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Transfer of bacteria-contaminated particles in a karst aquifer: evolution of contaminated materials from a sinkhole to a spring

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Abstract

The transport of particle-associated bacteria during rain events in karst waters has been investigated. In this aim, we studied the correlations between water turbidity and enumerations of sessile (attached) and planktonic (non-attached) bacteria. We monitored physicochemical, i.e. turbidity, electrical conductivity, size and nature of the transported particles, and bacteriological properties of waters since their infiltration on a karst plateau to their discharge at a karstic spring. Results showed a decrease of the concentration of sessile bacteria at the sinkhole for high turbidities. This phenomenon might be explained by the arrival of lower contaminated material. On the other hand, the amount of sessile bacteria at the spring was not influenced by the turbidity values. These data demonstrated that slightly contaminated larger particles were not recovered, whereas small-size particles, which exhibited a higher bacterial contamination, were directly transferred (i.e. not affected by intra-karstic deposition) through the aquifer. Our study highlighted some significant differences between the bacteriological time series at the sinkhole and at the spring, which characterizes the storage/resuspension function of the considered karst system. Moreover, we show a decrease of the concentration of planktonic bacteria after transport through the system whereas no reduction of the sessile population occurred. The present data confirm that turbidity does not constitute a good indicator for bacterial contamination: if high turbidity corresponds to high bacterial contamination, low turbidity does not systematically exclude a risk of contamination by sessile organisms.

Keywords: Geologic interface; Karst; Turbidity; Particle transport; Bacteria; Biofilms

1. Introduction

In Haute-Normandie (France), where the entire drinking water resources have a karstic origin, rain events generate turbid runoffs that might cause sanitary crises and recurrent interruption of water supply (Beaudeau et al., 1999). The chalky plateaus

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overhanging the Seine river are covered with quaternary aeolian silts. Owing to storm events, the erosion of these quaternary formations induces the occurrence of turbid streams which are introduced into the karstified chalk aquifer via sinkholes (Massei et al., 2002).

The transport of particles in karstic media is a complex process implying deposition and release phenomena (Massei, 2001; Massei et al., 2003). The observed turbidity would have two potential origins: (i) the direct transfer of particles from the inlet to the outlet of the karstic system (allochthonous origin) and (ii) the resuspension of previously deposited sediments (sub-autochthonous origin). Even though some studies have been performed to analyse the particle size distribution (Lacroix et al., 1998, 2000; Mahler and Lynch, 1999; Rodet, 1996), few works have been devoted to compare the bacterial flora attached on the different particles (Mahler et al., 2000).

However, during the past decade, it became evident that aquifers harbour large populations of physiologically active micro-organisms which interact with the abiotic environment by adsorption and/or biofilm formation on surfaces (Costerton et al., 1995). The more recent definition of biofilms does not only take account of readily observable characteristics (i.e. cells irreversibly attached to a surface or interface, embedded in a matrix of extracellular polymeric substances which these cells have produced) but also considers other physiological attributes of these organisms, i.e. altered growth rate and transcription of genes different than those transcribed by planktonic organisms (Donlan and Costerton, 2002). Recently, Lehman et al. (2001) showed that 99% of the total biomass was found attached to the geologic medium in packed columns. Consequently, turbid runoffs induce microbial contamination associated to suspended particles.

In the present study, we describe an original approach to characterize the functioning of a karstified chalk aquifer. The assessment of physicochemical and bacteriological data at a sinkhole and a spring after three storm events provides information about contaminant spreading and groundwater quality in the karst system. We focus on the sanitary significance of the water turbidity (indirect indicator of the bacterial contamination) compared with enumerations of sessile and planktonic bacteria. We show that turbidity is not always correlated with the numbers of particle-associated bacteria and consequently cannot be used as the sole indicator of sanitary risk.

2. Study site and methods

2.1. Study area

The study area is a karstified chalk aquifer in Norville (Haute-Normandie, France) that is located in the chalky catchment of the Seine river. The Norville system (Fig. 1), that has been widely studied (Massei, 2001; Massei et al., 2002), is composed of a sinkhole and a spring (at the named place ‘Le Hannetôt’).

The sinkhole is the swallowing point of Bébec Creek waters, which drain a small watershed of about 10 km^2. Soils on the plateau, consisting of silts approximately 10 m thick, are highly susceptible to crusting, compaction, and erosion, particularly during autumn and winter sowing. The Bébec Creek discharge is variable, from 3 l s^{-1} in summer dry periods up to 15 l s^{-1} in winter, and close to 500 l s^{-1} in response to major winter storms (Massei et al., 2002). Water from the creek recharges the chalk aquifer via a sinkhole. During storm periods, the water in Bébec Creek is very turbid, up to 2000 NTU, consequently to soil erosion.

Hannetôt Spring discharges from the foot of a karstified chalk cliff (located 2.2 km far from the sinkhole). After storms, the turbidity of the water discharging at the spring can exceed 600 NTU (Massei et al., 2002).

2.2. Physicochemical data

Physicochemical data at both sinkhole and spring were monitored from the 18th to 25th of February 2002 during different storms. Field equipment consisted of multi-parameter 6820 YSI datasonds, each comprising a turbidity probe, an electrical conductivity/temperature probe and a pressure sensor. An ISCO 674 rain gauge is settled on the watershed. Turbidity, electrical conductivity and precipitation are recorded according to a 15 min time step. Data are downloaded by means of the ISCO Flowlink 3 software (ISCO Inc.). The distribution of electrical conductivity frequencies was used to assess the karstic character of the system: three main types of water

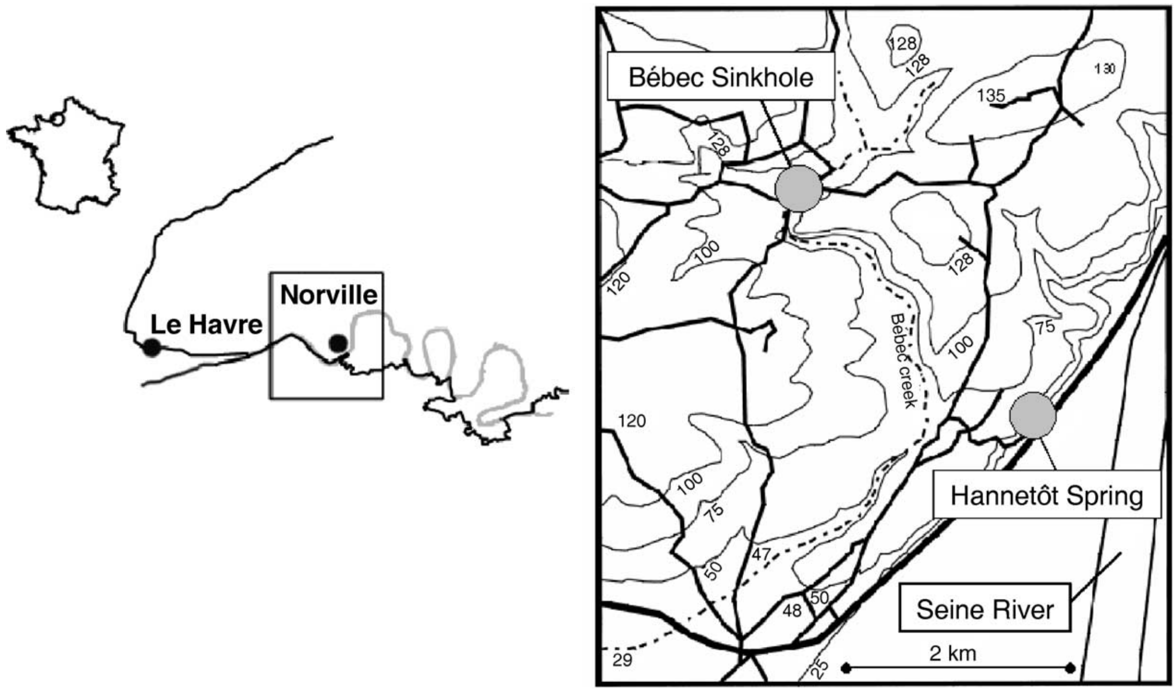

Fig. 1. Location of the study site.

could be distinguished over one hydrological cycle (typical matricial chalk water, storm-derived water and intermediate-conductivity water). Water level (pressure) values were not available at the sinkhole for the period of concern due to recording trouble. The pressure sensor was calibrated using an ISCO 4150 Doppler flowmeter which gave a rating curve for Hannetôt spring (Massei, 2001).

Samples were collected with autosamplers (ISCO 6700s at the sinkhole and the spring) in response to rain events and then filtered through pre-weighted Millipore filters (0.45 μm pore-size). The concentrations of suspended particles (SPM) were correlated to the measured turbidities. The particle size distributions, which allow measurement in the 2–63 μm range, were determined with a Coulter Multisizer particle counter, using a 100-μm aperture. The amount of particles larger than 63 μm was determined by successive sieving of 100 ml of water on filters of 63, 125 and 200 μm pore size.

Particles introduced into the sinkhole and those discharging from the spring were compared by scanning electron microscope observations (Au–Pd coating, secondary electrons-based method, Cambridge S200).

2.3. Enumeration of planktonic and sessile bacterial flora

For enumeration of planktonic cells, particles were recovered by filtration of 40 ml of water under vacuum using cellulose acetate membrane filters (1.2 μm pore size, Millipore). The number of Colony Forming Units (CFU) was determined by plating 0.1 ml of suitable decimal dilutions of the filtrate onto Plate Count Agar (PCA, Difco, Detroit, MI, USA) and incubating plates for 48 h at 25 °C.

For sessile bacteria enumeration, 20 ml of water were sonicated at 4 °C (Deltasonic bath, Meaux, France, 4 min at 50 W). Aliquots (0.1-ml volume) of appropriate decimal dilutions of the water were then spread onto PCA. Dishes were incubated for 48 h at 25 °C. Sessile flora were evaluated by subtracting the number of planktonic cells, which

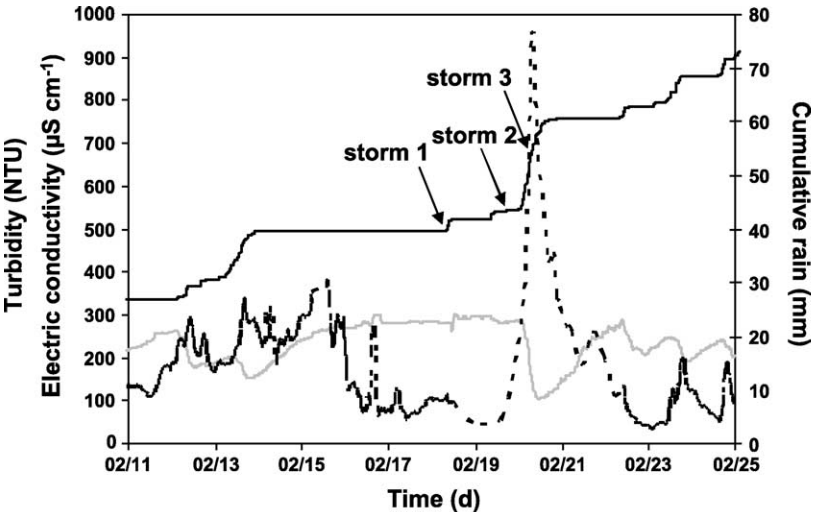

Fig. 2. Turbidity and electrical conductivity in response to rainfall at the sinkhole. – – –, turbidity; ▬, electrical conductivity; —, cumulative rain.

have been evaluated as described above, from the obtained cell population.

All counts were performed in duplicate.

3. Results

Responses to the three storms (labelled in Fig. 2) were studied by analysing the variations of turbidity, conductivity and bacterial concentrations, in relation to the occurrence, amount and intensity of the rain events.

Storm 1 (intensity of 0.75 mm h^{-1}) did not induce any significant change in turbidity while the second storm (intensity of 0.54 mm h^{-1}) induced the beginning of the turbidity peak (Table 1 and Fig. 2). A large increase of turbidity occurred during the third storm that was characterized by strong precipitations (intensity of 1.24 mm h^{-1}). The peak reached a maximum of 900 NTU 6.5 h after the beginning of the storm.

Table 1
Enumeration of planktonic and sessile florae at the sinkhole

Date (y/m/d/h)[a]	Turbidity (NTU)	SPM (mg l^{-1})	SPM > 63 μm (%)	Planktonic bacteria (CFU l^{-1}) × 10^6	Sessile bacteria (CFU mg^{-1}) × 10^6	R^b
02/02/18/11	40	51.4	–	10	5.3	27
02/02/18/19	19	24.6	–	112	7.6	2
02/02/19/03	15	17.2	–	1.8	3.67	3
02/02/19/15	22	24.3	–	3.8	9.3	59
02/02/20/03	240	429	14.81	2.2	0.04	8
02/02/20/07	900	2605	18.75	4.3	0.004	3
02/02/20/11	560	5619	54.37	4.6	0.05	58
02/02/20/23	200	969	25.97	3.6	0.09	24
02/02/21/09	100	556	57.43	1.9	0.03	9

–, undone.
[a] Year, month, day, hour.
[b] R, ratio of adherent bacteria versus planktonic cells.

Table 2
Enumeration of planktonic and sessile florae at the spring

Date (y/m/d/h)[a]	Turbidity (NTU)	SPM (mg l^{-1})	SPM > 63 μm (%)	Planktonic bacteria (CFU l^{-1}) × 10^6	Sessile bacteria (CFU mg^{-1}) × 10^6	R^b
02/02/18/11	18	35	–	0.1	1.6	455
02/02/18/19	14	36	–	1.0	0.1	5
02/02/19/03	16	23	–	0.005	0.3	1400
02/02/19/15	12	16	–	0.005	0.1	330
02/02/20/07	14	16	–	0.005	0.1	340
02/02/20/19	290	580	11.50	2.0	0.4	126
02/02/21/01	250	508	6.83	2.0	0.2	52
02/02/21/11	120	570	7.64	0.7	0.05	40
02/02/21/23	68	265	6.23	0.1	0.05	99

–, undone.
[a] Year, month, day, hour.
[b] R, ratio of adherent bacteria versus planktonic cells.

The decrease of the electrical conductivity points out a dilution phenomenon of surface waters by rainfalls. The phenomenon, less visible but existing for storms 1 and 2, was obvious during storm 3 (Fig. 2).

Only one hydrodynamic event associated to turbidity was observed at the spring: storms 1 and 2 were probably too low to produce a significant particle transport through the karst system contrarily to storm 3 which corresponded to higher rainfall. The lag in turbidity response between the spring and the sinkhole was 14.5 h which corresponds to an apparent modal velocity of 152 m h^{-1} (Tables 1 and 2, Fig. 3). At the peak, turbidity undergoes an apparent dilution of about 60% at the spring (maximum of 370 NTU) compared to that observed at the sinkhole (maximum of 900 NTU) (Tables 1 and 2, Figs. 2 and 3). The arrival at the spring of less mineralized water was also much more noticeable for storm 3 (strong decrease of electrical conductivity, Fig. 3).

Scanning electron microscope examinations showed a marked difference between particles discharging at the spring and those entering the sinkhole during rain events (Massei et al., 2002). At both the sinkhole and the spring, the particles

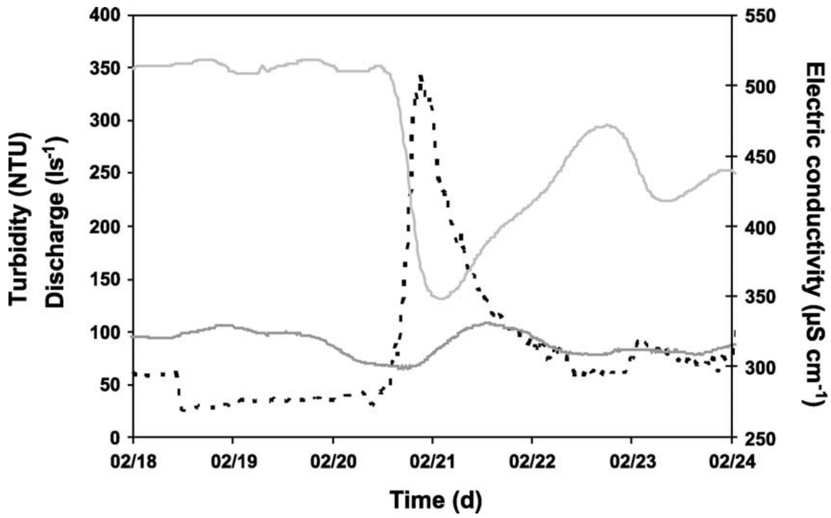

Fig. 3. Time-evolution of the turbidity, electrical conductivity and discharge at the spring. – – –, turbidity; ——, electrical conductivity; ——, discharge.

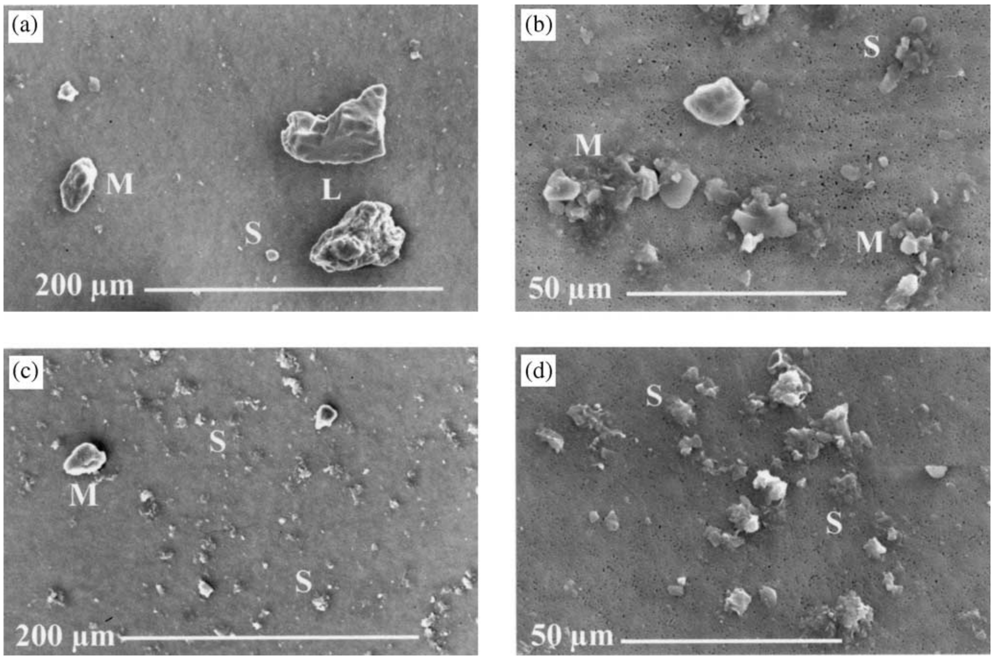

Fig. 4. Scanning electron micrographs of particles from the sinkhole (a, b) and the spring (c, d). L, large, M, medium, S, small particles.

consisted of organic-mineral flocs and quartz grains of about 5–10 μm in diameter, and aggregates and grains varying in size from 15 to 35 μm (Fig. 4). This material, characteristic of the erosion on the Bébec Creek watershed, was introduced into the aquifer via the sinkhole and served as a reference to which the material discharging from the spring can be compared. At the spring, particle size was equally variable and the ratio of the two size ranges was reversed as compared to that observed at the sinkhole (Fig. 5). This microgranulometric trend is typical of all the samples taken during the overall period (storms 1, 2 and 3). Sieving showed that 15–57% of the particles introduced at the sinkhole, and 6–11% of those recovered at the spring, exhibited a size superior to 63 μm (Tables 1 and 2).

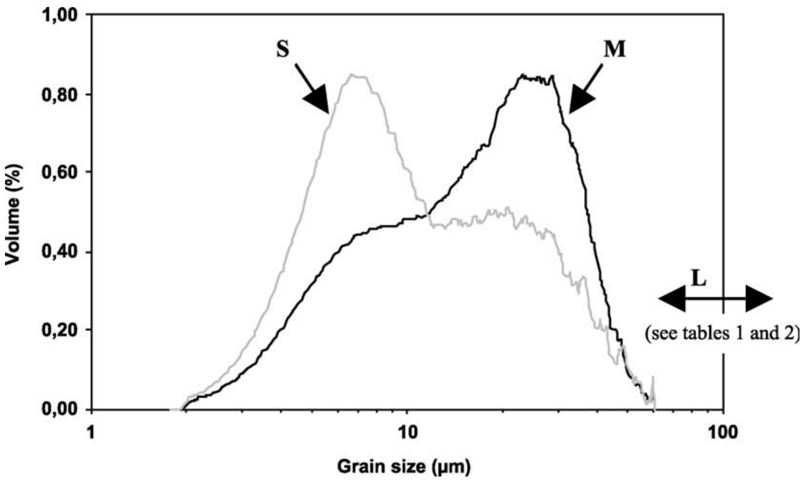

Fig. 5. Particle size distribution at the sinkhole and the spring. —, sinkhole; ——, spring; L, large, M, medium, S, small particles.

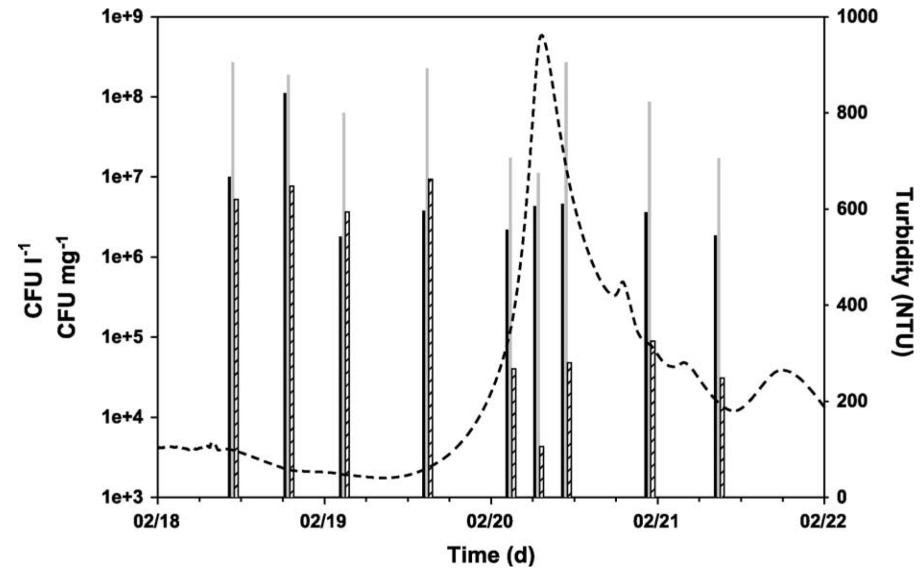

Fig. 6. Evolution of the amount of planktonic and sessile bacteria at the sinkhole. – – –, turbidity; —, planktonic bacteria; ▬▬, sessile bacteria (CFU l^{-1}); ▨▨, sessile bacteria (CFU mg^{-1}).

The concentration of planktonic bacteria remained constant at the sinkhole during the sampling period (in the range of 10^6 CFU l^{-1}), except after the first storm where a peak of contamination was observed (Table 1 and Fig. 6). Storms 1 and 2 did not change the concentration of bacteria associated to particles, that stabilized at about 5×10^6 CFU mg^{-1}. On the other hand, a strong decrease of the concentration of adherent bacteria was observed during storm 3.

At the spring, the concentration of planktonic cells was significantly lower than that measured at the sinkhole (Table 2 and Fig. 7). It slightly increased

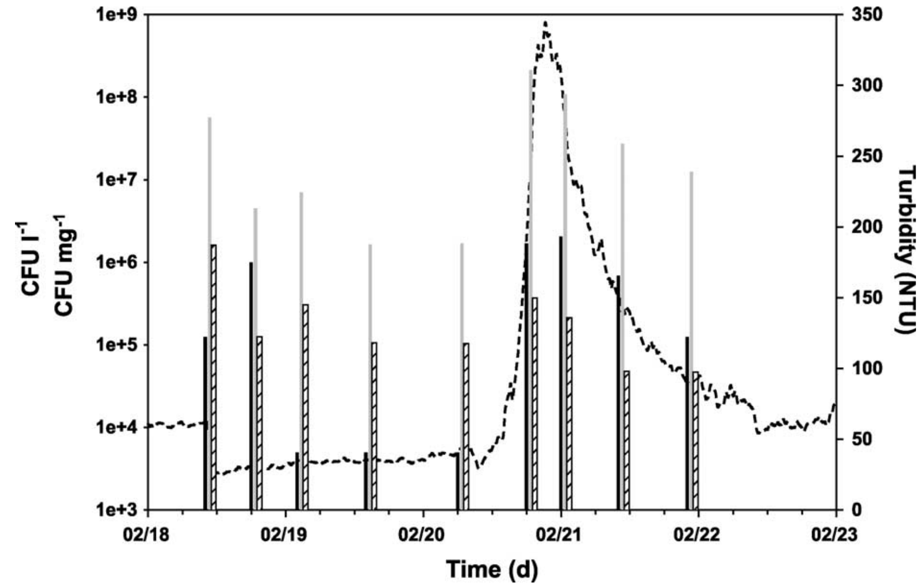

Fig. 7. Evolution of the amount of planktonic and sessile bacteria at the spring. – – –, turbidity; —, planktonic bacteria; ▬▬, sessile bacteria (CFU l^{-1}); ▨▨, sessile bacteria (CFU mg^{-1}).

Table 3
Dye tracing results according to hydrological conditions

Period of the tracer test	Oct-99	Feb-00	Jan-01 (1)	Jan-01 (2)
Mean spring discharge (l s^{-1})	34	47	64	63
Mean transit time (m h^{-1})	107.0	139.8	227.0	173.2
Tracer recovery rate (%)	93	99	94	95

during the third storm to reach 2×10^6 CFU l^{-1}. The concentration of sessile bacteria (about 10^5 CFU mg^{-1}) was slightly lower than that measured at the sinkhole but was not modified by the rainfall events.

4. Discussion

Various responses in turbidity were observed according to the storms at the sinkhole. The absence of turbidity change after the first storm was probably due to the dry period (1 week) that occurred prior to rain events. Consequently, rainfalls were not sufficient to allow soil erosion (infiltrations were predominant with respect to runoff). The low decrease of the electrical conductivity during storms 1 and 2 suggested a runoff beginning. Ground getting waterlogged, the second storm induced the beginning of the turbidity peak. The great increase of turbidity and decrease of electrical conductivity during storm 3 suggested an erosion process.

The modal velocity observed between the sinkhole and the spring is in accordance with those previously measured by tracer tests (Massei, 2001; Massei et al., 2002) and is typical of karstic systems where groundwater flow velocities may even exceed 200 m h^{-1} (Table 3). The similarity between the transit times for both tracer and turbidity suggests that particles follow the same pathway as the tracer. The apparent dilution of the turbidity at the spring might be due to a strong dilution of surface water within karstic groundwater (40% surface water/60% groundwater, based on peak turbidities ratio).

Even though an experimental artefact could not be ruled out, the appearance of a bacterial contamination peak at the sinkhole during the first storm might reveal the runoff beginning. The strong decrease of the concentration of adherent bacteria observed during storm 3 was due to the arrival of less contaminated large particles probably originating from soil erosion on the watershed. The low contamination of these particles explains the negative slope of the regression line correlating the concentration of sessile bacteria and the turbidity (Fig. 8).

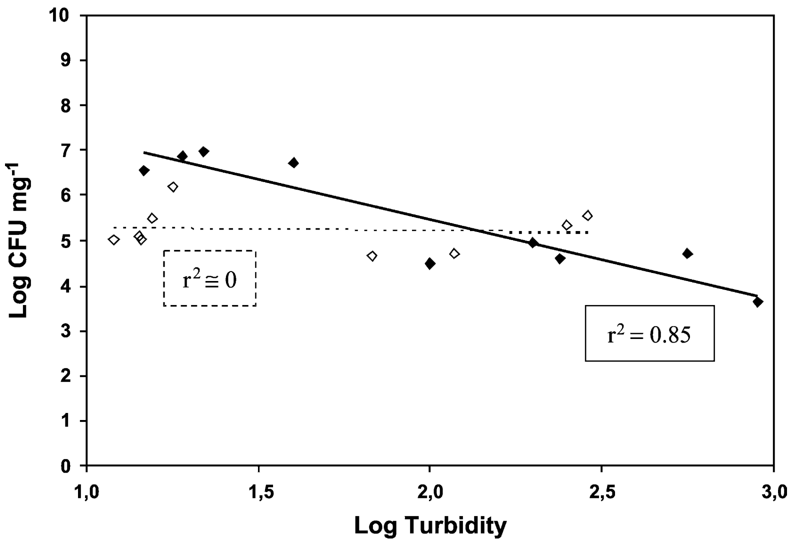

Fig. 8. Evolution of the amount of sessile bacteria over the turbidity at the sinkhole and the spring. ◆, sinkhole (— linear regression); ◇, spring (··· linear regression).

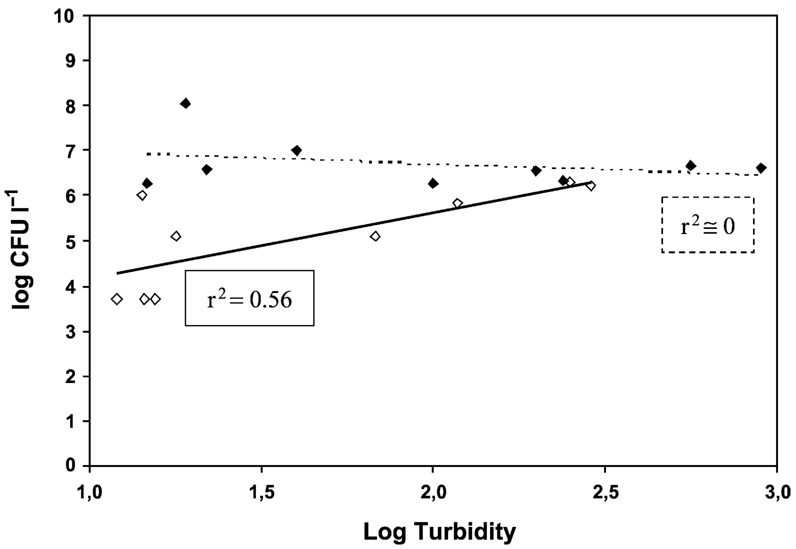

Fig. 9. Evolution of the amount of planktonic bacteria over the turbidity at the sinkhole and the spring. ◆, sinkhole (··· linear regression); ◇, spring (— linear regression).

At the spring, low concentrations of planktonic micro-organisms were measured for low turbidity values, i.e. for low hydrodynamic flows (Fig. 9). This phenomenon points at a storage property of the aquifer and/or a dilution by intrakarstic waters. However, the amount of planktonic organisms increased with the turbidity and tended towards the contamination level observed at the sinkhole (Fig. 9). In contrast, the concentration of sessile cells remained constant for all turbidity values (Fig. 8). This discrepancy might be explained by the re-suspension of previously deposited contaminated sediments. Moreover, for high turbidity values (i.e. larger than 120 NTU), the concentration of sessile bacteria exceeded that measured at the sinkhole. This observation and the presence of a great amount of bacteria attached to suspended sediments even for low turbidity values point out a major problem in the sanitary survey of groundwater outlets of karstified aquifers. In fact, the most striking feature of biofilms is their high resistance to stresses, in particular against antimicrobial agents (Stewart and Costerton, 2001). This reduced susceptibility of sessile organisms to inhibitors is a crucial problem for the treatment of chronic infections of implanted medical devices (Stickler and McLean, 1995; Habash and Reid, 1999) or chlorine disinfection (Samrakandi et al., 1997). In a recent paper (Dussart et al., 2003), we described the presence of a *Pseudomonas oryzihabitans* strain (an uncommon pathogen that may cause opportunistic infections) adhering on suspended particulate matters recovered from the Hannetôt spring. Adherent *P. oryzihabitans* cells (biofilms on clay beads) displayed a high resistance to chlorine as compared with the same organisms cultured in the planktonic mode.

Differences in the *R* values, i.e. ratios of adherent bacteria versus planktonic cells, measured at the sinkhole and the spring might be due to the fact that (i) the larger particles were not swallowed in the aquifer but might settle within the cone of the sinkhole (doline) or (ii) the larger particles might settle within the karstic network (Massei, 2001). The particle size distribution of suspended solids suggested the deposition of high-size particles, large particles (i.e. 20–200 μm) were in majority at the sinkhole whereas a population of small-size (i.e. 2–12 μm) particles dominated at the spring. Fig. 7 points out the high material contamination at the spring. The *R* values ranged from 5 to 1400, i.e. strongly higher than those observed at the sinkhole (from 2 to 59). The *R* values, strongly superior to 1, are in accordance with the paradigm stating that bacteria preferentially survive in the ecosystems under the sessile status (Zobell, 1943; Costerton et al., 1995; Dunne, 2002) and were higher than those described by Mahler et al. (2000) who reported that the proportion of fecal coliforms associated with sediment particles varied from 0 to 60% in wells. This discrepancy might be explained by the experimental protocol used by these authors to recover contaminated particles, i.e. membrane filtration. In fact, some experiments performed by our group pointed out a great underestimation of the sessile population when membrane filters were used to recover particles (data not shown).

5. Conclusion

One of the difficulties concerning the transport of the solid phase through aquifers is the difficulty to distinguish the resuspension of previously deposited sediments and the direct transfer of particles. It is in fact difficult to find markers characterizing the origin of turbidity. Thus, Mahler et al. (1998a,b) used tagged particles to trace particle transport. In this paper, we present an original approach for a better understanding of the turbidity phenomena that occur during rainfall periods in karst waters. We demonstrated that investigation on the transport and bacterial contamination of particles allows a better understanding of the response of a karst aquifer to rain events.

Our study confirmed the intrakarstic storage/resuspension notion that was previously advanced by Massei (2001). Moreover, we showed that the groundwater transfer through the chalk karstic aquifer induced a high decrease of the concentration of planktonic bacteria whereas no reduction of the sessile population occurred. The present data confirmed that a high turbidity obviously reflects a bad sanitary quality of the water but also demonstrated that low turbidity does not systematically exclude a risk by sessile organisms. These results and the presented data point out the role of aquifer biofilms as potential environmental sources for water-borne infections and confirm that karst terrain, by its nature, is vulnerable to bacterial contamination.

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