with a light source of 370 nm (UV LED) for the TOC excitation. The fluorescence was measured between 440 nm and 540 nm using a photodetector.

These three new methods for continuous measurment of $^{222}Rn$, $CO_2$ and TOC have allowed to investigate flood events with different hydrological conditions at high temporal resolution with relatively little cost. Moreover the monitoring devices have the advantage of being small in size and thus can be installed in caves for local scale studies.

## *4.2. Conceptual model*

The long term discharge and natural tracer ($^{222}Rn$, $CO_2$, TOC, electrical conductivity) measurements at different locations in the karst system provide insight into the high spatial and temporal variability of the pathway and transit time of water and solutes. In particular, the studies illustrate the critical importance of water storage, preferential flow paths and buffer capacity in the epikarst and the fractured LPV of the saturated zone. The data obtained during this study provide the basis for the development of a conceptual model of storage and transport of solutes in the unsaturated and saturated zones of karst system for base flows and flood events of variable intensities.

The conceptual model is based on observations made at Milandre cave (Milandrine river) characterized by diffuse infiltration. Three sub-systems are considered in the conceptual model (Fig. 4.1), the soil zone, the epikarst zone and the low permeability volumes (LPV). The LPV are subdivided in unsaturated LPV (vadose zone) and saturated LPV (phreatic zone). The epikarst splits the water into two flow components, the seepage flow through the unsaturated LPV and the quick flow in the vertical conduits connected to the phreatic zone. The Milandrine river presents the following particular hydraulic specificity:

- water from the saturated and unsaturated zones with contribution of the saturated LPV to the discharge. Quick flows and seepage flows from the epikarst zone to the river are observed. Thick soil cover.

Other complementary sampling locations presenting different hydraulic caracteristics than the Milandrine river were used in order to refine the conceptual model:

- Vers-Chez-le-Brandt and Grand-Bochat caves: water from the epikarst and soil zones without marked hydraulic influence of the unsaturated LPV. Quick flows in the conduits from the epikarst zone are observed. The Vers-Chez-le-Brandt test site is characterized by a 30 meters thick unsaturated zone and the Grand-Bochat by a 15 meters thick unsaturated zone.
- Gratte-Roche gallery (Milandre cave): water from the soil and epikarst zones with marked hydraulic influence of the unsaturated LPV. Essentially seepage flows in the LPV from the epikarst zone are observed.

### 4.2.1. Base flow and small flood events

During base flow conditions, the discharge in the main conduit essentially consisted of seepage flow from the saturated LPV (0 in Fig. 4.1.a,d). The saturated LPV are recharged through the unsaturated zone by the diffuse infiltration as already observed by Charmoille (2005). The $^{222}Rn$, $CO_2$, TOC and EC levels are constant. Discharge and chemistry are stable in the saturated zone. In Perrin's model, the base flow discharge is sustained by the epikarst reservoir.

During small rainfall events, an increase of the discharge is observed in parallel with an increase of the $CO_2$ concentration (1 in Fig. 4.1.b,d). The soil water is pushed into the epikarst reservoir, and the increasing water level in the epikarst causes an increase of the seepage flow through the unsaturated LPV but the recharge is not enough sufficient to create an overflow of the epikarst reservoir. The water pushed in the unsaturated LPV causes a hydraulic stress on the saturated LPV and a discharge increase is observed in the saturated zone as observed by Mudry (1987) and Charmoille (2005). The water contributing to the flood is the same as during base flow period. In Perrin's model, the system is fed by epikarst water mainly but this hypothesis does not explain the immediate $CO_2$ increase and the delayed TOC increase.

The water pushed out from the saturated LPV during piston flow is not equilibrated with the cave atmosphere unlike the water measured during base flow and a $CO_2$ increase is observed (see paragraph 2.1.4, small flood events). During the piston effect, the less permeable part of the saturated LVP may likely contribute to the discharge. For the Milandre site, between one and three days after the discharge had reached base flow values again (2 in Fig. 4.1.c,d) a TOC and EC increase is observed, due to the delayed contribution of the epikarst through the unsaturated and saturated LPV.

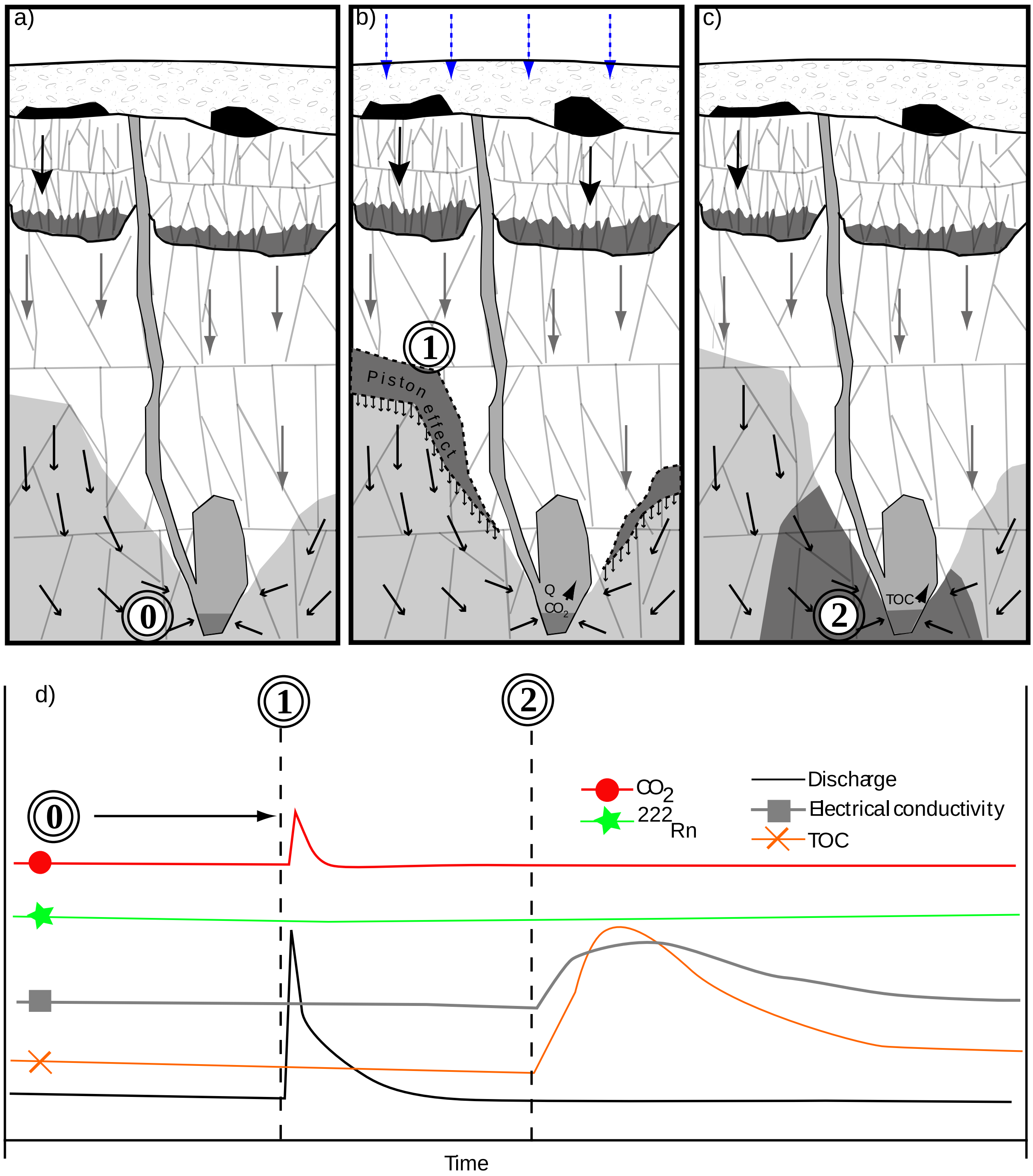


*Figure 4.1: a) During base flow discharge consist of seepage flow from the saturated LPV (0). b) The initial discharge increase is due to a piston effect (1) on the saturate LPV. c) A delayed TOC increase is observed in the river(2) due to the delayed contribution of the epikarst through the LPV. d) Schematic hydrograph and chemograph for base flow and small flood events.*

### 4.2.2. Medium flood events

During medium flood events, as for small flood events, an increase of the discharge is observed in parallel with a $CO_2$ increase due to the piston effect on the saturated LPV (1 in Fig. 4.2.a,d). In contrary to the small flood events, a TOC and EC increase is already observed during the flood peak (2 in Fig. 4.2.b,d). The relatively small offset and similar peak shape of discharge and TOC compared to the delayed TOC increase for small flood events suggest that the water from the epikarst zone rapidly transited across the unsaturated zone along conduits as already observed by Perrin (2003). A delayed increase of the $^{222}Rn$ compared to the discharge is also observed due to the delayed contribution of water stored in the soil zone. The more elevated water levels in the conduits induce an inversion of the hydraulic gradient. The pressure in the karst conduits is higher than the one in the fractured LPV of the saturated zone and prevents the contribution of this sub-system to the discharge (3 in Fig. 4.2.b,d). Once the discharge had reached base flow values again, a second TOC and EC increase is observed (4 in Fig. 4.2.c,d). The hydraulic gradient returns to base flow conditions leading to a delayed epikarst contribution to the discharge through the LPV.

During flood events, the flow velocity in the unsaturated and saturated LPV is comprised between 19 m/day and 55 m/day when water transit through the LPV (small flood events) and higher than 10 m/h when water transit though preferential flow paths (higher flood events). The flow velocity is obtain by plotting the delay of TOC, $^{222}Rn$ and $CO_2$ increase versus the maximum discharge for the flood events measured during the period of observation (see paragraph 2.3.4) Perrin (2003) found a value on the order of 50 m/h in the unsaturated zone for base flow and flood events. This velocity is similar to the velocity found for the higher flood events in this study (preferential flow paths). The elevated velocity through the unsaturated zone was necessary to explain the contribution of the epikarst zone to small flood events. However, an immediate contribution of the saturated LPV (piston flow) could also explain the discharge increase as already postulated by Charmoille (2005).

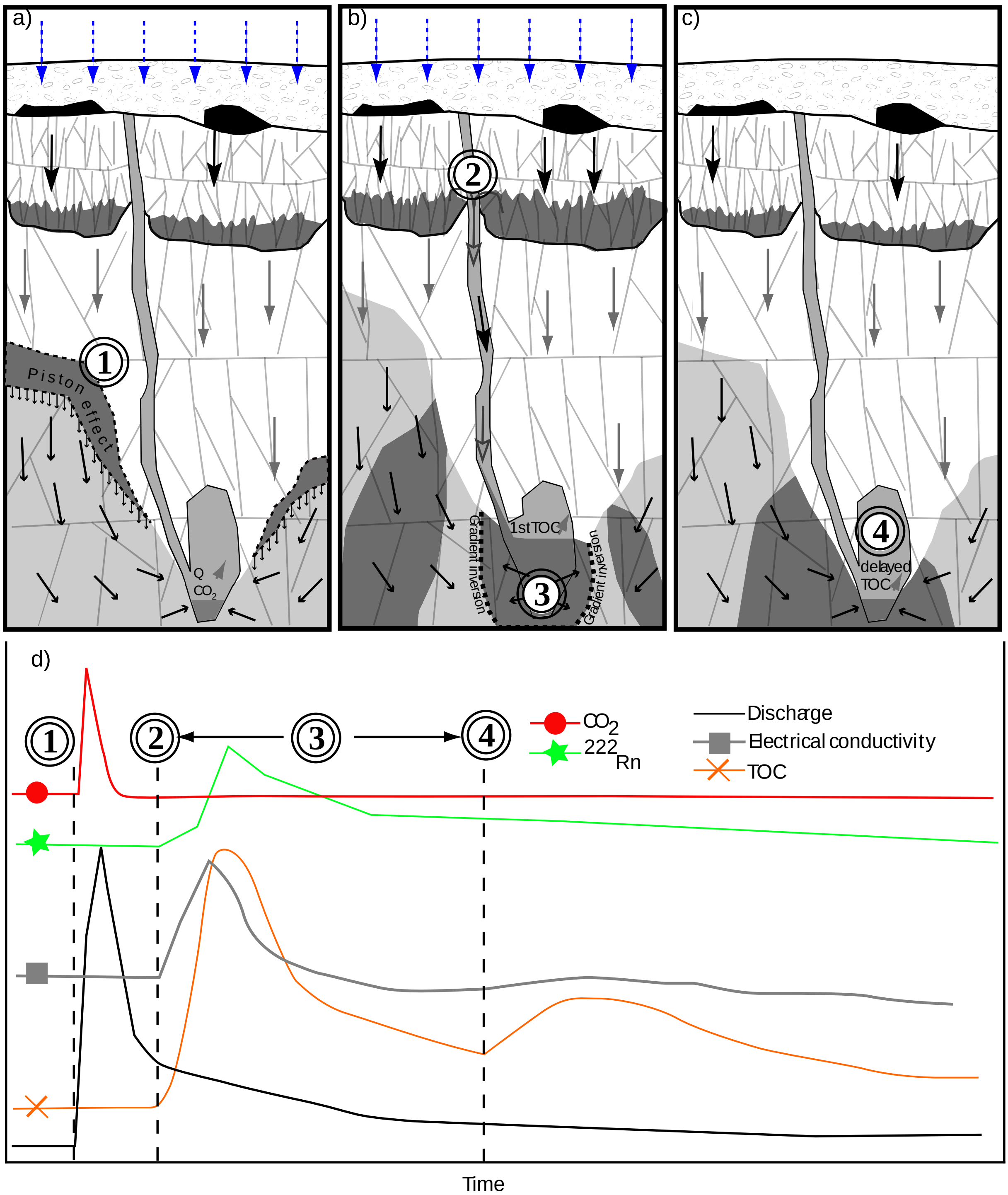


*Figure 4.2: a) The initial discharge increase is due to a piston effect on the saturate LPV. b) An inversion of the hydraulic gradient in the conduits blocks the contribution of the saturated LPV to the discharge. A first increase of the TOC concentration is observed due to the direct contribution of the epikarst to the discharge. c) A second delayed TOC increase is observed due to the delayed contribution of the epikarst through the LPV. d) Schematic hydrograph and chemograph for medium flood events.*

### 4.2.3. Large flood events

During large flood events, the beginning of the discharge increase is due to the piston effect on the saturated LPV (1 in Fig. 4.3.d) as for small and medium flood events. The elevated recharge rate of the epikarst reservoir by percolating water from the soil zone, results in an overflow of the epikarst into the vertical conduits with a $^{222}Rn$ and TOC increase in the main conduits or spring (2 in Fig. 4.3.a,d). The prolonged rainfall leads to a direct contribution of rainwater to the discharge as indicated by strong decrease of the EC is observed at the spring or main conduits. (4 in Fig. 4.3.b,d). The contribution of fresh rainwater (20-30%) estimated based on electrical conductivity variations is similar as estimated by Perrin (2003). The contribution of the saturated LPV to the discharge is blocked during several days by inversion of the hydraulic gradient (3 in Fig. 4.3.a,d). Due to the long duration of the larger flood events, the delayed contribution of the epikarst reservoir by seepage flow through the unsaturated and saturated LPV can usually not be observed (5 in Fig. 4.3.c,d) because generally other floods occur before baseflow conditions are reached again.

During medium and small flood events, the saturated LPV contribute to the discharge at the beginning of the floods and are then stopped by an inversion of the hydraulic gradient caused by the rapid contribution of soil and epikarst water through preferential flow paths

During the recession phase after rainfall has stopped, the soil releases water into the epikarst reservoir. The system is mainly fed by epikarst and saturated LPV water, but soil water can still be present (sustained $^{222}Rn$ concentrations, see paragraph 2.1.4 and Fig. 4.3.c,d).

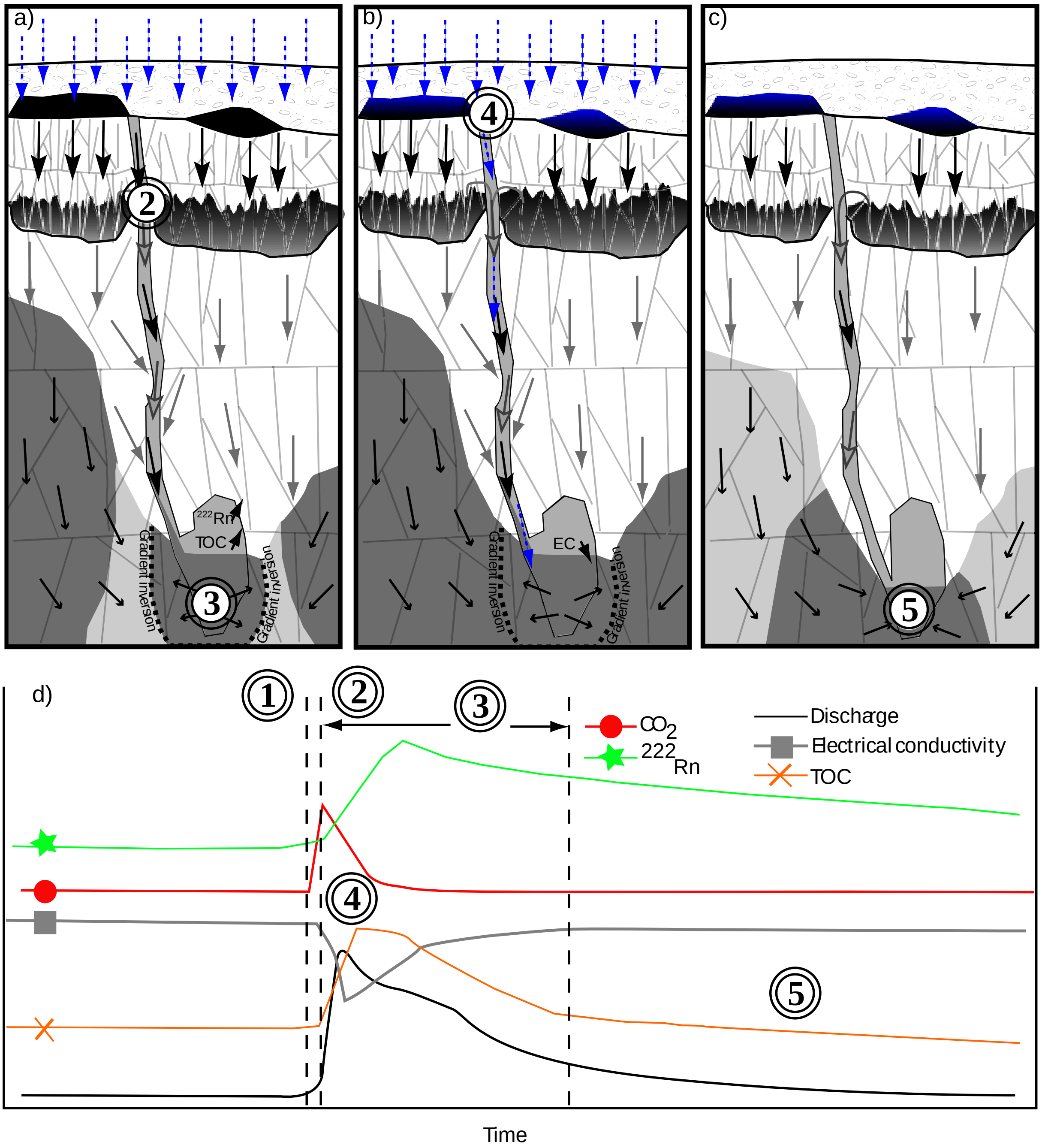


*Figure 4.3: The initial discharge increase is due to a piston effect on the saturate LPV (see figure 4.3.a). a) An inversion of the hydraulic gradient in the conduits blocks the contribution of the saturated LPV to the discharge. A first increase of the TOC concentration is observed due to the direct contribution of the epikarst to the discharge. b) The strong decrease of the EC indicates a contribution of the rainwater to the discharge. c) No delayed TOC increase is observed due to the long time extend of the flood events. d) Schematic hydrograph and chemograph for large flood events.*

According to this conceptual model, an elevated $^{222}Rn$ together with an elevated TOC concentration indicates a rapid transfer of water from the soil zone with a possible short duration storage in the epikarst. High concentration of both tracers coupled with a strong decrease of the EC indicates a significant contribution of fresh rainwater to the discharge. A low $^{222}Rn$ with an elevated TOC indicates the arrival of water that was stored for more than 20 days below the soil zone. Finally water with low $^{222}Rn$ and low TOC must have resided in the subsurface for weeks to months.

The proposed conceptual model is in agreement with existing conceptual models. One of its specific characteristics is to combine epikarst storage models and phreatic storage models:

1) Perrin (2003) proposed a conceptual model with water storage mainly in the upper part of the unsaturated zone (soil and epikarst zones). Several other authors proposed an important storage in the epikarst zone and a funnelling of the flow towards conduits (Williams, 1985; Smart and Friederich, 1986; Klimchouk, 2000; Klimchouk, 2004). The results of the TOC, $^{222}Rn$ and $CO_2$ measurements strengthen the conceptual model proposed by Perrin (2003). As for Perrin's model, the storage is mainly located in the soil and epikarst zones but the LPV play an important role, as they are necessary for explaining the observed hydraulic responses and TOC - $CO_2$ observations during base flow and small flood events. The used of additional tracers than Perrin during this study (TOC, $^{222}Rn$ and $CO_2$) allowed the detection of the soil water contribution during flood events and the detection of the saturated LPV contribution during base flow.

2) In contrary to the models implying important water storage in the epikarst, the phreatic storage in Jura karst systems was described by Jeannin (1996) and Charmoille (2005). Jeannin (1996) calculated based on hydrodynamic observations (base flow evolution, piezometers levels, water balance) that 50% of the infiltrated water during rain events participates to the recharge of the fractured LPV of the saturated zone in the Milandre test site. Charmoille (2005) demonstrated the importance of the fractured LPV in the Jura karst systems for the prolonged storage of groundwater and their possible

participation to the discharge. Several other authors (Jeannin et Grasso, 1995; Guglielmi et Mudry, 2001; Maloszewski et al., 2002; Lee et Krothe, 2003) described the importance of the phreatic storage in the LPV in karst systems.

To summarize, the principal characteristics of the conceptual model are:

- Storage located in the saturated LPV and the soil epikarst zone.
- Epikarst splits the water into seepage flow across the unsaturated LPV and quick flow in the vertical conduits depending of the recharge intensity.
- Elevated buffer effect of the soil, epikarst and the unsaturated LPV (unsaturated zone).
- An increase of vertical flow velocities through the unsaturated LVP with an increase of the recharge; For Milandre site the vertical flow velocity is less than 20 m/day for base flow and 55 m/day for the larger flood event.
- During base flow and small flood event seepage flow is dominant and discharge essentially sustained by the saturated LPV.
- During larger flood events, conduit flow is dominant and discharge sustained by overflow of the epikarst and soil zones in conduits. An inversion of the hydraulic gradient blocked the contribution of the saturated LPV to the discharge.
- The intensity of the recharge events controls the contribution of the different subsystems (soil, epikarst, saturated LPV) to the discharge.

These conclusions have important repercussion for the transfer of dissolved contaminants in the karst systems. In case of large recharge events a large amount of the contaminant can be flushed out the soil and the epikarst in the vertical drainage conduits and reaches the saturated zone by preferential flow paths. In case of smaller recharge events a part of the dissolved contaminant is pushed into the LPV and can be stored for prolonged time in the less permeable parts of the system.

## *4.3. Extension of the model to other karst systems or subsystem*

The conceptual model of solute transfer across the unsaturated and saturated zones of the karst systems with diffuse infiltration can be extended to other karst systems. Moreover the conceptual model can be extended to specific sampling points at the base of the unsaturated zone (unsaturated LPV or conduits).

### 4.3.1. Unsaturated zone

***Unsaturated LPV with dampened hydraulic response, low tracer recovery***

At sampling points located at the base of the unsaturated zone where seepage flow is dominant, the discharge is generally not correlated with rainfall events except in case of elevated rainfall events associated with low PET.

An important seasonal effect of the discharge with base flow during summer period in parallel with the increasing PET and high flow during winter is observed. This effect is also observed in the phreatic zone but partially masked by the elevated discharge peak of flood events.

The storage is mainly located in the upper part of the unsaturated zone, in the soil and epikarst zones. In case of large recharge events, preferential flow paths through the unsaturated LPV are activated due to an exceeding of epikarst storage capacity. The initial discharge increase is due to a piston effect linked to the expulsion of water stored in the unsaturated LPV. The volume of the groundwater storage in the epikarst is important and allows sustaining base flow during long periods. The effect of storage is also observed for artificial tracing experiments where very low recovery rate were observed (< 1%) despite high amount of water infiltrated.

***Vertical conduits with quick hydraulic response and elevated tracer recovery***

Observations done in conduits located at the base of the unsaturated zone and connected with the epikarst indicate a low contribution of the unsaturated LPV to the discharge. The measured discharge is very low or null during base flow periods. The discharge essentially consists to an overflow of the soil and epikarst reservoir during rainfall events. In contrary to observations done at the base of the unsaturated zone where seepage flow is dominant, tracing experiments and discharge measurements in conduits connected to the epikarst indicated an elevated hydraulic response and elevated recovery rate (>50%).

As for the conceptual model developed above for sampling points in the phreatic zone but on a different scale, two peaks of tracers are observed during large flood events. The first peak is observed during the recharge event and the second peak once the discharge has reached base flow value again. In this case, the first peak is due to a direct contribution of the epikarst reservoir through preferential flow paths in the more permeable parts of the unsaturated LPV (quick flow in conduits for the conceptual model) and the second delayed peak to a contribution of the epikarst reservoir through less permeable parts of the unsaturated LPV.

## 4.4. Biodegradation of solutes contaminants in karst systems

The natural tracing studies demonstrated that a part of the water can be stored in the unsaturated zone for a prolonged period probably in the soil and epikarst zone and the fractured LPV. To evaluate the relevance of storage on the fate of organic contaminants, it is important to gain information on the microbial activity in these zones. For this purpose, a reactive tracer method was developed and tested at the Vers-Chez-le-Brandt and Gänsbrunnen test sites. The experiments indicated that biological activity in the soil and epikarst zone is high. However the results of the experiments cannot be generalized. The reactive tracers used during the experiment were highly degradable and different results would be expected with other less biodegradable compounds. The experiment was carried out under artificially imposed steady state conditions which regard to flow. For quantification of biodegradation, the epikarst zone was approximated by a Continuously Stirred Tank Reactor (CSTR) that overflows to the conduit where measurements were made. The tracer solution injected on the soil surface migrates through the unsaturated soil and epikarst and reaches the saturated epikarst reservoir (Fig 4.5, t1 and t2). The epikarst reservoir presents a relative uniform tracer concentration (Fig 4.5, t3) and overflows in the vertical drainage conduits. Steady state conditions allow an exponential decrease of the tracer concentration in the soil and epikarst reservoir as postulated in the theory of Continuously Stirred Tank Reactor (see paragraph 3.1.2) and biodegradation of the reactive tracer (Fig 4.5, t4). At the end of the experiment (Fig 4.5, t5), reactive tracer is totally degraded or pushed out of the epikarst.

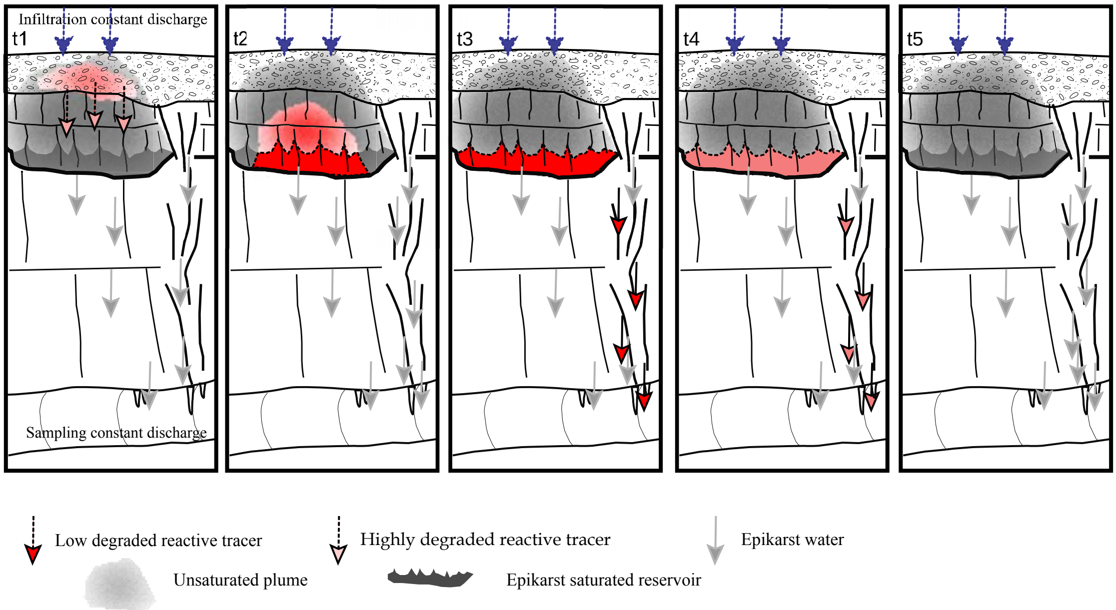


*Figure 4.5: Model of biodegradation of solutes in the unsaturated zone of karst based on the theory of the CSTR.. t1 and t2) Injection of the contaminant and migrations though the soil and epikarst zones. t3) The contaminant is stored at the base of the epikarst and overflows in the vertical drainage conduits. t4) the concentration decreases due to dilution and biodegradation. t5) the contaminant is totally degraded or evacuated.*

Based on this study, it is not possible to assess the variability of the degradation rates in each subsystem of the unsaturated zone. The mean degradation rate of the contaminant in the whole system is considered as constant but each subsystem of the unsaturated zone (soil, epikarst and unsaturated LPV) can have a variable degradation rates. The final recovered mass of contaminant (amount of not degraded contaminant) corresponds then to the duration of the storage in each subsystem. The natural tracer studies demonstrated that a prolonged storage of water in the unsaturated zone can occur and the reactive tracer experiments indicated a high biological activity but the specific location of the biodegradation in the unsaturated zone (soil, epikarst or LPV) can not be determined. Based on the $CO_2$ measurments, the soil zone is probably the subsystem presenting the most elevated biological activity, as expected in paragraph 2.1.4. In summary these findings indicate that in the absence of large precipitation events, a substantial biodegradation of organic contaminants can be expected. However, during large rainfall events contaminants can likely breakthrough due to the short transit time in the unsaturated zone of some of the water. Depending on the condition of the contamination (diffuse or concentrated, initial contaminated subsystems), the development of the unsaturated zone (soil cover or not, seepage or conduit flows), and the recharge events intensities and successions, a multitude of biodegradation scenarios can be developed. The following example describes the case of a recent diffuse contamination of the soil zone (for substance with limited soption) in a karst system without prior contamination. In the presented case, the unsaturated zone is well developed with water stored in the epikarst contributing to the discharge by quick flow through vertical conduits and seepage flow through unsaturated and saturated LPV. This geomorphology corresponds to the most of the Jura karst systems.

Base flow

During base flow, the discharge is essentially sustained by the saturated LPV. Contaminant should not be observed in the saturated zone due to is location in the soil zone (Fig. 4.6, a). The saturated LPV are slowly recharged by the epikarst zone.

Small floods

In case of small recharge events, there is no direct contribution of the soil and epikarst reservoirs to the discharge as explained in the conceptual model (see paragraph 4.2). The recharge is not enough large to create an overflow of the epikarst reservoir in the vertical conduits. The contaminant is partially flushed is the epikarst and reaches the saturated LPV by seepage flow through the unsaturated LPV (Fig. 4.6, b). It can be stored in the less permeable parts of the LPV for weeks to months with extended period available for biodegradation. After the rainfall event, the remaining contaminant is stored in the soil and epikarst zone leaving time for biodegradation until the next rainfall event (Fig. 4.6, c). If not completely degraded, the contaminant stored in the saturated LPV will be detected at the spring several days after the peak discharge, once the discharge has reaches baseflow values again. In this case, the overall duration of the storage is large and the recovered mass of the contaminant will be low depending of the degradation rate.

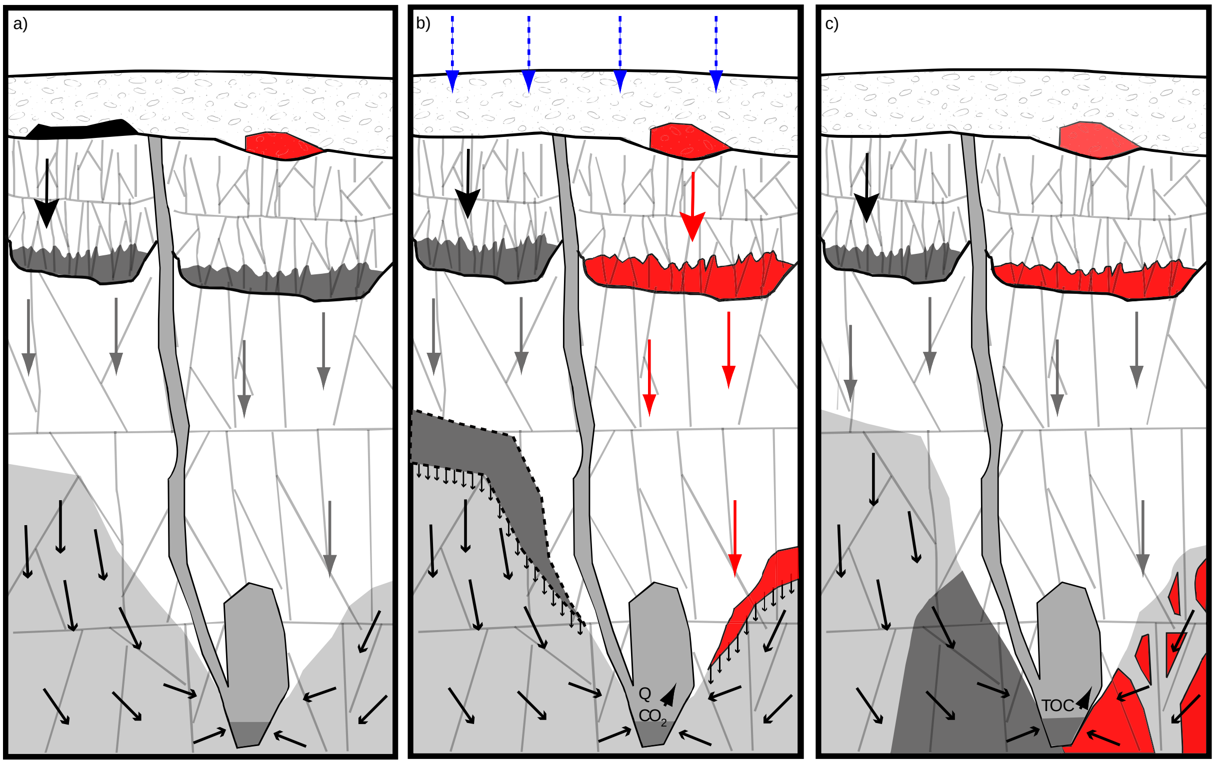


*Figure 4.6: a) Base flow conditions. The discharge is sustained by the saturated LPV. No contaminant is detected. b) Small flood event. The epikarst contaminated and piston effect through the saturated LPV with contamination. c) After the flood, all the subsystems are contaminated and a delayed increase of the contaminant is observed in the river.*

Larger floods

For the larger flood events, as for the small flood events an initial piston effect with contamination of the saturated LPV is expected (Fig. 4.7, a). Subsequently, an overflow of the epikarst reservoir into the vertical conduits likely occurs with an elevated contribution of this reservoir to the peak discharge. Elevated concentration of the contaminant will be measured in the saturated zone (Fig. 4.7, b). In this case, the duration of the storage is small and the recovered mass of the contaminant will be higher than for small flood events depending of the degradation rate. The contaminants flushed into the LPV will be detected later in the saturated zone once the discharge had reaches base flow values again (Fig. 4.7, c). In case of elevated recharge, a direct contribution of the soil zone to the discharge will be observed resulting in an elevated contaminant concentration in the saturated zone.

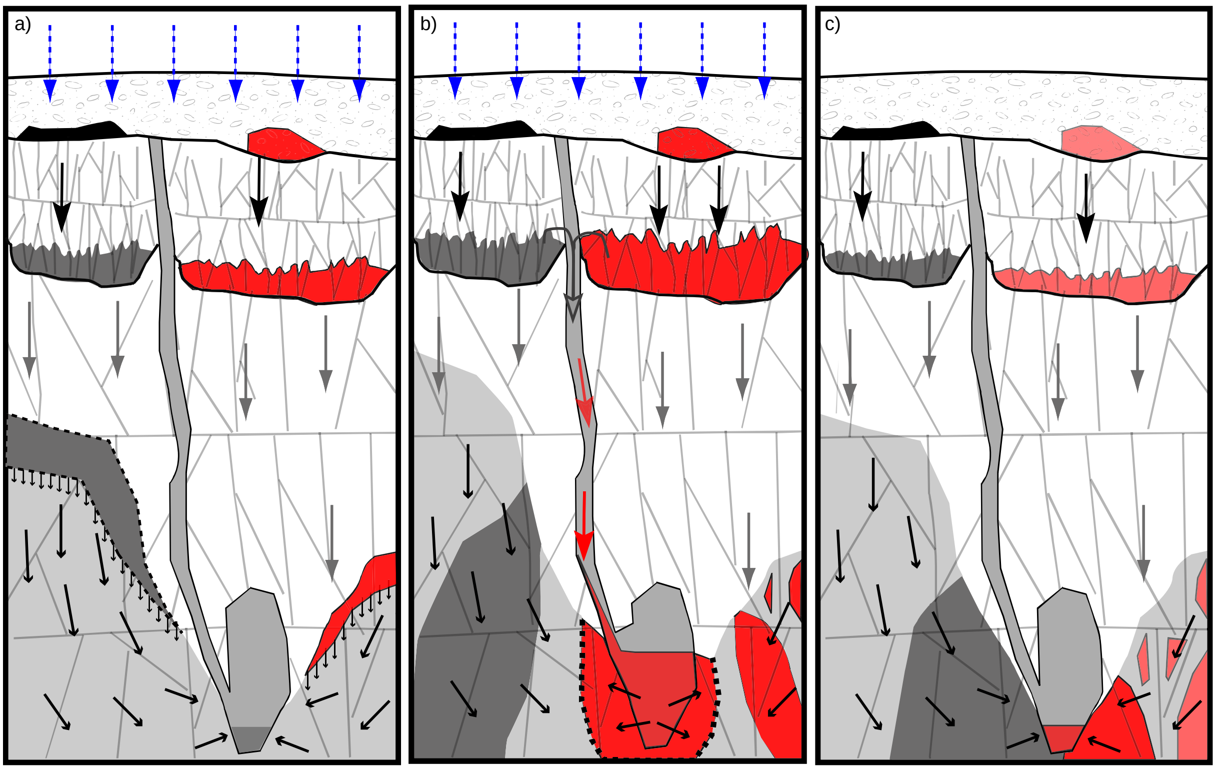


*Figure 4.7: a) Large flood event. The epikarst contaminated and piston effect through the saturated LPV with contamination. b). Overflow of the epikarst reservoir with high contaminant concentration in the saturated zone. c) After the flood, all the subsystems are contaminated and a delayed increase of the contaminant is observed in the river.*

## 4.5. Implications for applied investigation

### 4.5.1. Contamination assessment of karst aquifers

Based on discharge, EC, TOC and contaminant measurements during flood events and baseflow, the contaminated subsystems of the unsaturated and saturated zones of the karst system can be determined. This method is only valid for non retarded (e.g. sorption) compounds. Table 4.1 illustrates how the contaminated subsystems can be identified. At first, the presence or absence of the contaminant has to be determined during baseflow. In case of contaminant detection, the LPV have to be considered as contaminated. Secondly, during flood events, the presence or absence of contaminants has to be determined during the initial discharge increase. In case of an immediate contaminant increase without detection during base flow, the epikarst and the saturated LPV have to be considered as recently contaminated (see paragraph 4.2). And finally depending of the intensity of the flood event, the presence or absence of contaminant in the epikarst and soil subsystem can be determined. During a small flood event, if a peak of contaminant is observed once the discharge has reaches base flow values again, the epikarst is likely contaminated. In case of larger flood event, a less delayed peak of contaminant will be observed.

*Table 4.1: Determination of the contaminated reservoirs based on discharge EC and TOC measurement in parallel with the contaminant for different hydraulic conditions: base flow, piston flow and flood events.*

| | **EC and TOC** | **Contaminant** | **Contaminated reservoir** |
|---|---|---|---|
| Step 1: Base flow | Constant<br>Constant | -<br>Detection | LVP not contaminated<br>LVP contaminated |
| | | | |
| Step 2: Piston | Constant<br>Constant | -<br>Detection | LVP not contaminated<br>LVP contaminated |
| | | | |
| Step 2a: Small flood | Delayed increase once discharge reaches base flow | -<br>Detection | LPV and epikarst not contaminated<br>LPV and epikarst not contaminated |
| | | | |
| Step 2b: Large flood | Delayed increase<br>Delayed increase | -<br>Detection | LPV, epikarst and soil not contaminated<br>LPV, epikarst and soil contaminated |

### 4.5.2. Soil cover assessment based on natural tracers

The buffer effect of the soil zone was clearly demonstrated in the preceding chapters (soil water contribute to the discharge only during large flood events) and the production of the natural tracers (TOC, $^{222}$Rn and $CO_2$) will be directly influenced by the presence or not of the soil cover. Thus a study of the natural tracers produced in the soil zone in parallel to the hydraulics comportment of the karst systems can provide useful information about the vulnerability of the systems to infiltration of contaminants.

***$^{222}$Rn***

Elevated concentration of $^{222}$Rn at the spring during base flow will generally indicated a thick soil cover in the catchement area of the karst system or a thin soil cover with elevated $^{226}$Ra and $^{222}$Rn concentrations. Low $^{222}$Rn concentration at the spring is generally due to the absence of soil cover. The $^{222}Rn$ background concentration is due to the limestone activity. An elevated $^{222}Rn$ concentration at the spring indicates generally a lover vulnerability of the system compared to low $^{222}Rn$ concentration (infiltration is buffered). However, in karst systems with thick unsaturated zone and prolonged storage of water in this zone, a decay of the $^{222}Rn$ from the soil zone can occurred and lower concentration at the spring be measured. Low $^{222}Rn$ concentration at the spring could also simply be due to a lack of soil in the catchment area. For this reason, the water for $^{222}Rn$ analysis has to be sampled during flood events with contribution of soil water to the discharge and then a good knowledge of the floods amplitude of the systems is necessary.

***$CO_2$***

As for $^{222}Rn$ an elevated $CO_2$ concentration at the spring will indicate the presence of a thick soil cover in the catchement area of the karst system. Low $CO_2$ concentration at the spring will indicate the presence of a thin soil cover in the catchement area or the absence of soil cover and then a more elevated vulnerability.

***TOC***

Without good knowledge of the catchment area of the karst systems, TOC has to be used carefully for vulnerability assessment of karst system. Indeed an elevated TOC concentration can indicate a thick soil cover and low vulnerability but can also indicated a local concentrated rich TOC water infiltration (e.g. contaminated sinkhole, farm effluents) indicating a local elevated vulnerability

## 4.6. Further work

### 4.6.1. Numerical simulation

The presented conceptual model of storage and transport of solutes in the unsaturated and saturated zones of karst system for base flow and flood events of variable intensities could be used as a basis for mathematical simulations to evaluate the hypothesizes of the conceptual model in a quantitative manner. The soil, epikarst and unsaturated zone should be modelled as partially and locally saturated depending on the recharge. The soil zone buffers the infiltration and should be modelled as a porous media. It should be included that the epikarst splits the water into two flow components depending of the recharge intensity. The epikarst should be modelled as a fractured media recharged by the water percolating from the soil zone. The unsaturated LPV should be modelled by a combination of fractured/karstified media drained by conduits. Indeed for small recharge, seepage flow through the unsaturated LPV is observed. During high recharge, unsaturated LPV are recharge by seepage flow but an overflow of the epikarst in the vertical conduits connected to the phreatic zone is observed.

### 4.6.2 Use of other reactive tracers

In order to better study the biological activity of the unsaturated zone of karst systems, use of reactive tracers with smaller first order biodegradation rate than EAc must be used. These compounds could be used for long term experiments with prolonged storage in the unsaturated LPV. The reactive tracer could be artificially injected in the karst system during sprinkling experiments and in order to store the tracer in the unsaturated zone, the sprinkling is interrupted before the tracer reaches the base of the unsaturated zone or the saturated zone. Therefore a very good knowledge of the hydraulic reactivity of the studied karst system is needed including preliminary tracing experiments with variable infiltration rates.

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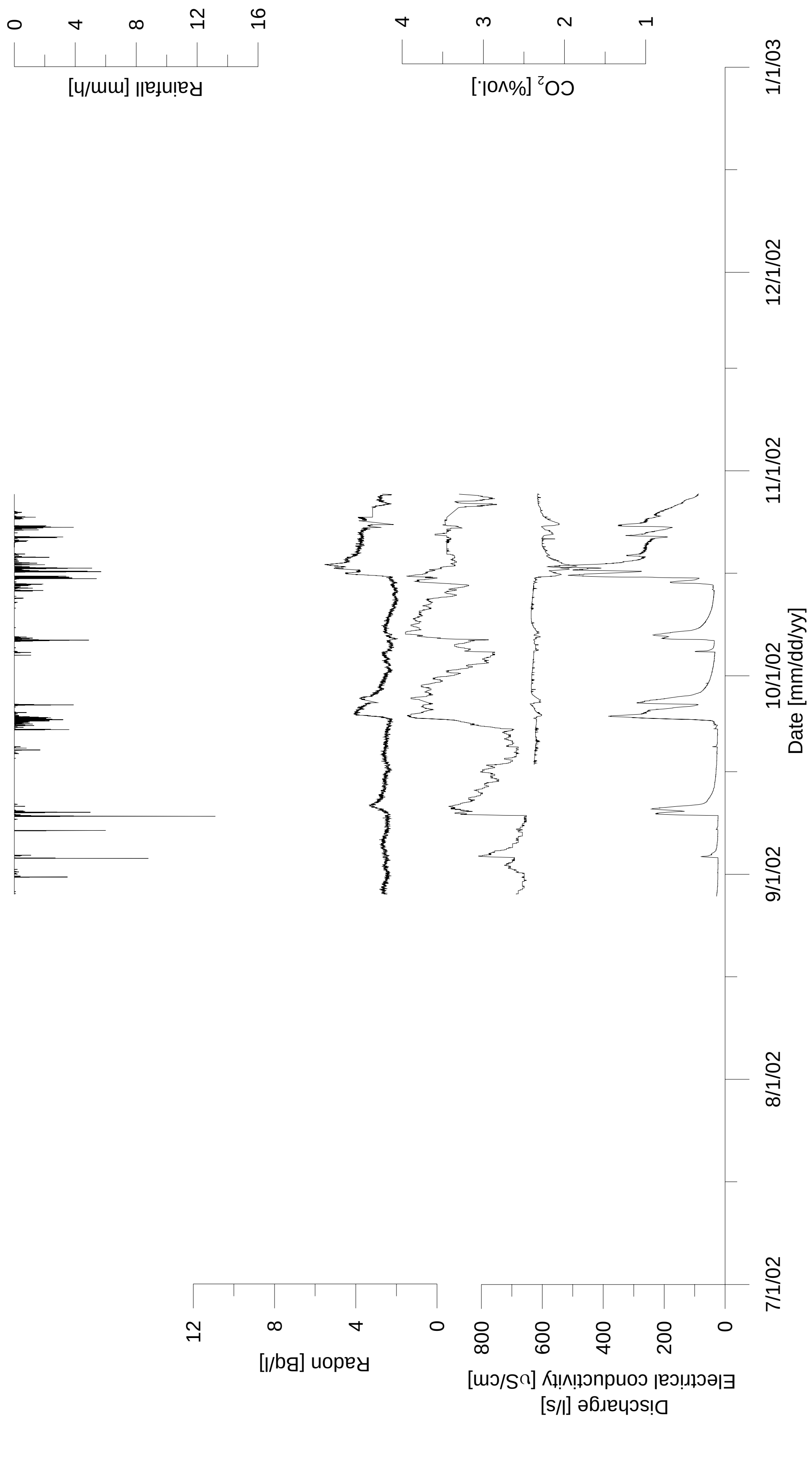
# Annex 1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Radon [Bq/l]
12 8 4 0
Electrical conductivity [µS/cm]
Discharge [l/s]
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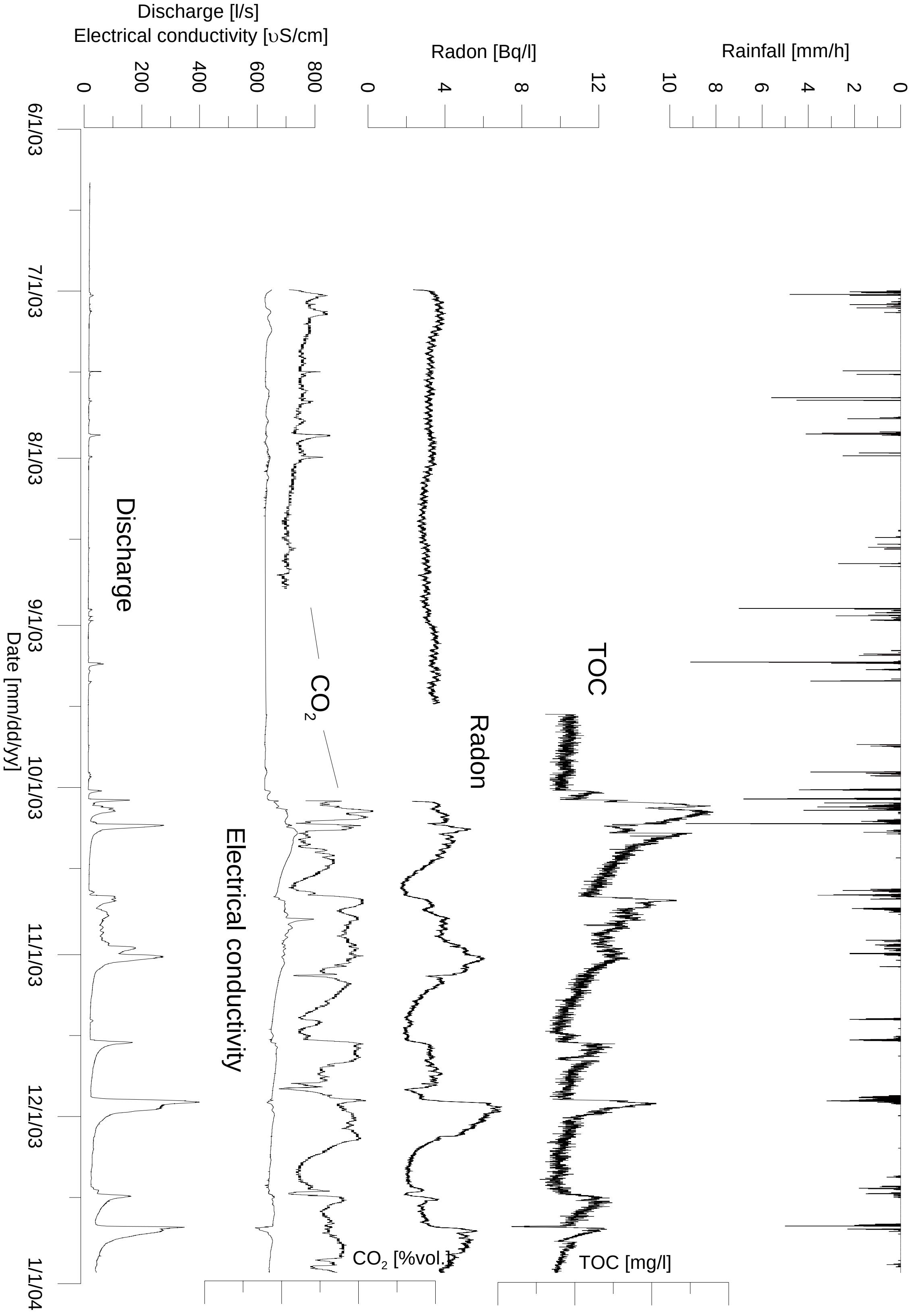
7/1/02 8/1/02 9/1/02 10/1/02 11/1/02 12/1/02 1/1/03
Date [mm/dd/yy]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Electrical conductivity [υS/cm]
Radon [Bq/l]
Rainfall [mm/h]
TOC
Radon
CO2
Discharge
Electrical conductivity
CO2 [%vol.]
TOC [mg/l]
Date [mm/dd/yy]
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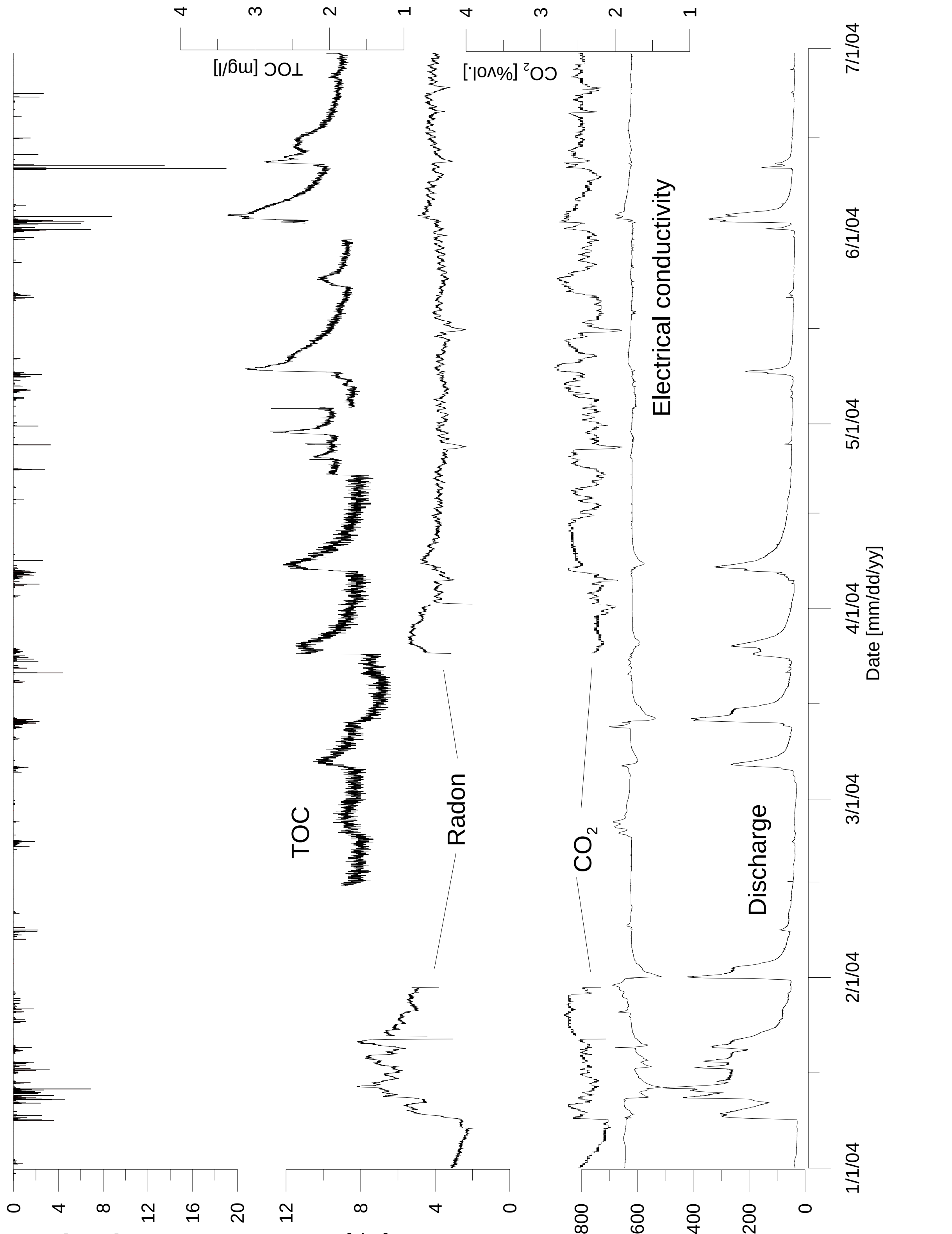

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12
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Radon [Bq/l]
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4
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Electrical conductivity [µS/cm]
Discharge [l/s]
800
600
400
200
0
TOC [mg/l]
4
3
2
1
CO2 [%vol.]
4
3
2
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TOC
Radon
CO2
Discharge
Electrical conductivity
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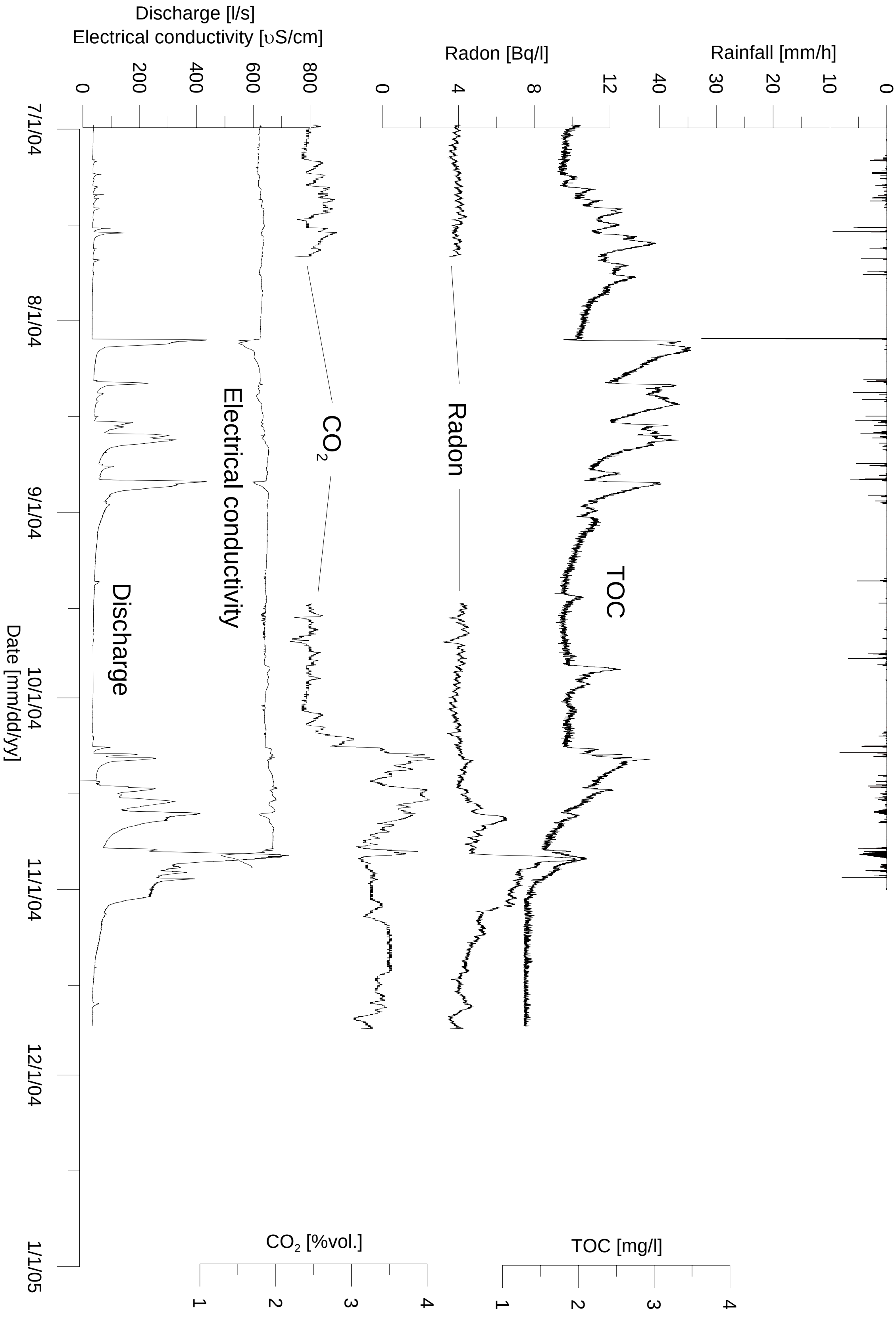

Rainfall [mm/h]
Radon [Bq/l]
Discharge [l/s]
Electrical conductivity [υS/cm]
TOC [mg/l]
$CO_2$ [%vol.]
Date [mm/dd/yy]
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TOC
Radon
$CO_2$
Electrical conductivity
Discharge