Characterization of natural colloids from a river and spring in a karstic basin

O. Atteia · D. Perret · T. Adatte · R. Kozel · P. Rossi

Abstract Colloids are known to transport contaminants over long distances in natural media. Despite this potentially harmful effect, very few studies have been undertaken in subsurface aquifers. This paper presents the first results of a study of natural colloids and particles in a karstic aquifer. The site was chosen for its coverage by clay layers and peat which deliver various and numerous particle types in water. The methodological part describes three methods used for size determination and sample fractionation of surface water and spring water. These methods have been adapted for the treatment of multiple samples due to the rapid discharge variation typical of karstic aquifers. The analysis of many particle size distributions (PSD) shows that they can be described by a Pareto law. The variation of the slope of the PSD at the spring is mainly dependent on discharge. This behavior is interpreted as a washing of the karstic drains during the first phase of high flow events. Fractionation of the samples allowed application of various characterization techniques to particle size classes. X-ray diffraction (XRD) patterns showed that most of the mineral particles originated from Quaternary deposits and limestones. However the use of scanning electron micrsocopy with energy-dispersive spectrometer (SEM-EDS) detailed the composition of individual particles and revealed particles not found by XRD. These techniques also showed the high complexity of the natural particles and the important place of coprecipitation in their formation. Consequences on the fluxes of particulate matter and its potential role as a carrier of contaminants are discussed.

Key words Colloids · Particle size distribution · Karstic aquifer · Analytical techniques · Bacteria

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O. Atteia (✉) · R. Kozel
[01]Centre d'Hydrogéologie de Neuchâtel, Neuchâtel University, 11 rue Emile-Argand, 2007 Neuchâtel, Switzerland

D. Perret
[02]Institut de Chimie Minérale et Analytique, 1015 Lausanne, Switzerland

T. Adatte
[03]Institut de Géologie, Neuchâtel University, 11 rue Emile-Argand, 2007 Neuchâtel, Switzerland

P. Rossi
[04]Laboratoire de Microbiologie, Neuchâtel University, 9 rue Emile-Argand, 2007 Neuchâtel, Switzerland

Introduction

Particles are generally considered as colloids when their size ranges from some nm to some μ depending on the flow conditions. This size range implies a long settling time in water, typically from days to months, and therefore a potential for colloids to carry contaminants over long distances (McCarthy and Zachara 1989). In natural waters colloids are usually clays, iron (oxyhydr)oxides, silica, organic matter and bacteria. Concerning their chemical properties, colloids are often defined as charged species and solids having large specific areas (Buffle and Leppard 1995). This implies a high reactivity which explains their ability to transport contaminants. These properties also allows the colloids to aggregate under the influence of varying physical and chemical conditions. Colloids have mostly been studied in two types of aquifers: deep fissured rocks for radionuclide repositories (Smith and Degueldre 1993) and contaminated subsurface porous rocks (Gschwend and others 1990). Despite the lack of studies on karstic aquifers they are of major interest for two main reasons: (1) they are highly vulnerable to contaminants (Doerfliger and others 1996) and therefore any potential contaminant carrier must be studied, and (2) the discharge variations are very large (Kiraly 1988), thus enabling the study of colloidal behavior in very different hydrodynamic conditions. The uniqueness of karst and the developing concern over colloids incited the study presented here.

This paper presents one of the first attempts to characterize colloids from a karstic aquifer. We are mainly concerned here with (1) the methodology development of colloidal analysis which was needed; (2) the characterization of the particle size distribution (PSD); and (3) the type, origin, and evolution of particles sampled in these waters. In fact PSD and particle type are both necessary to understand the role of colloids on contaminant transport.

Site description

Site location

The Noiraigue basin is located in the Swiss Jura Mountains at an altitude ranging from 1000 to 1350 m (Fig. 1). Average rainfall, over the last 70 years, was about 1500 mm/year. The catchment area is represented by (1) a flat valley ('Vallée des Ponts') covered after the last glaciation by peat-bogs, and (2) the surrounding calcareous anticlines. Peats and agricultural soils are drained by the 'Bied' Brook which flows to a main sinkhole. Noiraigue spring, single outlet of the closed basin, lies at an altitude of 740 m, at about 4 km from the main sinkhole, and gathers waters from the valley (ca. one-third) and from the calcareous anticlines (two-thirds).

Geological setting

During the late Miocene the alpine deformation led to the folding of the Jura, with anticlines and thrust faults trending in a general SW–NE direction. The general structure of the Vallée des Ponts syncline can be described as a triangular flatlying basin bordered by thrusted anticlines. A major N–S trending fault has been recognized within this bend zone. The Noiraigue spring flows close to this tectonic contact (Gretillat 1995). The anticlines are mainly composed of Jurassic limestones overlain by Cretaceous limestones and marls. Controlled source audio magnetotellurics (CSAMT) measurements conducted in the Vallée des Ponts syncline showed an unexpected thickness of about 300 m of Tertiary molasse overlying the Mesozoic carbonates. This result has been confirmed by preliminary reflection seismic sounding measurements.

The main Quaternary deposits identified in the valley are (1) tills, characterized by small hillocks; (2) alluvial fans, originating from lateral valleys perpendicular to the axis of the anticlines; (3) peats; and (4) soils. Marly deposits, thicker than 10 m, underlying the soils were detected by shallow boreholes drilled in the southern and central part of the valley. No marly deposits were found on slightly more elevated zones of the valley, i.e., on till deposits or on fans which represent a minor area. The large area covered by these very low permeable deposits explains peat formation and its large extent before agricultural development.

Water circulation

The hydrogeological details can be found in Atteia and others (1997). The Jurassic and Cretaceous limestones constitute the major part of the aquifer in which the karstic network is developed. Tracer tests showed the connection between the sinkholes and Noiraigue Spring, and the high water velocities within the aquifer (50–150 m/h) suggest large karstic drains. It is hypothesized that the major water passways occur in the fractured zones of the aquifer, i.e., along the anticlines.

The meteoric water entering the peat flows laterally within the very permeable ($K = 10^{-2}$–10^{-3} m/s) top peat layers. The water trapped in the deep layers flows very slowly due to compaction of the peat ($K = 10^{-7}$–10^{-8} m/s). Most of the peats have been converted for agricultural use by draining. The drainage network is very tight due to the low permeability of the presently mineralizing topsoil layers. It quickly drives the water to Bied Brook or to other sinkholes located on the border of the valley (Fig. 1), where the Quaternary sediments and the subvertical limestone are in contact.

Potential sources of colloids

The basin should, a priori, be able to deliver the main types of natural colloids: clays, grain minerals, bacteria, viruses, organic matter and oxyhydroxides. The Quaternary marly layers covering the valley are potential sources of clays. The action of bacteria releases organic matter in the form of humic and fulvic acids, which are important colloid builders in the environment (Stumm 1987). Peat areas should thus be able to deliver very large amounts of fulvic and humic acids as they contain more than 90% organic matter (Matthey 1986). Agricultural soils will also deliver humic and fulvic acids to the water because they still contain a high percentage of organic matter. These molecules should be more mature than peat ones due to the oxygenation and neutral pH of these soils. As water flows very slowly within the deep peat layers, these levels are often anoxic. This leads to the presence of Fe in ferrous (Fe^{2+}) form which is soluble and can thus be transported as solute. When ferrous iron enters in contact with oxic waters, it will precipitate and can thus form colloids. This process may be retarded if Fe^{2+} is protected from oxidation by forming complexes with humics.

Methods

The methodology for sampling and characterization of colloids has been set up according to the following objectives and constraints:

1. Particle size distribution (PSD) – particle size is a very important parameter for the transport of colloids and varies within a very broad range in natural samples (Buffle and Leppard 1995).
2. Numerous samples – due to the very fast and important hydrographic variations in karstic aquifers (Tripet 1972) numerous samples must be taken.
3. Chemical and mineralogical characterization are necessary as calcite, organic matter and clays do not adsorb contaminants in the same way.

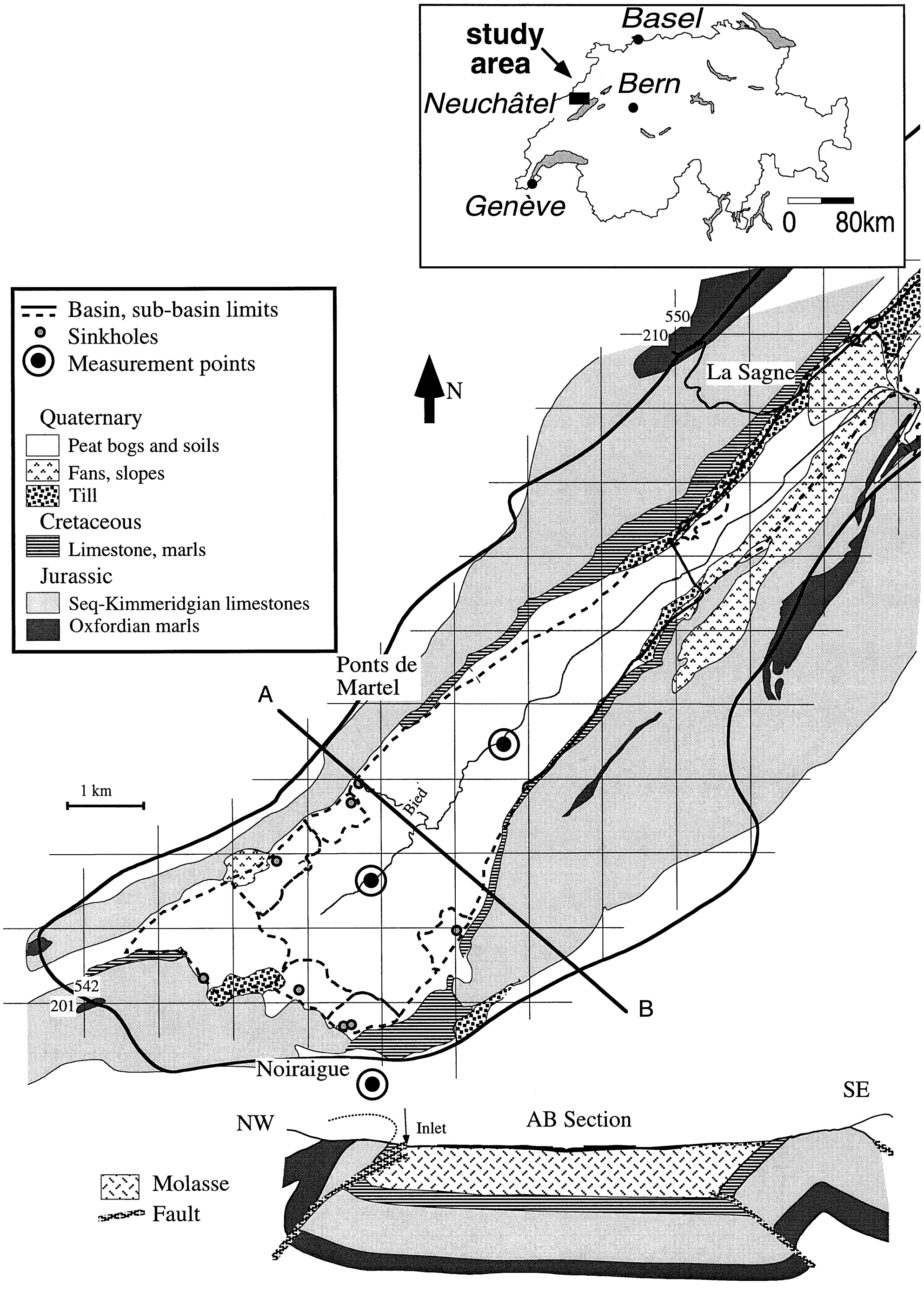

Fig. 1
Simplified geological map and section of the Noiraigue aquifer

4. Different and complementary methods – numerous techniques exist for the characterization of colloids (Barth and others 1987), but they all present nonnegligible artifacts and few of them have been applied to natural suspensions.
5. Quick analysis – colloidal suspensions were shown to evolve quickly in some cases (Perret and others 1994), even if the mechanism of coagulation is not totally understood for natural samples. Only very few studies have successfully used preservatives (Gallegos and Menzel 1987), and none of them is universally accepted.

Chemical analysis of solutes

Suspensions were taken each week for one year; one 15-day-long event was sampled at shorter time steps. Samples were analyzed, after addition of 0.6% supra-pure HNO_3, for cations such as Ca, Mg, Na, K, and Fe by atomic absorption flame spectroscopy (AAS, Perkin Elmer 5100 PC); Al, Si, Sr, and Ba concentration were determined on some samples by inductive coupled plasma (ICP, Perkin Elmer Plasma 1000). Anions were analyzed by flow injection analysis (FIA, Tecator Fiastar 5010), total organic carbon (TOC) and dissolved organic carbon (DOC) obtained by filtration on glass fiber filters (ca.

Table 1
Average concentration of solutes in sampled waters

	Ca^{2+}	Mg^{2+}	Na^{+}	K^{+}	Si	Al^{3+}	NO_3^-	Cl^-	SO_4^{2-}	TOC	DOC	Fe^{2+}	Mn^{2+}	Ba^{2+}	Sr^{2+}
	mg/l											µg/l			
Bied	79.4	2.8	1.4	1.4	3.6	0.3	3.4	3.6	8.8	20.2	20.6	741	88	17	218
Noiraigue	84.0	3.1	1.8	1.3	1.9	0.3	7.9	3.7	5.4	4.7	5.0	114	7	12	416

0.3 µ) were measured by a Dohrmann Xertex DC-180. Mean concentrations in Bied Brook and at Noiraigue Spring are presented in Table 1.

Laser light blocking for the determination of PSD

The apparatus used (CIS, Galai) counts individual particles from 0.5 to 60 µ by measuring their shadow on a laser light detector. There is no need for any pretreatment or fractionation of the sample. This technique has been applied under continuous flow (50 ml/min) and stirring conditions. The analysis was always repeated three times (Jantschik and others 1992) and the analysis was done until the replicability was judged sufficient.

Different tests were performed to assess the quality of the PSD determined by CIS. Three cultures of bacteria were counted by epifluorescence and by CIS; the standard deviation between the two techniques was lower than 20% for a range of more than one order of magnitude. A second comparison, between a single particle counter using scattering and diffraction (Grimm system, EAWAG, Switzerland) and the CIS, showed differences of less than a factor of 2 for counting over a range of 5 orders of magnitude. However, the results were less precise for the poorly represented particles, larger than 10 µ, due to dilution differences between the two techniques.

For one sampling event, CIS data were compared with particle counting on Nuclepore filters of 2, 0.8, 0.2 and 0.05 µ. After filtration the filters were dried and three pictures of each filter where taken by scanning electron microscopy (SEM). Using an image analysis software, 1500 particles were then measured and counted. The two techniques agree well, with a difference less than 25% for measurements over several orders of magnitude.

Filtration

Filtration is a widely used technique (Laxen and Chandler 1982). Its main advantages are its ease of use and the possiblity of performing fractionation directly on site.

The main drawback, described by several authors (Buffle and others 1992; McDowell-Boyer and others 1986), is the accumulation of particulate material close to the filter, which enhances the coagulation effect and further induces clogging. This can be diminished by using a slow flow rate, with a flux of approximately 1 ml min^{-1} cm^{-2} depending on the sample (composition, concentration).

For a more accurate size cut-off, Nuclepore filters are commonly used (Gschwend and Reynolds 1987; Laxen and Chandler 1982). Cascade filtration is advised to separate different size classes and to avoid clogging of small pore-size filters.

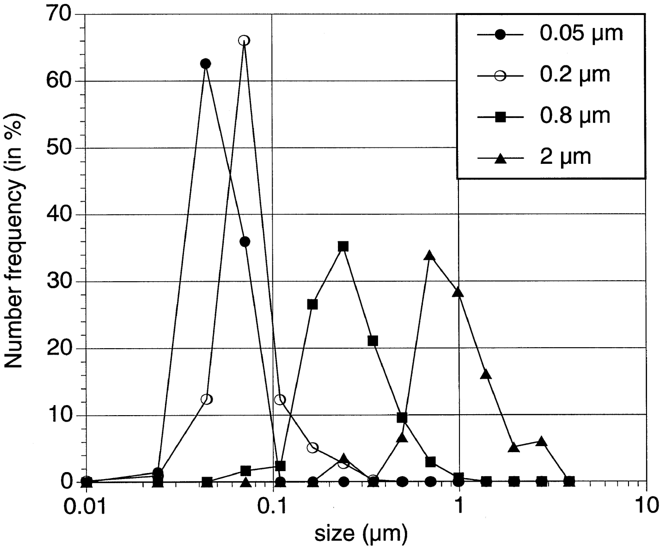

Fig. 2
Particle size distributions of particle collected by the cascade Nuclepore filtration stack and determined by SEM

Knowing these important constraints we developed a cascade stack with Nuclepore 2.0, 0.8, 0.2, and 0.05 µ filters (47 mm ∅). For the three initial filtration steps, the suspension is filtered by gravity using a 20 cm water pressure; then pressure is imposed on the system to flow through the 50 nm filter. The whole filtration procedure lasts 2–4 h to get the 50 ml of each fraction necessary for chemical analysis. The water velocity, the hydrophobicity of the filter and the irregular shape of the particles decrease the real cutting size (Fig. 2). However this diagram shows that the technique used allows a clear fractionation without clogging, no artifacts are observed as the PSD is conserved (as compared with CIS Galai).

Centrifugation for fractionation

The mineral composition of the first 40 sampling events for water samples was determined by XRD using centrifugation at 1800 g for 20 min. This allows the deposition of the >1 μ fraction. Deposited matter was treated ultrasonically in a small tube containing an XRD glass plate at its bottom. After 3 days of sedimentation, the glass plates were air dried and analyzed with a Scintag 2000 diffractometer (Kübler 1987).

To perform fractionation of the colloidal matter, we used ultracentrifugation which separates particles according to their *size and density*. Different authors suggest that ultracentrifugation should lead to fewer artifacts than filtration and used it to estimate PSD (Buffle and Leppard 1995; Perret and others 1994). To avoid the artifacts generated by repeated centrifugation and resuspension (Salbu and others 1985; Salim and Cooksey 1981), we used continuous ultracentrifugation.

The continuous ultracentrifuge (Sharpless T1) consists of a vertical cylinder in which the sample is introduced at the bottom. During rotation, a 0.5-cm-thin film of water flows up on the cylinder wall at a velocity given by the imposed water flux. Particles submitted to the horizontal centrifuge force (up to 40,000 g) are collected on the cylinder wall. The particle accumulation shows a vertical gradient defined by size and density of particles. Particles are recovered onto a plastic sheet previously inserted in the cylinder cut at given distances to obtain size classes. Using a sedimentation step for particles larger than 5 μ, and applying 20,000 rpm (13,000 g) and 275 ml/min, we obtained classes of the following calculated sizes: 0.2–0.4, 0.4–0.65, 0.65–1.3, and 1.3–5 mm. The limit of the classes are approximate due to the uncertainty in density and to turbulent motion existing in the tube. Due to its low density, organic matter is not collected by this procedure, except for large organisms. Ten liters of a suspension containing 0.5 mg/l of colloids were necessary to weigh the recovered material and to record reliable XRD spectra on all size classes. The difference between the mass of particle classes obtained from the ultracentrifuge and those obtained by using a laser sizer on four samples was lower than a factor of 2 despite the errors in density estimation.

Mineralogy

Clay mineral analyses are based on the methods of Kübler (1987). For rock samples, ground chips were mixed with deionized water (pH 7–8) and agitated. The carbonate fraction was removed with the addition of 10% HCl (1.25 N) at room temperature for 20 min, or longer until all the carbonate was dissolved. Ultrasonic disaggregation was accomplished in 3-min intervals. The selected fraction was then pipetted onto a glass plate, air-dried at room temperature and analyzed by a Scintag XRD 2000 diffractometer (Scintag 1994). The height (in counts per second, CPS) of selected XRD peaks characterizing each clay mineral present in the size fraction (e.g., chlorite, mica, kaolinite, smectite) were measured for a semiquantitative estimate of the proportion of clay minerals present in the size fractions <2 μ and 2–16 μ (given in percentages; error 5%).

Electron microscopy

The composition of individual particles was determined with a SEM-EDS (Link analysis Si(Li) detector with Be window, mounted onto a Jeol JSM-35 microscope operated at 20 keV). Particles from Bied Brook and Noiraigue Spring were recovered from 4 sampling dates (19 and 26 July, and 09 and 16 August 1995) onto Nuclepore polycarbonate filters (2 and 0.8 μ). Approximately 1000 particles were analyzsed after carbon coating of the membranes; they were randomly chosen and analyzed under identical conditions. Particles below 0.5 μ were not detected because of poor signal-to-noise ratio. In the 0–10 keV region, special attention was given to the x-ray emissions of Mg, Al, Si, P, K, Ca, Ti, Mn and Fe. The results are expressed as the intensities of emission for the given elements; only peaks with a detection limit >2 were considered. As a rough estimate, and because the analyzed surface was identical for every particle (5 μ × 5 μ), the absolute intensities of recorded peaks are related to the particle size.

Bacteria counting

The bacteria flora was enumerated by using the following two techniques:

1. Liquid cultures by serial dilution of samples in microtiter plates or incubation of filters on solid media.
2. Epifluorescence using a photonic microscope with fluorescent light source (Stoecker and others 1989). The nucleic acid stain used is DAPI (4′-6′ diamino-2-phenylindole). Counting is done on a square glass slide.

Typical size distributions

Table 2 shows the large variation of amount and size of particles in different types of natural waters. The lack of standardization or comparison of methods for this difficult analysis suggests that one should only focus on orders of magnitude. For the amount of colloidal/particulate matter, this study is intermediate between deep groundwaters and large rivers. The peaks of the volume weighted distributions occur at fairly large sizes compared to any other groundwater. A preliminary explanation may be the high water velocity in karst which allows the transport of large particles.

The PSDs are analyzed elsewhere (Atteia and Kozel 1997). The average particle number distributions are very similar at Bied and Noiraigue (Fig. 3). A difference appears only on volume distributions: (1) the 4–5 μ peak is higher for Noiraigue than for Bied, and (2) the Bied samples show greater particle numbers between 12 and 15 μ. The Noiraigue samples show larger variability in particle number than the Bied ones (Fig. 3). Briefly, at Noiraigue the maximum size reached by the particles increases with discharge values, whereas the total number of small particles is mainly determined by water pH. In the Bied brook samples, no relation between PSD and any of the measured parameters has been discovered.

Table 2
Some selected examples of colloidal particles observed in natural water samples

Location	Techniques	Number #/ml	Amount (mg/l)	Size observed (μ)	Peaks (μ)	Reference
Rainwater Darmstadt (D)	Quartz Balance PCS		0.006	0.08		Malyschew and others 1994
Ocean Northern Pacific	Filt, PC, epifluorescence			0.38–1		Koike and others 1990
Ocean Gulf of Mexico	Filt, TEM			0.02–8		Harris 1977
Ocean, Biscaye Bay	PC	10^4–10^6		0.5–150		Jantschik and others 1992
Lake Bret (CH)	Filt, TEM, PC			0.04–0.4	0.1	Buffle and others 1989
Chuckawa Creek (USA)	Sieving, PCS		50	0.3–1.3	0.2	Gallegos and Menzel 1987
River Mississipi (USA)			350		0.3	Rees and Ranville 1988
River Yarra (Aust.)	Sed-FFF		1–10	0.1–0.5	0.22	Beckett and others 1988
River Rhine (CH)	Cent, Filt, TEM, PCS			0.004–4	0.015 0.4 2 5	Newman and others 1994; Perret and others 1994
Noiraigue Spring (CH)	PC, Filt, Centr.	10^7	0.5–10	0.5–60	0.8–1 4–5	This study
Groundwater	LS, PCS		1–30		0.1 0.6–0.8	Gschwend and others 1990
Groundwater Gorleben (D)	LIPAS	5×10^{10}			0.005–0.01	Dearlove and others 1991
Groundwater Grimsel (CH)	Filt, Grav, SEM, TEM, PC	6×10^6–4×10^7	0.1	0.04–1		Degueldre and others 1989
Groundwater Markham Clinton (UK)	Filt, SEM, LIPAS			0.04–1		Longworth and others 1990

Filt filtration; *Cent* centrifugation; *Grav* gravimetry; *SEM* scanning electron microscope; *TEM* transmission electron microscope; *PC* particle counting; *PCS* photon correlation spectroscopy; *TOC* total organic carbon; *FFF* field flow fractionation; *LIPAS* laser induced photoacoustic spectroscopy

Particle mineralogy

Mineralogy of rocks and suspended matter

The mineralogy of *rocks* and of *Quaternary deposits* representative for the studied region is given in Table 3. In the Mesozoic rocks, the most abundant minerals are calcite, quartz, and dolomite, which can occur in ankeritic form; secondary minerals are feldspars, mica, smectite, and kaolinite. In the Quaternary deposits, the marly layers are mainly composed of calcite, phyllosilicates, and quartz; feldspars are secondary minerals. Dolomite has been recognized in one sample. Samples taken near the molasse outcrop contain larger grains and have higher quartz and feldspar contents. Phyllosilicates present consist mainly of mica, kaolinite, and chlorite with possible additions of smectite and illite-smectite mixed-layer clays (I/S). Boreholes near the molasse outcrops show significant amounts of serpentine and paragonite (Fig. 4).

The *suspensions* from Bied Brook and Noiraigue River contained mainly quartz, calcite and dolomite grain minerals; and mica, chlorite, and kaolinite phyllosilicates (Table 4). Due to preferential orientation during the recovery process, the diffraction patterns are not quantitative and therefore intensities should theoretically be compared for the same mineral only. However, assuming a similar deposition for all phyllosilicates, estimates of per-

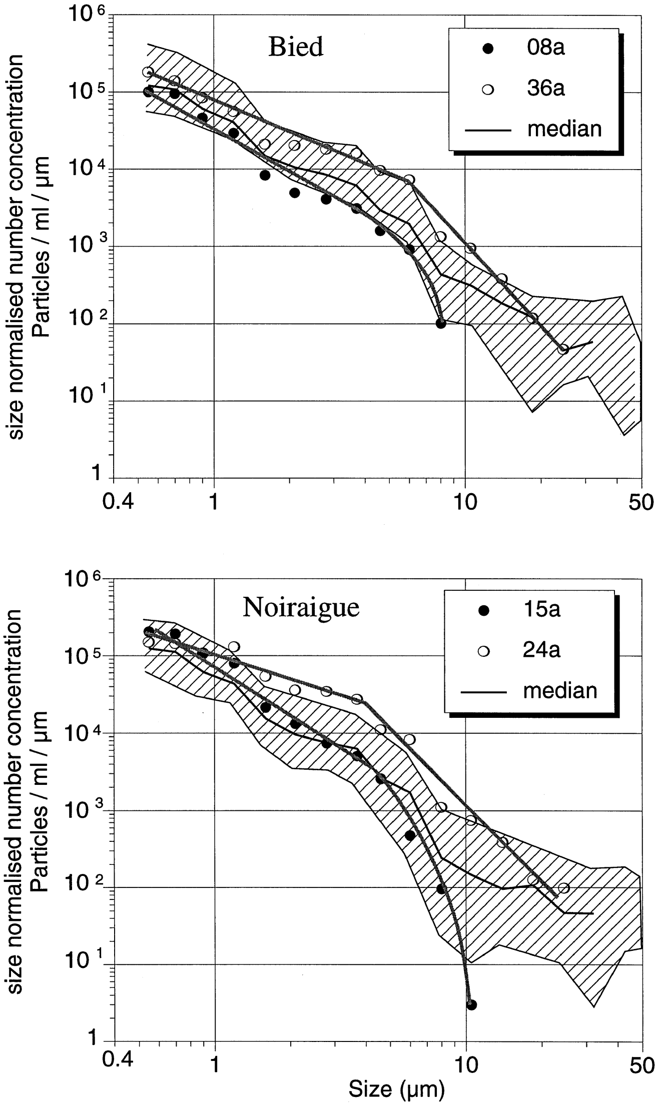

Fig. 3
Typical PSD curves for Noiraigue Spring and Bied Brook (08a, 36a are sampling event numbers), including fitted Pareto or exponential laws (—), and median (–) plus 10 and 90% percentiles (▨) of the number values for 50 (Noiraigue) and 35 (Bied) samples

centage of each type to the sum of phyllosilicate can be obtained. Both locations show similar composition of the phyllosilicate fraction, i.e., ca. 50% mica, ca. 25% kaolinite, and ca. 25% chlorite.

The Bied Brook and Noiraigue Spring suspensions include phyllosilicates also existing in the Quaternary deposits. However smectite and mixed-layer clays, well represented in the deposits (Table 3), are absent from the sampled waters. Ratios of grain minerals to quartz have been used to compare different sites (Fig. 4). Suspensions are enriched in feldspars relative to deposits. This might be due to a comparison between deposited, and thus oriented, particles (suspensions) and randomly oriented powders (deposits). Bied Brook contains a higher percentage of feldspars than Noiraigue Spring, no artifact affects these results. On the contrary, the content of calcite relative to quartz is higher in the Noiraigue waters than in the Bied ones.

Mineralogy and size

The diffraction patterns of one typical sample fractionated by sedimentation and ultracentrifugation are shown on Fig. 5. The sedimented fraction contains only quartz, calcite, dolomite and sometimes feldspars. Ultracentrifuged fractions show phyllosilicates, quartz, dolomite in some samples, and scarce and small peaks of feldspars. Quartz peaks decrease with decreasing particle size, and dolomite peaks almost disappear down to ca. 0.65 μ. Mica, kaolinite, and chlorite are not significantly differentiated by size, as opposed to mixed-layer clays and talc which appear only at small particle size. Mixed-layer clays were detected only after fractionation: they might have been hidden by larger particles on raw samples.

Chemical characteristics of individual particles

Approximately 1000 particles deposited on 2 and 0.8 μ Nuclepore filters for four sampling events at Bied and Noiraigue were analyzsed by SEM-EDS. Although large particles collected on the 2 μ filters induce higher EDS peak intensities, no significant difference was found between the composition of particles deposited on the two filter sizes. Table 5 shows that the main difference between Bied and Noiraigue are the Fe intensities being more than two times higher in the Bied Brook.

A principal component analysis on the EDS peak intensities did not allow a distinction of groups of particles. A differentiation could however be obtained using only Al and Si intensities. Numerous points show an Al/Si ratio similar to the one typical of clays (ca. 2). We therefore differentiated four groups of particles existing in Bied Brook:

1. "Clays" which have $Si/Al \approx 2$ and $Si + Al > 50\%$ of the total intensities.
2. "Quartz" with $Si > 50\%$ of the total intensities.
3. "Fe-Ca particles" with $Fe + Ca > 50\%$ of the total intensities.
4. "Mixed" particles which do not match any of the previous categories.

At the Bied, Fig. 6a shows that this differentiation is evident on the Al/Si graph. However if these groups are plotted on a Ca–Fe graph (Fig. 6b) they cannot be differentiated because they have the same range of Ca and Fe intensities and the same Ca/Fe ratio. Besides, a clear Ca/Fe correlation appears (except for sampling event 49) independently of the particle types when particles are dif-

Table 3
Composition of Mesozoic to Quaternary deposits in the folded Jura

Layers		Mica	Smectites	Mixed-layer clays	Kaolinite	Chlorite	Ankerite	Dolomite	Goethite	Pyrite	K-Feldspar	Plagioclase
Quaternary Marls		+++	–	+++	+++	+	+	+	–	–	++	++
Tills		++++	++	–	–	++++	++	+	–	–	++	++++
Molasse	III	++++	+++	+	–	++++	+	++	–	–	++	++++
Siderolithic		+	+	–	++++	–	–	–	+++	–	–	–
Pierre-jaune-Urgonian		+++	++	++	++	+	++	–	++	–	++	–
Marnes Bleues	Cretaceous	++	++++	+	+	+	+++	–	–	+++	+	–
Calcaire roux		++	++	++	++	+	+	–	++++	–	+	–
Marbre Bâtard		+++	+++	++	+++	+	–	–	+	–	++	–
Purbeckian		+++	+	+++	–	+	+	++	+	+	+	–
Portlandian		+++	++	+	++	–	+	++++	–	–	+	–
Sequanian-Kommeridgian	Jurassic	++++	++	++	+++	+	+	+++	+	–	+	–
Oxfordian-Argovian		++++	++	++	++++	++	++	–	–	++	++	–
Dogger		+++	+	++	+++	++	++	+	+++	+	+++	+
Lias		++++	–	++	+++	+	+	–	–	+++	–	–

++++ very abundant; +++ adunbant; ++ common; + rare; – absent

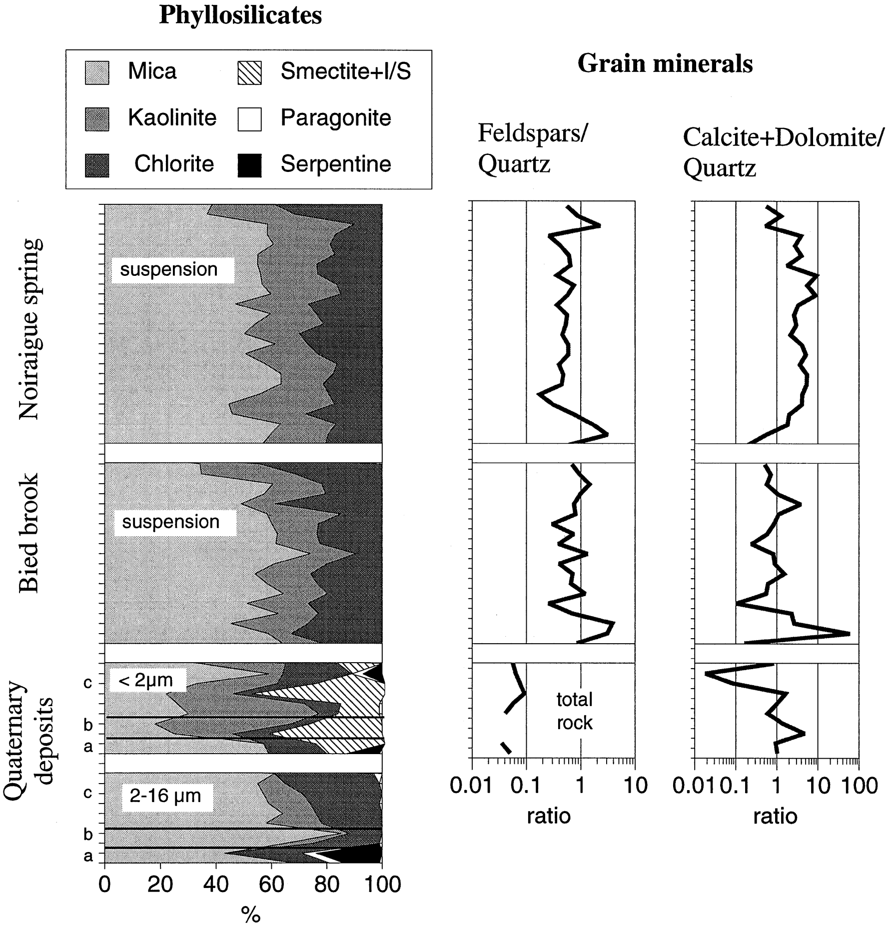

Fig. 4
Mineralogy of boreholes drilled in surface sediments (a: molasse, b: tills, c: marls) and suspensions from Bied Brook and Noiraigue Spring (horizontal ticks show different sampling events). Phyllosilicates are presented in proportions of total phyllosilicate and grain mineral as a ratio to quartz in CPS (counts/s)

Table 4
Average mineralogy of the suspensions, in counts/s (cps) and percentage of total phase

Site	Mica	Kaolinite	Chlorite	Quartz	K-Felspar	Plagioclase	Calcite	Dolomite
Bied	112.5	35.3	47.5	86.8	30.5	31.6	120.2	16.6
Noiraigue	118.0	48.3	45.8	108.8	32.0	35.5	181.3	183.3

	in % of phyllosilicates			in % of grain minerals				
Site	Mica	Kaolinite	Chlorite	Quartz	K-Feldspar	Plagioclase	Calcite	Dolomite
Bied	58	18	24	30	11	11	42	6
Noiraigue	56	23	22	20	6	7	34	34

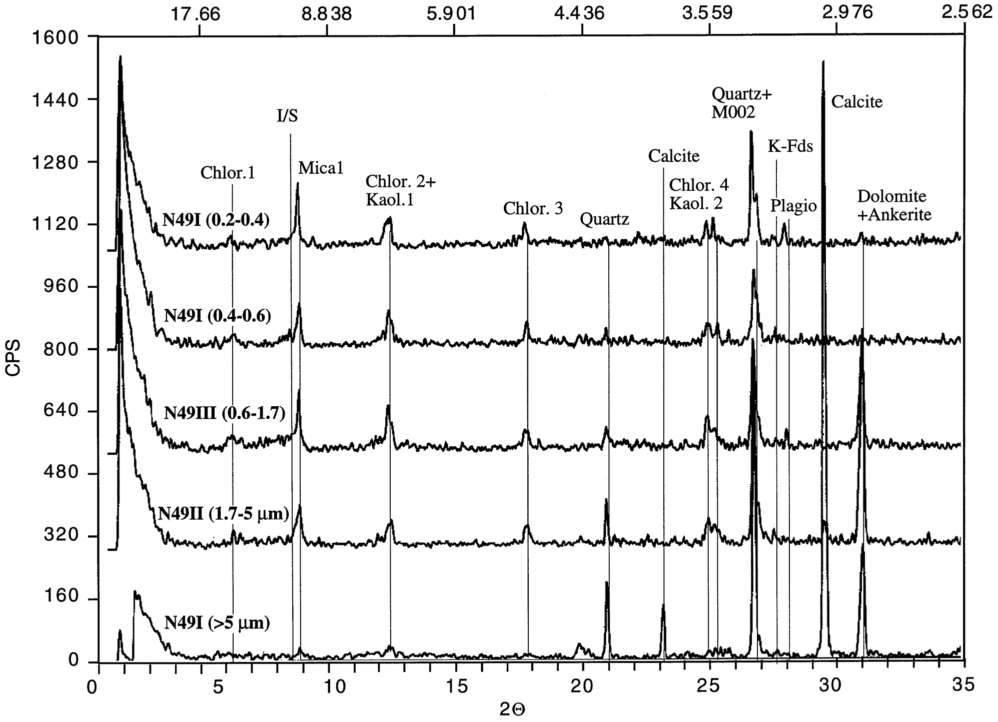

Fig. 5
Mineralogy of different fractions obtained by ultracentrifugation of one sample from Noiraigue Spring (CPS)

Table 5
Average intensities measured by SEM-EDS on collected particles (differentiated by pore size of the filters used)

	Fe	Al	Si	P	Ca	Mn
Bied						
2 μ	4.27	4.10	15.0	0.67	5.15	0.06
0.8 μ	1.69	1.48	4.42	0.45	2.68	0.003
Noiraigue						
2 μ	0.80	2.96	9.94	0.31	3.91	0.016
0.8 μ	0.95	2.59	8.83	0.17	2.11	0

ferentiated according to campaign number (Fig. 7). The Ca/Fe ratio varies greatly between the sampled events; however no relation between this ratio and the measured parameters has been found. At Noiraigue the particles present very similar Si/Al ratios but very small amounts of Fe. No correlation between Fe and Ca content has been found, even when discriminating between sampling events.

Bacterial populations

The statistics of the number of bacteria counted for 20 sampling events at each site are presented on Table 6. The total number of bacteria varies by 2 orders of magnitude whereas the total number of particles varies by only 1 order of magnitude. The ratio of the bacteria to total number of particles varies from 1% to 100% at the Bied with a median at 30%. At Noiraigue, the figures are completely different as the ratio varies from 0% to 22% with a median at 2%. However these ratios must be considered as overestimates because the smallest observed bacteria had a size close to 0.2 μ and the particles counted with CIS Galai were larger than 0.5 μ.

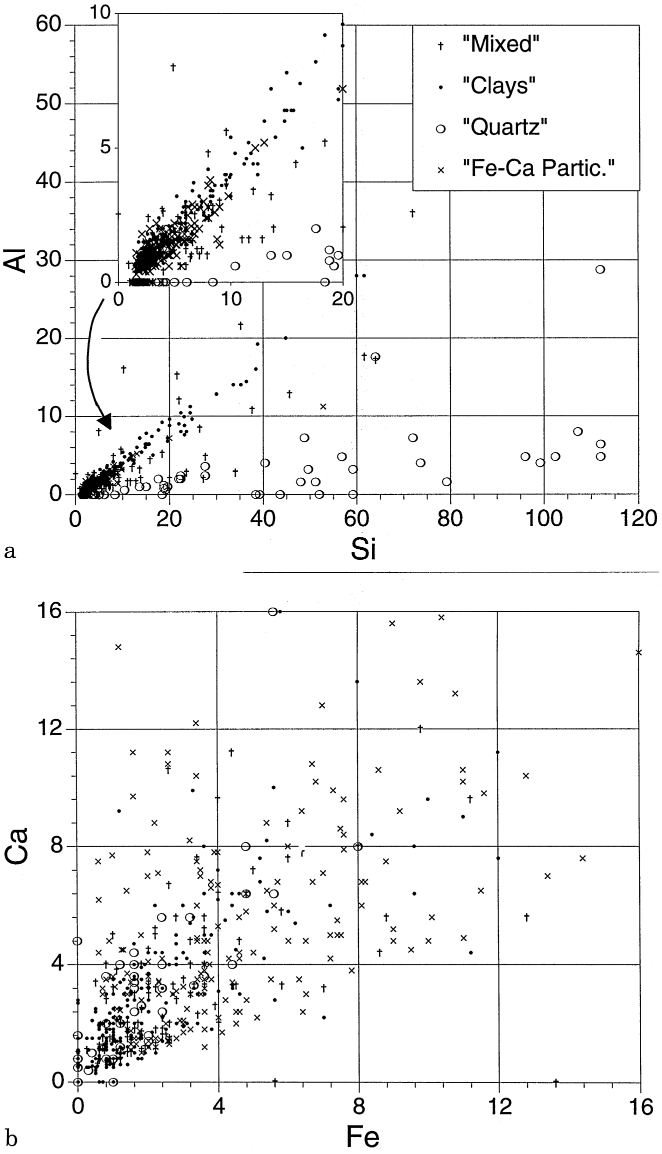

Fig. 6a, b
SEM-EDS intensities (relative units) measured on particles from Bied Brook: **a** Si and Al, **b** Fe and Ca

Table 6
Comparison of number of bacteria and particles at Bied and Noiraigue (ratio is the number of bacteria divided by the number of particles)

	Bacteria [ml^{-1}]	Particles [ml^{-1}]	Ratio (%)
Bied			
Min	2.22×10^3	1.30×10^5	1
Median	1.81×10^5	3.62×10^5	31
Max	5.18×10^5	1.35×10^6	100
Noiraigue			
Min	1.56×10^3	1.77×10^5	0
Median	2.70×10^4	3.81×10^5	2
Max	1.01×10^5	2.40×10^6	22

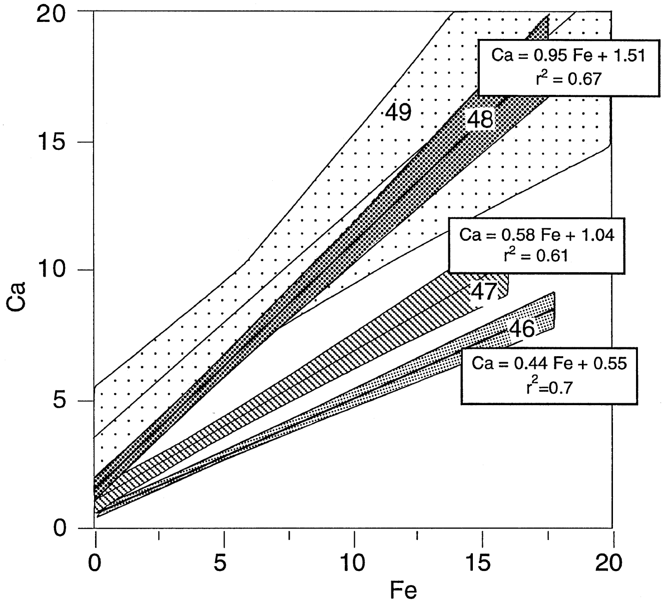

Fig. 7
Correlations between Fe and Ca at Bied Brook for different sampling events; shaded area represents 95% confidence limits

One major cause of the variability in number is the effect of temperature, as seen in Fig. 8. This effect can easily be understood due to the general influence of temperature on the development of living organisms. It seems to be the only measured environmental factor playing a significant role on the number of bacteria, while the total number of particles is not influenced by this parameter. Approximately 10% of all bacteria were identified by cultivation methods. Within this 10% the large majority consist of heterotrophic bacteria while a negligible proportion corresponds to *enterococci.*

Discussion

Size

The particle size distributions were shown to depend on the evolution of discharge at Noiraigue (Atteia and Kozel 1997). The observed evolution of PSD could be explained in a first attempt by the 'washing' of the aquifer during the first discharge rise. This washing can result from the turbulent flow occurring at high discharge in the karstic conduits which resuspends the previously deposited particles. This could also explain the quick decrease in particulate matter found after the highest flow. The number of small particles (<5 μ) does not depend on discharge but on pH. These types of particles therefore present a real colloidal behavior as they can be transported over long distances whatever the hydrological conditions are. This size, attributed to colloids, is surprisingly large but can be explained by the high water velocities encountered in the karst conducts.

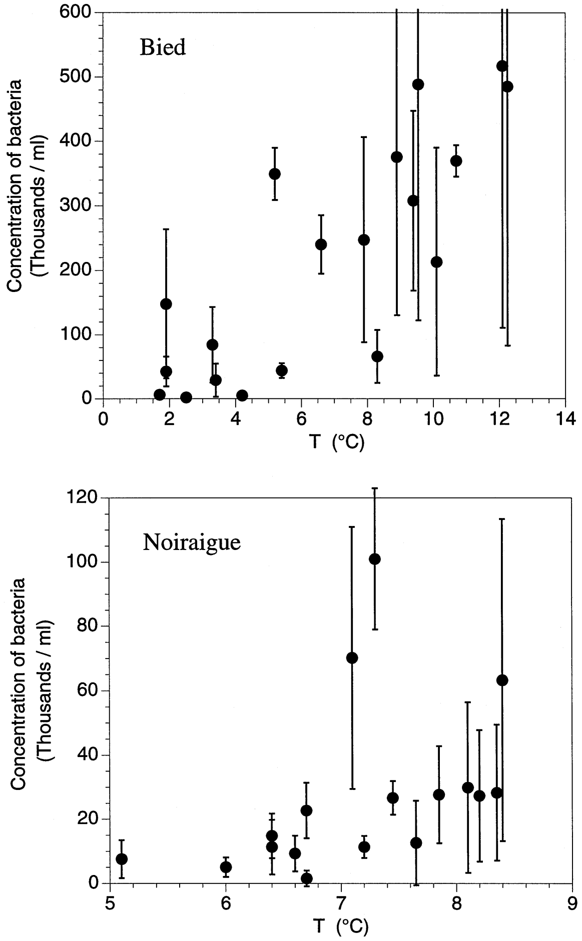

Fig. 8
Evolution of the number of bacteria in relation with water temperature at Noiraigue Spring and Bied Brook

Estimation of fluxes of suspended matter

The estimation of the suspended matter fluxes must take into account the role of discharge. It has been possible to fit the total volume of particulate material measured with Galai CIS by an exponential law of discharge in the following form:

$$V = a \times \exp\left(\frac{Q}{b}\right) \qquad (1)$$

with $a=0.5$ and $b=4$, V in ml of particles/l of water, Q in m^3/s. Therefore the yearly flux of suspended matter can be estimated by the formula:

$$\Phi = \sum_i a \times \exp\left(\frac{Q_i}{b}\right) \times \rho \times 86400 \qquad (2)$$

with Φ in kg/year and ρ in kg/l (taken equal to 2). Q_i is the value of the discharge obtained on the cumulative frequency curve of the spring discharge, for the frequency i. The factor 86400 is applied to transform discharge per second to discharge per day. In our aquifer the estimate of Φ is ca. 7.5×10^4 kg/year.

Table 7
Percentage of Fe in the different fractions to the total Fe, Bied Brook

%	<0.05 μ	0.05–0.2	0.2–0.8	0.8–2	>2 μ
Min	24.1	2.1	0	0	6.9
Median	46.6	12.5	4.5	3.4	34.3
Max	66.7	37.5	11.7	14.2	44.7

The soluble salt concentrations are much less dependent on discharge at a daily scale and rough estimates can be obtained by multiplying the average mineralization (200 mg/l) by the yearly water flux (8×10^7 m^3), which leads to a value of 1.6×10^7 kg/year. Therefore volume or mass of transported matter in particulate form represents only 0.5% of the soluble salts. There is a large difference between Al being almost entirely in particulate form and Ca or Mg which are mainly soluble. Between these two extremes the element presenting the largest variation in size is Fe. As can be seen on Table 7, Fe is present in all the analyzed fractions. The fraction <50 nm, which includes dissolved Fe too, contains the highest proportion of Fe. However the 50 nm-0.2 μ and >2 μ also include large, but highly variable, proportions of Fe. These proportions do not show a simple dependency on any measured factor (T, pH electric conductivity).

Types of particles transported through the Noiraigue basin

Minerals transported by water (Table 4) include quartz and phyllosilicates, as suggested above, and also some feldspars, calcite, dolomite, and ankerite. In Bied Brook waters, the existence of these minerals can be explained by a similar composition of the marly layers covering the valley (Fig. 4). Amounts of kaolinite, quartz, calcite, and dolomite significantly increase from Bied Brook to Noiraigue Spring (Table 4). Calcite and dolomite evidently originate from the thick karstified limestone layers; quartz is also found in limestones and marls. The ankerite form of dolomite occurs mainly in Cretaceous marly deposits. The siderolithic material deposited in karst cavities (Adatte and others 1991) contains a high percentage of kaolinite and can thus explain the increase of this mineral content during its travel through karst. The absence of smectite from the suspensions is striking when related to the amounts in the Quaternary deposits. These clays may be present in the very small size fraction, as seen after fractionation; thus they may be (1) difficult to collect on the plates, and/or (2) easily altered when in contact with water.

Excluding one sample containing a very small amount of goethite, crystallized oxides were not found by XRD. However these oxides can easily occur in natural suspensions in poorly crystallized (ferrihydrite) or even amorphous forms. These forms are difficult to detect by XRD (Tipping and others 1981) due to a high background around the Kα radiation of Cu. In Bied Brook, the evident association of Ca and Fe in a ratio varying from 2/1 to 1/1 (EDS peak intensities, Fig. 6), often containing 5–15% P (Table 5), does not correspond to any XRD identified mineral. These oxides do not exist as individual particles because iron particles always contain more than 40% Si, Al, or Ca; they could occur as coatings on other particles. This phase can be amorphous Ca–Fe–P particles which are known to exist in lakes (Buffle and others 1989; Leppard and others 1989). Furthermore, the analysis of the SEM-EDS peak ratios showed the presence of Al and Si with a specific Al/Si ratio thus they are recognized as clays. These clays contained Ca and Fe in proportions similar to the oxyhydroxides cited above. These Ca–Fe coatings seem to be deposited on every particle independent of type. This coating could depend on the hydrodynamic and chemical conditions as the Ca/Fe ratio is similar for all particles of the same date, on the one hand, and varies from date to date (Fig. 7) on the other hand.

As suggested in the first section the presence of peats and marl layers easily explains the presence of Ca and Fe in waters. The water flows laterally through the peat and enters in contact with the marly layer adjacent to Bied Brook. These waters contain Fe, in its ferrous form, from the peats or organic soils, and Ca from the marl. Upon reaching the Bied, oxygenation would induce Fe precipitation. A coprecipitation of Ca, oversaturated in solutions, could occur simultaneously. The nucleus of these particles could consist of organic matter.

The amount of Fe particles drastically decreases from Bied to Noiraigue, which could be interpreted as a deposition process of the Ca–Fe particles within the aquifer. It must be noted that, as the pH is similar at Bied and Noiraigue, it is not a simple effect of calcite precipitation. The number of bacteria also decreases strongly from inlet to outflow. The biochemical processes taking place in the aquifer must have rapid kinetics since the water flows quickly.

Role of colloids as carriers

As described above, the particulate matter represents a small proportion of the whole of the dissolved elements, but can yet act as a carrier of contaminants. The particles smaller than 5 μ, which were defined as the colloidal phase, constitute about 10% of the total particle mass. In terms of surface area, assuming that colloids are spheres (if they have 1 μ radius, this leads to approx. 3 m^2/g), this proportion reaches 40%. But taking into account the clay nature of these colloids, with an actual specific surface area of 50–300 m^2/g, the proportion of available surface on these colloids reaches more than 95% of the total surface area of particle matter. As adsorption is highly related to specific surface, even small amounts of colloidal matter can play an important role in contaminant transport. Nevertheless this large carrier capacity should be studied in more details because the aggregation can reduce the actual area and the coatings can increase it.

Conclusion

This study of a karstic aquifer shows that colloid and particle distributions are entirely multidispersed and must be analyzed in terms of PSD. A combination of different methods was necessary to obtain reliable PSD and to analyze fractions. The study of the PSD, detailed elsewhere (Atteia and Kozel 1997), enabled us to distinguish particles and colloids. As the PSD curve parameters vary with hydrodynamic conditions, this approach should help in defining the water velocities in the aquifer.

The second major characteristic of the studied colloidal populations is their complex chemistry and mineralogy. We found almost all types of natural and intimately associated particles. This has a consequence on the choice of appropriate analytical methods: different methods must be applied to obtain an insight into the real organisation of the particles. Moreover, if the evolution of colloids is of interest, the processes of precipitation and aggregation of particles must be taken into account. This aspect requires advanced research to describe the surface area and charge of the colloids. Finally the capacity of contaminant transport by colloids is high, yet their heterogeneity limits realistic estimations of adsorption capacities.

The study of this karstic aquifer shows that Fe and bacteria present the most specific behavior. Iron is typically in the colloidal size range and is related to the evolution of Ca and organic matter. A clear evolution of Fe particles has been evidenced within the aquifer whereas pH only shows minor changes. Further aspects of Fe chemistry within this karstic aquifer are currently being studied by means of electron microscopy and particle synthesis. This should allow the determination of the chemistry conditions prevailing on the colloid formation at the Bied Brook limits and on the colloid maturation in the aquifer. In order to understand the evolution of bacteria in a karstic aquifer the spatial distribution of bacterial populations is being studied in another aquifer which is accessible.

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