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Formation of a distinct class of Fe–Ca(–C_{org})-rich particles in a complex peat–karst system

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Abstract

The Vallée-des-Ponts in the Jura mountains (Switzerland) is characterized by a complex hydraulic system, where drained peat bogs are crossed by a river (Bied Brook) which sinks into a karstic aquifer prior to rise as a spring (Noiraigue spring). A general chemical and microscopic survey of the particulate species throughout the basin highlighted the complex behaviour of iron-rich entities. Bulk chemical analyses show that iron is present as its dissolved and humics-complexed ferrous form in the anoxic–acidic peat porewater; the oxidation step of ferrous iron mainly occurs into drains, where conditions change from anoxic–acidic to oxic-neutral. Analytical electron microscopic characterization of individual colloids in the different compartments of this complex system suggests that iron is slowly oxidized to an abundant particulate form while it is transported by drains to the river, but these particles are then mostly retained when travelling through the karst. Small organic-rich granules, present as dominant species in the peat porewater, could act as condensation nuclei for the formation of iron-rich globular colloids containing significant amounts of calcium. These distinct particles have sizes in the 100–500 nm range and eventually contain a carbon-enriched core surrounded by a Fe–Ca-enriched outer layer. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Iron particles; Humics-iron interactions; Colloid formation; Peat; Karst; Analytical electron microscopy

1. Introduction

Mineral colloids and particles have been identified for decades as scavengers of toxic substances in aquatic systems (Buffle, 1988; Buffle and Leppard, 1995; Filella and Buffle, 1993; Honeyman and Santschi, 1992; McCarthy and Zachara, 1989; Salomons and Förstner, 1984; Stumm, 1993; Tessier, 1992). Amongst these, iron particles (hydroxides, oxyhydroxides and/or oxides) play a dominant role. Nevertheless, little is known on their formation pathways and intimate physico-chemical characteristics, especially in subsurface waters.

Most past studies have relied on data obtained from bulk chemical analysis of natural water samples which were fractionated by simple-to-complex filtration and/or centrifugation schemes (Buffle et al., 1989; Degueldre et al., 1989; Perret et al., 1994a; Salbu et al., 1985). However, in order to extract targeted information at the level of the individual

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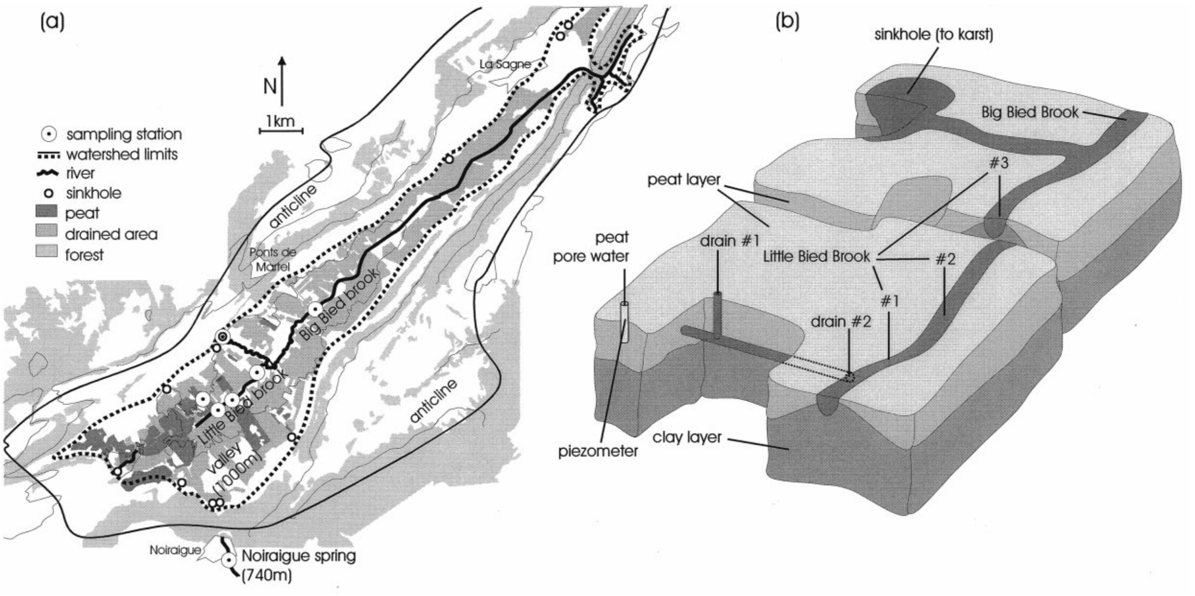


Fig. 1. (a) Site description of the watershed of the Vallée-des-Ponts (Neuchâtel, Switzerland) and location of the sampling stations. (b) Schematic of the sampling points between the peat bog, the Little Bied Brook and the sinkhole to the karstic aquifer.

particles (e.g. associations between elements within a single colloid or aggregate), bulk chemical analyses must be supplemented by analytical electron microscopic techniques (AEM; Leppard, 1992a,b; Nomizu et al., 1987; Perret et al., 1994b; Couture et al., 1996). AEM can not only produce illustrative information on the morphology and size of particles, but also specific and quantitative information on their intimate composition.

The combination of chemical and microscopic analyses is thus an alternate route which is capable of solving the complex problem of formation and transformation of authigenic particulate material in heterogeneous aquatic systems. Under these circumstances, the behaviour and fate of colloids and particles as pollutant carriers may be better understood in relation to their size, morphology, composition, associations and reactivity.

In this study, we adopted an interdisciplinary sampling and analytical strategy to characterize the particulate species in the different compartments of the complex watershed (peat, drains, river, karst spring) of the Vallée-des-Ponts (Switzerland). We show that analytical electron microscopy evidences the genesis of an abundant class of Fe–Ca(–C_{org})-rich colloids, which are then scavenged during their transport into karst. The formation pathway of these colloids from peat to river is strongly supported by bulk chemical analyses.

2. Site description

The site and associated sampling points are depicted in Fig. 1. The Vallée-des-Ponts (Neuchâtel, Switzerland) is a flat triangular valley formed during the folding of the Jura mountains in the late Miocene (Atteia et al., 1998a). The valley (altitude: 1000 m; ca. 23 km^2) is characterized by a thick (ca. 300 m) layer of molasse overlying carbonates and covered by peats, agricultural soils and alluvial fans; it is surrounded by anticlines (altitude: 1000–1350 m; ca. 68 km^2) mainly composed of limestones and marls. The anticlines-valley interface presents tectonic faults in direct contact with the karstic aquifer.

The central valley is densely networked by agricultural drains which drive meteoric waters to the Bied Brook. This river gathers waters from the drained peats and organic-rich soils, and flows through the valley into a main sinkhole entering the faults; after a ca. 4 km travel distance (50–150 m/h) through the karst, the water rises as a spring (Noiraigue; altitude: 740 m). The Noiraigue spring thus collects waters from the anticlines (ca. 2/3 of the total surface of the watershed) and from the valley (ca. 1/3).

3. Sampling and analytical strategy

In order to comprehend the behaviour of particulate material in the basin, waters from peat pore waters, drains, the Bied Brook, the main sinkhole, and the Noiraigue spring were sampled and size fractionated between 1995 and 1998 at monthly intervals and analysed by a combination of bulk chemical methods and analytical electron microscopic techniques. Fig. 2 summarises the multi-method approach which was adopted for the study.

Chemicals used throughout were of the *pro analysis* grade (Merck, Fluka). Dilutions, standards and blanks were prepared with ultrapure, ultrafiltered, UV-irradiated water (Elgastat UHQ-II).

A piezometer was inserted in the peat to sample pore waters. Drain waters were collected from a manhole and at the outlet of the drain. Samples from the Little Bied Brook were collected within a few meters after the outlet of the drain, 2 km downwards, and at the main sinkhole (see Fig. 1b). Waters from the Noiraigue spring were sampled 200 m away from the outlet of the karst. Raw waters were fractionated for bulk chemical analyses by filtration on 0.2 μm membranes (Nuclepore polycarbonate). Samples were acidified in the field (HCl 2% or HNO_3 2%) except those for microscopy, which were processed within 4–8 h after sampling.

3.1. Bulk chemical analyses

Dissolved oxygen (Orbisphere 27141), pH (Metrohm 704) and conductivity (WTW LF323) were measured in situ at the sampling stations.

Cations were determined by Inductively Coupled Plasma-Atomic Emission Spectroscopy (ICP-AES; Perkin–Elmer Plasma 1000; $\lambda_{Al} = 328.068$ nm, $\lambda_{Ba} = 455.403$ nm, $\lambda_{Ca} = 393.366$ nm, $\lambda_{Fe} = 259.940$ nm, $\lambda_K = 769.896$ nm, $\lambda_{Mg} = 279.553$ nm, $\lambda_{Mn} = 257.610$ nm, $\lambda_{Na} = 588.995$ nm, $\lambda_{Si} = 251.611$ nm, $\lambda_{Sr} = 407.771$ nm). Prior to analyses, raw water

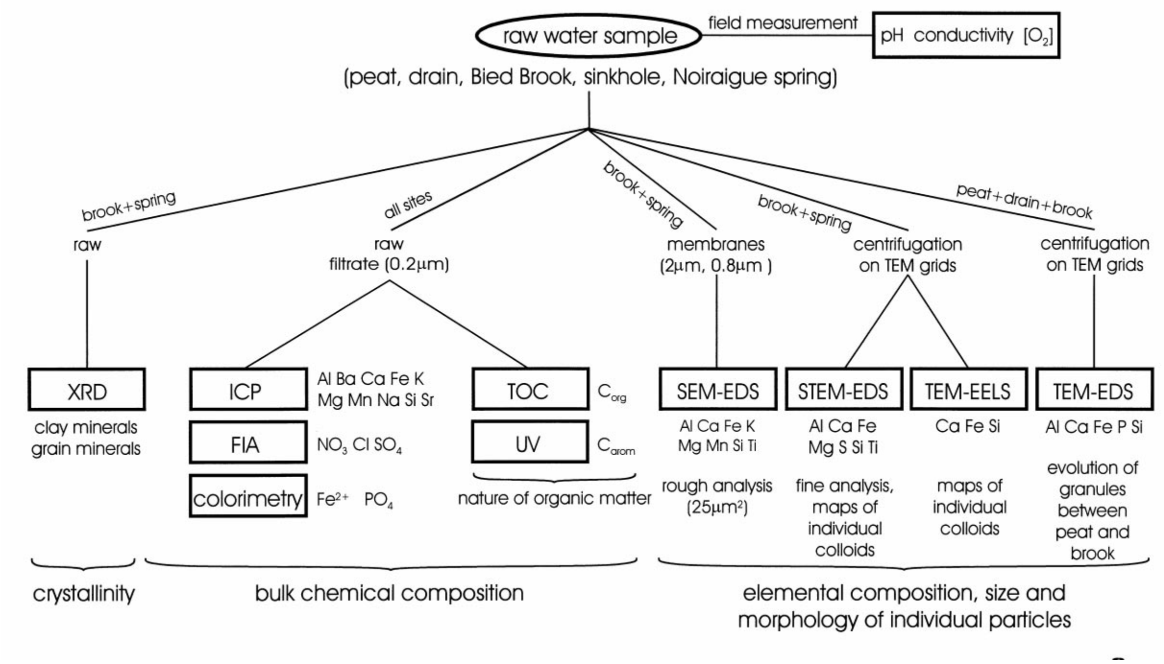


Fig. 2. Multimethodological approach adopted for the characterization of particulate material in the samples.

samples were mineralized in 12.5% HCl:12.5% HNO_3 for 24 h at 100°C; these samples were then diluted to obtain 5% HNO_3:5% HCl solutions.

Anions (NO_3^-, Cl^-, SO_4^{2-}) were measured on raw water samples by Flow Injection Analysis (FIA; Tecator Fiastar 5010). In addition, colorimetry (Hach DR2000) was used in the field and in the laboratory to analyse Fe^{2+} after the reaction with ferrozine (Stookey, 1970; $\lambda_{abs} = 562$ nm), and PO_4^{3-} after reaction with molybdate (APHA, 1976; $\lambda_{abs} = 890$ nm).

Total Organic Carbon (TOC) of filtered samples was measured by high-temperature combustion on a Shimadzu TOC 5000 after acidification by 25% H_3PO_4. Total Inorganic Carbon (TIC; ΣCO_2) was measured on the same device by analysing the CO_2 which evolved by the addition of 25% H_3PO_4 to the samples.

The nature of minerals present in raw water suspensions was obtained by X-Ray Diffractometry (XRD; Scintag 2000) after deposition of the >1 μm fraction by centrifugation (1800 g, 20 min), resuspension of the collected material by sonication, and sedimentation onto XRD glass plates (Kübler, 1987).

3.2. Analytical electron microscopy

For Scanning Electron Microscopy (SEM), particles were collected by cascade filtration onto membranes of decreasing porosity (Nuclepore polycarbonate; 2, 0.8 μm). After recovery, these membranes were glued with a conductive carbon paint (SPI Supplies) onto SEM stubs and coated with a carbon film.

Specimens for Transmission Electron Microscopy (TEM) were prepared by direct ultracentrifugation onto TEM grids (SPI Supplies; Cu 200 mesh, collodion-coated, carbon-covered) to retain the native aspect of colloids (Perret et al., 1991; Mavrocordatos and Perret, 1995; Lienemann et al., 1998).

Analytical Electron Microscopy (AEM), either in Scanning (SEM), Transmission (TEM) or Scanning-Transmission (STEM) mode, allowed to gain accurate morphological and compositional information at the *per particle* level, but at different degrees of refinement in spatial and spectral resolution.

For a preliminary survey of particles from the Bied Brook and the Noiraigue spring recovered onto filtration membranes, ca. 1000 randomly selected particles or groups of particles with sizes >0.8 μm were analysed with a SEM (Jeol JSM-35, W source, 20 keV) equipped with an Energy Dispersive Spectrometer (EDS; Link). The analysed area (25 μm^2) and the operating conditions were kept constant for all measurements to allow for a semi-quantitative comparison of EDS spectra and an estimate of the particle concentration in the specimens. The K_α emissions lines of Al (1.49 keV), Ca (3.69 keV), Fe (6.39 keV), K (3.31 keV), Mg (1.25 keV), Mn (5.89 keV), P (2.01 keV), Si (1.74 keV) and Ti (4.51 keV) were considered as relevant when their signal-to-noise ratio (SNR) was higher than 2.

Colloids recovered onto TEM grids were analysed by STEM–EDS, both in imaging and spectrum modes. EDS spectra of ca. 600 randomly selected colloids (Bied Brook, Noiraigue spring) with sizes >50 nm were recorded with a STEM (Jeol TEMSCAN 1200 Ex II, LaB_6 source, 80 keV) equipped with an EDS detector (Princeton Gamma Tech). The analysed area was adapted to the size of the colloids in order to extract their elemental composition with accuracy. Element maps of Ca, Fe and Si in these specimens were obtained with another STEM (Jeol 2010F, field emission gun, 200 keV) equipped with an EDS detector (Link). Colloids collected from the peat, the drains and the Bied Brook were also analysed with a TEM (Philips CM12, W source, 80 keV) equipped with an EDS detector (EDAX).

For some specimens of the Bied Brook, preliminary investigations were undertaken on the analysis of individual colloids by Electron Energy Loss Spectrometry (EELS). This technique is highly complementary to EDS for the determination of the elemental composition of aquatic particles (Perret et al., 1995; Lienemann et al., 1997; Mavrocordatos and Perret, 1998), especially for identifying light elements with a high spatial resolution (Egerton, 1986). EELS spectra of colloids collected in the Bied Brook were recorded in imaging mode with an Energy Filtered TEM (EF-TEM; Zeiss CEM-902, W source, 80 keV, magnification: 30 000 ×, objective aperture: 90 μm, spectrometer entrance aperture: 100 μm, collection angle β: 2.5 mrad, acceptance angle α: 5 mrad) equipped with a Castaing–Henry filter. α was determined using the diffraction rings of a thin NiO film (Egerton and Cheng, 1994). Spectra were recorded with a photomultiplier, and filtered images (spectrometer entrance aperture removed) were recorded with a TV camera (Dage SIT 66) fitted under the energy filter.

4. Results and discussion

4.1. Characteristics of particles from the inlet to the outlet of the karstic aquifer

For most analysed species and physico-chemical parameters, temporal trends were observed in relation to discharge conditions; these variations are discussed in details in Atteia et al. (1998b) and are out of the scope of the present paper. For this reason, only the time-averaged concentrations of solutes in waters sampled upstream (Bied Brook) and downstream (Noiraigue spring) of the aquifer have been summarized in Table 1.

Waters from the Bied Brook represent only one third of the hydraulic recharge reaching the Noiraigue spring; thus, it is rather difficult to compare directly the concentrations of species at the two sampling stations. However, these data reveal that the analysed species behave differently within the aquifer and that no general figure can be drawn to explain the variability in the observed [upstream]:[downstream] concentration ratios.

The highest variations from brook to spring are found for TOC, Fe and Mn, which are apparently strongly retained during their travel through the fractured aquifer, at least in the absence of very high flow events. These species, under their particulate form, are powerful pollutant scavengers (Buffle, 1988), and their retention within the karst might thus significantly influence the transport pathways of toxic substances. Measurements of UV-absorption (285 nm) vs. TOC indicate that organic matter in the waters is aromatic-rich and has a dominant humics/fulvics character (Buffle, 1988; Zumstein, 1989), which is explained by the influence of the drained organic-rich soils in the valley.

To efficiently focus microscopic investigations of suspended material in the brook and in the spring, a set of randomly selected particles collected by cascade filtration during four sampling campaigns was analysed by SEM–EDS, irrespective of their size (ca. 0.5–10 μm) and morphology (i.e. the analysed area is maintained constant and large, 25 μm^2, for all measurements; individual particles and aggregates are thus analysed indistinctly).

Because of the analytical procedure chosen, no significant difference was found in the composition of particles collected on 2 and 0.8 μm membranes, although net EDS peak intensities were higher on

Table 1

Time-averaged raw concentrations of major solutes upstream and downstream of the karstic aquifer. Temporal variabilities observed over the 1995–1998 sampling period range between 10 and 50%. 'Balance' represents a significant (>30%) release (⇑) of species from, or retention (⇓) into the aquifer

Element	Bied brook	Noiraigue spring	Balance between inlet and outlet
Ca (mg/l)	79.4	84	⇒
Si	3.6	1.9	⇓
Mg	2.8	3.1	⇒
Na	1.4	1.8	⇒
K	1.4	1.3	⇒
Al	0.3	0.3	⇒
SO_4	8.8	5.4	⇓
Cl	3.6	3.7	⇒
NO_3	3.4	7.9	⇑
PO_4	120	90	⇓
TOC	20.2	4.7	⇓ ⇓
Fe (μg/l)	*741*	*114*	⇓ ⇓
Fe^{2+}	450	27	⇓ ⇓
Sr	218	416	⇑
Mn	88	7	⇓ ⇓
Ba	17	12	⇓

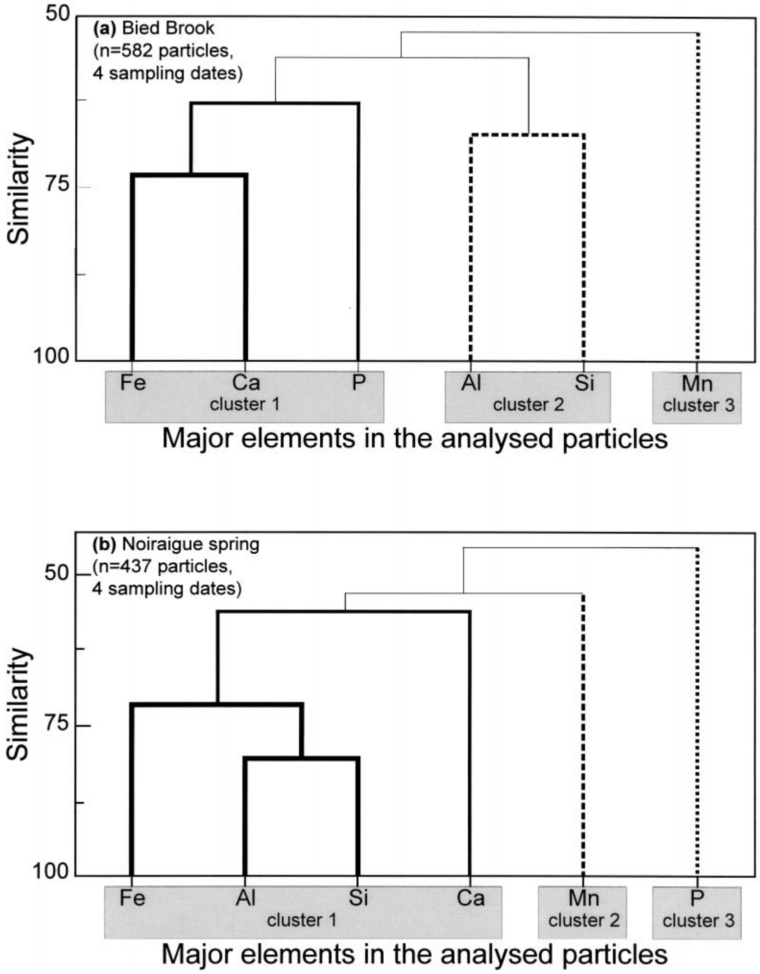


Fig. 3. Dendrograms resulting from the cluster analysis of the elements identified by SEM–EDS in particulate material (ca. 1000 measurements of randomly selected 25 μm^2 areas) collected onto filtration membranes (2, 0.8 μm) during four selected campaigns: (a) behavioural similarities in the Bied Brook (582 observations); (b) behavioural similarities in the Noiraigue spring (437 observations). The agglomerative clustering of variables Al, Si, Ca, Fe, P, Mn was performed by calculating the average linkage between clusters using a correlation distance method.

the former because of the larger sizes of the analysed entities. Qualitatively, the number concentration of particles is lower in the Noiraigue spring, the amount of poorly electron-dense organic material is smaller, and the relative ‘crystallinity’ of particles (geometrically shaped entities with diffraction patterns) is higher than in the Bied Brook.

A cluster analysis was performed on the whole set of SEM–EDS results. This hierarchical classification is aimed at highlighting the behavioural similarities between apparently heterogeneous variables. The resulting dendrograms obtained for the particles of the Bied Brook and the Noiraigue spring are depicted in Fig. 3 and allow us to determine which elements are most closely related to others.

In the Bied Brook (Fig. 3a), iron, calcium and eventually phosphorus belong to a class of elements (cluster 1) which is clearly distinct from the group of the clay-like elements (Al, Si; cluster 2). In the Noiraigue spring (Fig. 3b), by contrast, iron is primarily associated to the clay-like elements Al and Si, and to a lesser extent to

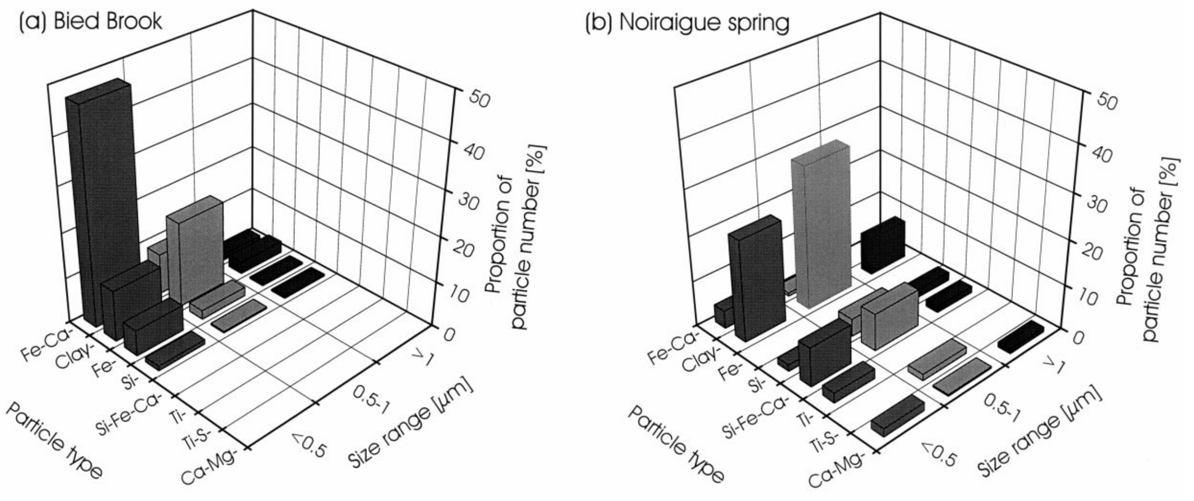


Fig. 4. Classification of individual particles (ca. 600 measurements for each site) in: (a) the Bied Brook; (b) the Noiraigue spring, identified by STEM–EDS. Particle types are given according to the most intense EDS peaks. The STEM–EDS probe size was fitted to the size of each individual particle.

Ca (cluster 1). Phosphorous exhibits a differentiated behaviour from the Bied to the Noiraigue, while manganese apparently exists as an individual entity at both sites.

An attempt to determine quantitatively the relationships between elements on the basis of the cluster analysis yielded element ratios with a weak statistical significance. This is explained by the analytical bias of the procedure chosen for SEM–EDS: most analysed areas (25 μm^2) contain more than one particle or aggregate, and generate results with an artificially high variability. This was however not the case for Al–Si-containing particles.

Particles containing Al and Si are the most abundant ones at both sampling stations (90% of particles in the Bied, $Al_{EDS} : Si_{EDS} = 0.35 \pm 0.17$; 85% of particles in the Noiraigue, $Al_{EDS} : Si_{EDS} = 0.39 \pm 0.37$). The Al vs. Si relationship is similar for the four sampling campaigns at both sites.

Clay-like particles ($Al_{EDS} + Si_{EDS} > 50\%$ of the recorded EDS peak intensities, and $0.4 < Al_{EDS}:Si_{EDS} < 0.6$) represent 20% (Bied Brook) to 30% (Noiraigue spring) of the particles containing Al and Si. This suggests that clay-like particles are often associated to other elements or aggregated with other particle types, at least within the resolution limits of the SEM–EDS analyses. However, XRD measurements of suspended material (Atteia et al., 1998a) show similar proportions of the dominant phyllosilicates (ca. 57% mica, ca. 20% kaolinite, ca. 23% chlorite) in the Bied Brook and in the Noiraigue spring, indicating that clay-like particles are not strongly affected during their transport through the karst.

Fe–Ca-rich particles ($Fe_{EDS} + Ca_{EDS} > 50\%$ of the recorded EDS peak intensities) represent 35% of the total number of analysed particles in the Bied Brook. In the Noiraigue spring, they do not exceed 10% of the particle number, and are most often associated to Al–Si-rich particles. The much lower proportion of iron-rich particles in the Noiraigue spring compared to the Bied Brook confirms the bulk chemical analyses. It is thus postulated that Fe–Ca-rich entities identified in the Bied Brook may undergo scavenging and/or transformation within the karstic fractures.

SEM–EDS analyses also show that phosphorus is often associated to iron within particles of the Bied Brook. This association, which is confirmed by the cluster analysis, is however rarely observed in the Noiraigue spring. In fact, phosphates, mostly released into surface waters of the valley by agricultural activities, may preferentially react with iron-rich entities (He et al., 1996; Lienemann et al., 1999).

SEM–EDS analyses proved to be helpful for a fast statistical description of the major associations between elements, but, because of the chosen

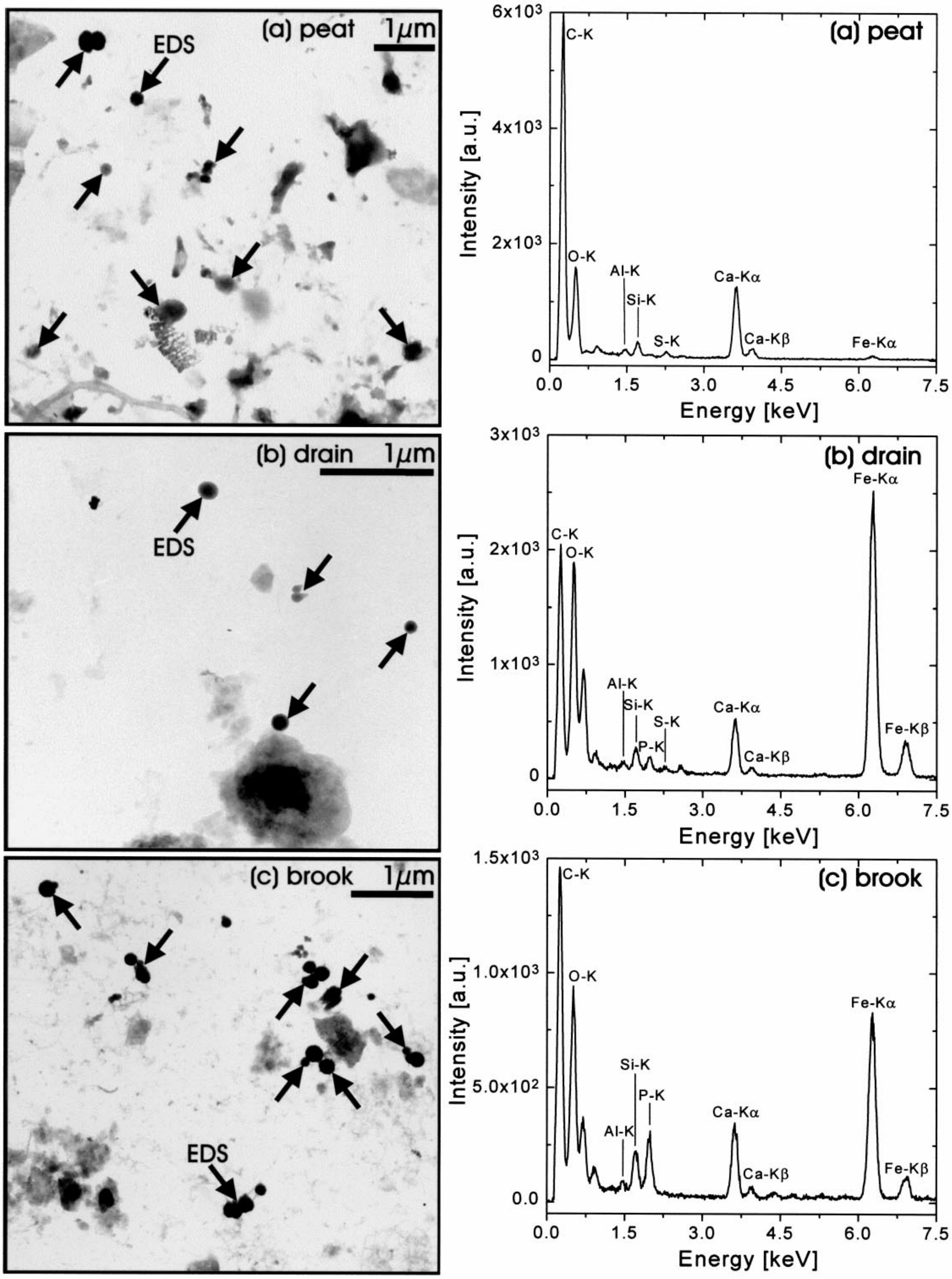


Fig. 5. TEM and EDS of typical globular entities collected in: (a) peat pore water; (b) drain water; and (c) water from the Bied Brook. EDS spectra correspond to the arrowed Fe–Ca(–C_{org})-rich colloids (drain, brook) and their precursors (peat).

approach (comparison of spectra recorded on 25 μm^2 areas), results were restricted to the analysis of large particles, aggregates, or groups of individual colloids.

For this reason, a more refined analysis was undertaken by STEM–EDS on ca. 600 randomly selected individual particles in the size range 0.05–2 μm. Particles were classified according to their size and composition (most important elements identified on EDS spectra). Results are presented in Fig. 4. It must be stated that organic-rich entities are highly abundant and easily identified under the TEM (ill-defined, poorly electron-dense); they were however not considered as a distinct class of particles during EDS analyses, because the carbon content of the supporting film of specimens (collodion + carbon) interferes on the C-K_α emission line of the natural organic entities.

The STEM–EDS approach does not allow comparison of results from particle to particle and from site to site, because, contrary to the SEM–EDS approach, the probe size is systematically fitted to the size of the particle of interest; this in turn enables an accurate determination of its composition, whatever particles are in its vicinity.

The size/composition classification of particles in the Bied Brook (Fig. 4a) shows that 47% of the total number of analysed particles are Fe–Ca-rich colloids in the <0.5 μm size range. Clay-like particles represent the second most important class of particles (11% in the <0.5 μm size range, respectively 19% in the 0.5–1 μm size range). Fe-rich and Si-rich particles (Fe_{EDS} respectively $Si_{EDS} > 50\%$ of the recorded EDS peak intensities) represent minor particle types in the Bied Brook.

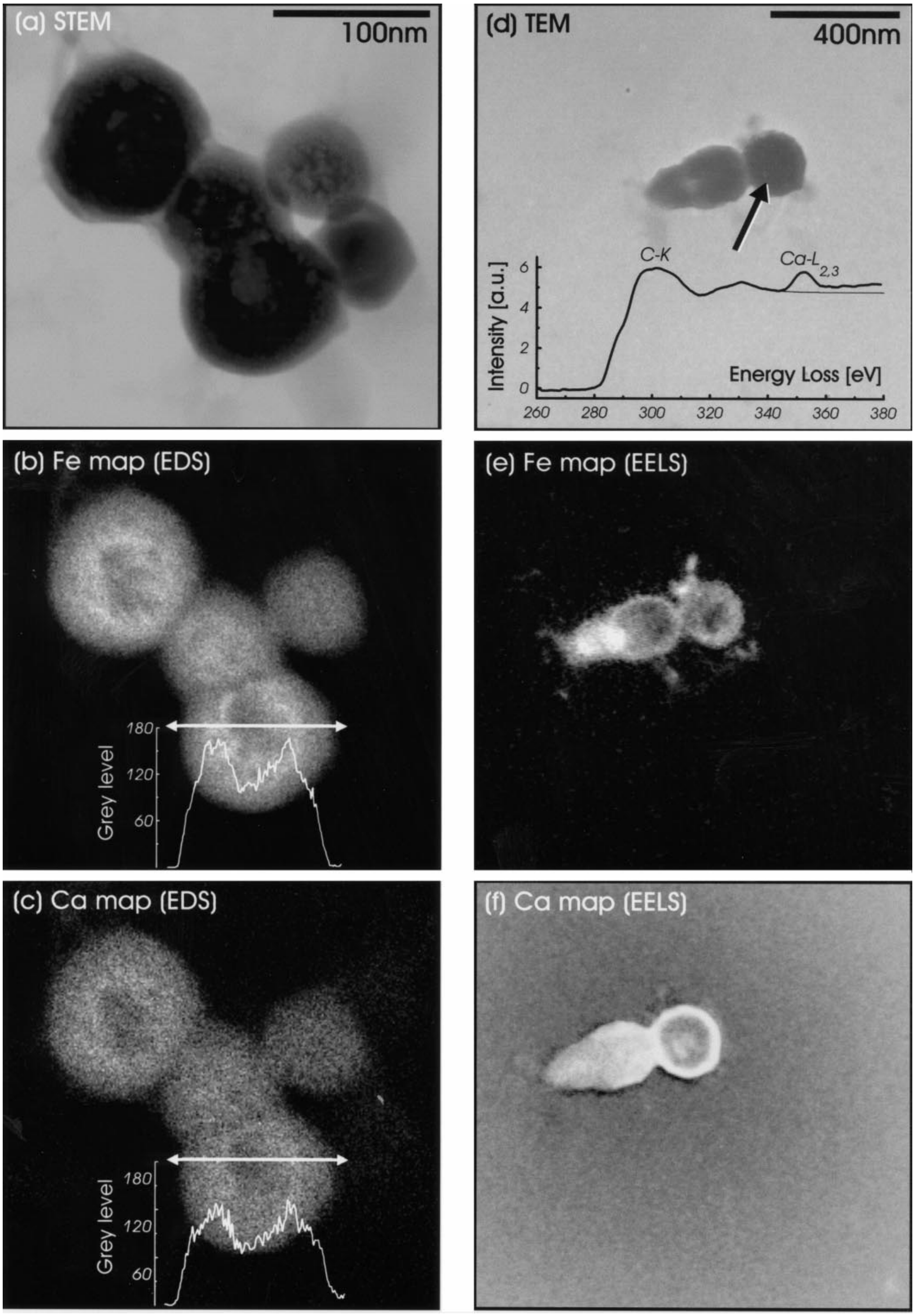
Fig. 4b identifies particles analysed in the Noiraigue spring. In this case, the diversity in terms of composition is higher. Clay-like particles in the <0.5 μm and 0.5–1 μm size ranges are the dominant entities (23%, respectively, 32% of the total number of analysed particles). Fe–Ca-rich colloids associated to Si appear as non negligible species (17% of total particle number). Other particle types (Fe–Ca-rich, Si-rich, Ti-rich, Ca- and Mg-rich) are found, though in much lower proportions.

Although not quantitatively comparable, Fig. 4a and b clearly show qualitative changes from the Bied Brook to the Noiraigue spring. It is remarkable that titanium oxides and sulphides, not found in the Little Bied, are identified in the spring. More importantly, the proportion of Fe–Ca-rich colloids strongly decreases from the inlet to the outlet of the aquifer, while Fe-rich particles are no longer found in the spring. As already stated, only individual particles were analysed by STEM–EDS; for this reason, the association between Fe–Ca-rich colloids and Al–Si-rich or clay-like particles within aggregates does not appear as a specific particle type in Fig. 4; however, these associations were often encountered.

The morphology of particles was also considered during their STEM–EDS analysis. In the Bied Brook, suspended material is mainly amorphous and organic, whereas particles present a higher crystallinity in the Noiraigue spring, i.e. angular-shaped entities generating electron diffraction patterns are proportionally more abundant than rounded, amorphous entities. Fe–Ca-rich colloids found in the Bied are mostly spherical and fairly sensitive to the electron beam of the microscope, while Si–Fe–Ca-rich particles in the Noiraigue, which are not observed in the Bied Brook, are not affected by beam damage, under normal conditions of operation.

From our microscopic observations, it emerges that Fe–Ca-rich entities identified in the Bied Brook dominate the number concentration of inorganic-rich particles in the surface waters of the valley. The majority of these colloids have sizes in the 100–500 nm range. Clay-rich and Fe–Ca-rich particles

Fig. 6. (a) STEM of an aggregate of globules in the Bied Brook; (b) EDS map of iron in the aggregate of globules; (c) EDS map of calcium in the aggregate of globules. The overlays in (b) and (c) show the grey level intensity of the transect marked by the white arrow to highlight the depletion of Fe and Ca in the core of the globule. Recording of STEM–EDS maps was processed under very low e^--dose to enhance the contrast caused by spatial differences in element concentrations, and to avoid beam damage; the resulting long acquisition time (30 min) required a live correction of the lateral drift of the specimen. (d) TEM of an aggregate of globules in the Bied Brook; (e) EELS map of iron (Fe-$L_{2,3}$ edge: 716 eV) in the aggregate of globules; (f) EELS map of calcium (Ca-$L_{2,3}$ edge: 350 eV) in the aggregate of globules. The overlay in (d) shows the EELS spectrum of the arrowed colloid in the C-K and Ca-$L_{2,3}$ region (260–380 eV), and exhibits a marked C edge. Recording of TEM-EELS maps was obtained by digitally subtracting an extrapolated background image at the edge of the element (Mavrocordatos and Perret, 1998); the total acquisition time was less than 30 s.


(a) STEM
100nm
(d) TEM
400nm
C-K
Ca-L2,3
Intensity [a.u.]
0
2
4
6
Energy Loss [eV]
260
280
300
320
340
360
380
(b) Fe map (EDS)
180
120
60
Grey level
(e) Fe map (EELS)
(c) Ca map (EDS)
180
120
60
Grey level
(f) Ca map (EELS)

are often aggregated, leading to entities larger than 1 μm. Clays show no apparent temporal trend and are qualitatively (types of clays) and quantitatively (size distribution, number concentration) unaffected during their travel through the karstic aquifer.

On the other hand, Fe–Ca-rich colloids may undergo important modifications from the inlet to the outlet of the aquifer: their number concentration drastically decreases, their Ca:Fe ratio significantly varies with time, and they apparently become more crystalline; in addition, these entities may scavenge phosphates in the Bied Brook, but the latter are probably released as soluble species in the Noiraigue spring, as a result of the physico-chemical transformation of the Fe–Ca-rich colloids. Ca-rich (calcite) and Mg-rich (dolomite) particles are rare in the Bied Brook, but are released from the karst into the Noiraigue spring, as are titanium oxide colloids.

TOC measurements indicate that the high concentration of organic matter in the waters upstream of the aquifer strongly decreases at its outlet. It is thus postulated that Fe–Ca-rich colloids and organic substances are strongly retained within the karstic fractures. During their retention, Fe–Ca-rich colloids may undergo ageing and/or partial reduction, provided that these species are scavenged into poorly oxygenated regions of the fractures, while organic matter may be decomposed. To confirm this hypothesis, further investigations are actually undertaken to establish the accurate mass balance of these particles under low and high flow events.

4.2. Characteristics of particles from the peat bogs to the Bied Brook

Samples were specifically collected in the peat pore water, in the drain and in the river in order to highlight the potential transformations of the colloids during their transport from the peat bogs to the Bied Brook. Chemical analyses of these samples indicate that the precipitation of iron hydroxide can occur in the drainage system. In the peat pore water, anoxic ($<1\%$ O_2 saturation) and acidic ($pH \approx 4.5$) conditions favour the stabilisation of ferrous iron ($[Fe^{2+}]_{peat} \approx 90\%$ of $[Fe]_{tot}$), whereas oxic ($>60\%$ O_2 saturation) and neutral ($pH \approx 6.5$) conditions lead to a decrease of Fe^{2+} in the drain and in the river ($[Fe^{2+}]_{drain} \approx [Fe^{2+}]_{river} \approx 50–55\%$ of $[Fe]_{tot}$). The high proportion of ferrous iron still present in the Bied Brook suggests that the high concentration of humic substances in the system (ca. 20 mg/l) stabilises Fe^{2+} by complexation.

Assuming that the Fe–Ca-rich entities would originate from the oxidation of dissolved and humics-complexed Fe^{2+} present in the peat pore water, we attempted to identify the process of formation of these globules on a sequence of peat-drain-brook TEM specimens (see Figs. 5 and 6).

In addition to a majority of typically organic ill-defined entities with micrometric sizes, electron-dense globules are identified in the peat pore water (Fig. 5a). Remarkably, these globules have sizes and shapes similar to the Fe–Ca-rich entities found in the drain (Fig. 5b) and in the Bied Brook (Fig. 5c). EDS measurements on peat globules indicate that they contain minute amounts of Fe and Ca. However, the nature of the supporting film for TEM specimens (collodion + carbon) does not allow unambiguous identification of carbon within these entities.

EDS element maps were thus recorded on selected globules in the Bied Brook, in order to characterize their intimate nature. Fig. 6b and c exhibit the Fe map, respectively the Ca map of an aggregate of Fe–Ca-rich globules identified by STEM–EDS (Fig. 6a). A careful examination of these maps reveals that the core of the globules is depleted in Fe and Ca (see the overlayed profiles of grey levels through one globule in Fig. 6b and c). The globules also contain carbon, but, as mentioned above, this element cannot be quantified. Element maps of Si (not shown) indicate that this element is strictly located at the surface of the globules, and that a Si-rich film might act as a cement at the interface between Fe–Ca-rich entities.

EELS maps (Fig. 6e and f) of the Fe–Ca-rich particles in the Bied Brook (Fig. 6d) suggest that their Fe–Ca-depleted core might contain significant amounts of carbon. In addition, EELS spectra (see the overlayed spectrum in Fig. 6d) indicate that the Fe–Ca-depleted core contains a non-negligible amount of carbon atoms. However, due to difficulties in the absolute quantification of carbon within these particles, further investigations using SiO supporting films are required to confirm this preliminary result. Nevertheless, our observations support the hypothesis that Fe^{2+} present in the peat pore water oxidizes into globular

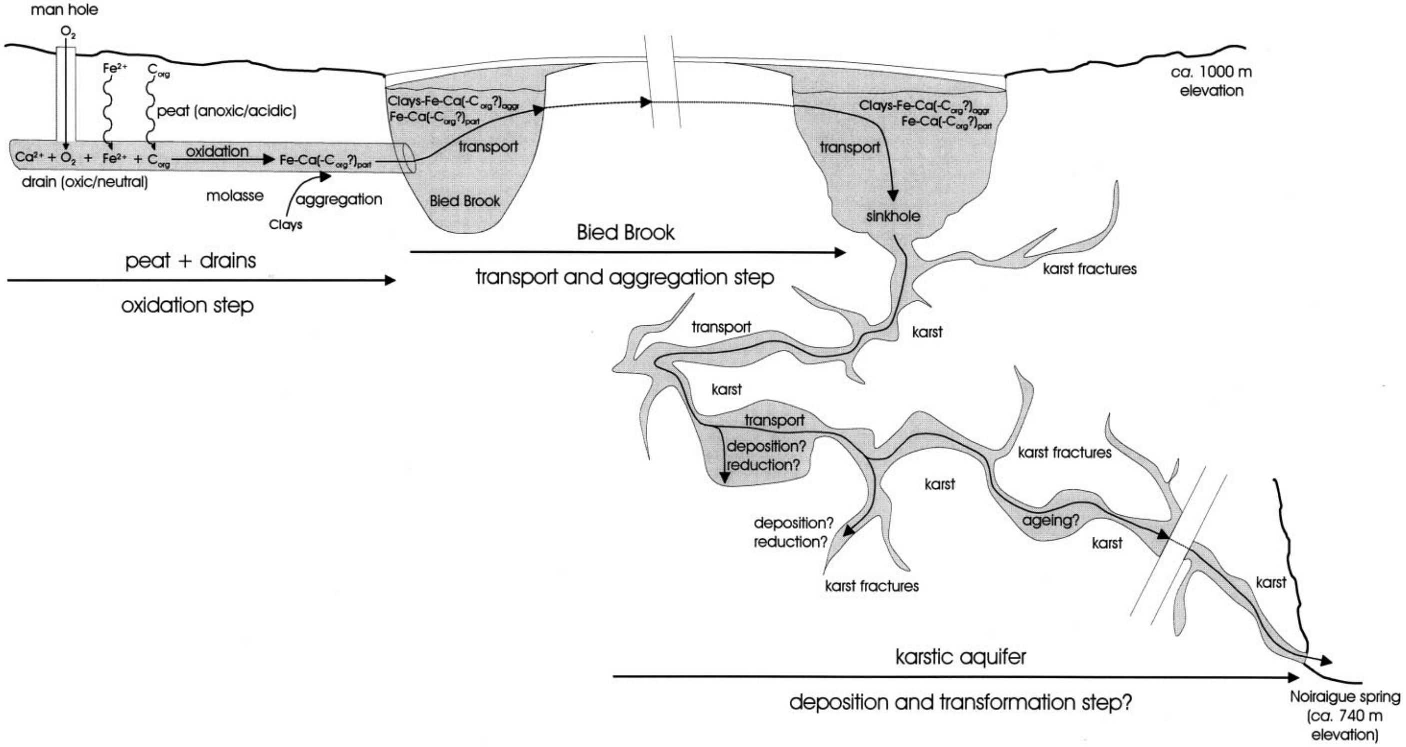


Fig. 7. Suggested pathway for the formation and evolution of Fe–Ca(–C_{org})-rich globules in the Vallée-des-Ponts.

Fe–Ca-rich entities. Peat humics may act as condensation nuclei during this process, leading to Fe–Ca(–C_{org})-rich particles which exhibit a Fe–Ca-depleted core.

5. Conclusions

Electron microscopy, used as an analytical tool (AEM) rather than for illustrative purposes, enables the qualitative and quantitative characterization of colloidal and particulate species in this complex water pathway. AEM and classical wet chemical analyses strongly complement to highlight the most probable pathway of diagenesis and transformation of Fe–Ca(–C_{org})-rich globules in the Vallée-des-Ponts, as proposed in Fig. 7.

Our results show that iron undergoes physico-chemical transformations during its transport through the aquifer. The dissolved and humics-complexed Fe^{2+} form present in peats is eventually oxidized at the surface of humics/fulvics-rich granules, with incorporation of calcium, during its travel through drains. Then, these colloids aggregate mostly with clay-like particles. In the surface waters, the diversity of particle types is fairly low, Fe–Ca(–C_{org})-rich entities representing the majority of colloids. Fe–Ca(–C_{org})-rich particles are then most probably retained by the karst, at least under normal discharge conditions; clay-like particles are apparently unaffected by karst, which, on the other hand, releases other types of colloids (Ca-rich, Mg-rich, Ti-rich).

While most studies discuss the transport of colloids or pollutants in karst from the point of view of hydrogeological considerations, obscuring the role of the individual toxic scavengers, our results focus on the physico-chemical characteristics of the reactive particulate entities upstream and downstream of the karstic aquifer. Thus, our approach is expected to have major consequences for the estimation of the vulnerability of karstic aquifers towards accidental pollution. Our present and future investigations should help verify this proposed scheme.

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