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Aquatic Sciences

Identification of stoichiometric iron-phosphorus colloids produced in a eutrophic lake

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ABSTRACT

Iron-rich colloids formed at the oxic-anoxic interface of a eutrophic lake (Lake Lugano; CH) were characterized by bulk chemical methods and analytical electron microscopy. Fractionation of raw waters showed that non-dissolved iron is particulate above the oxicline and mainly colloidal in the anoxic part of the hypolimnion, while non-dissolved ortho-phosphate is mostly colloidal through the water column. Because of these differences, filtration did not prove helpful for the determination of the role of iron-rich species in the scavenging of ortho-phosphate. On the other hand, analytical electron microscopy revealed that iron-rich nano-granules (ca. 50 nm) are associated to the surface of bacterially produced fibrillar polysaccharides. Iron colloids in these complex entities contain important and constant amounts of phosphorus ($[PO_4]_{part}$:$[Fe]_{part} = 0.48 \pm 0.11$, n = 1096 Fe-rich entities analyzed by electron microscopy), which suggests that phosphates are stoichiometrically incorporated into the hydrous iron oxide phase, with a tentative composition $Fe_2[OOH]_{1-x}[(OH)_3]_x[PO_4]$, during its genesis.

Introduction

Eutrophication problems have been studied in detail for more than half a century (Vollenweider, 1968) while many aquatic systems have changed their biology and chemistry as a consequence of the large quantities of phosphates added by human activities around the world. Since then, many research groups have focused their

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attention on the speciation of phosphorus in natural waters (Lijklema, 1977; Von Gunten and Lienert, 1993; Wheat et al., 1996). The influence of solid phases on the retention of phosphates has been evidenced in soils (Rodier and Robert, 1995), rivers (Stone and Mudroch, 1989), lakes (Stauffer and Armstrong, 1986) and sediments (Löfgren and Boström, 1989, Span et al., 1992), and particulate iron has been shown to efficiently scavenge phosphates in many instances (Gupta et al., 1979; Manning and Jones, 1982; Foy, 1985; McQueen et al., 1986).

Laboratory experiments have been conducted in order to understand the effects of pH, phosphate concentrations and ageing of particles on P-Fe interactions (Gupta et al., 1979), while the mechanism of PO_4 fixation onto Fe_{part} has been studied in parallel (Parfitt et al., 1975; Anderson et al., 1985, Rose et al., 1996, 1997) and modelized for different environments (Van Riemsdijk et al., 1984; Schneider and Schwyn, 1987; Goltermann, 1995). Some authors have hypothesized that PO_4 is adsorbed at the surface of Fe_{part} (Parfitt et al., 1975) while others showed the diffusion of PO_4 into aggregated particles (Willett et al., 1988). These studies were mostly processed with the use of synthetic particles. However information on the physico-chemical structure of natural hydrous iron oxide particles containing phosphates is still missing. Recently, He et al. (1996) demonstrated the change in morphology of laboratory produced iron hydroxide colloids with various phosphate concentrations, but no quantitative information was given at the *per particle* basis.

In this study, we have successfully attempted to fill this lack by collecting freshly formed hydrous iron oxides around the oxicline of Lake Lugano (TI, CH) and analyzing individual colloids with minimum perturbation by analytical electron microscopy. Results of microscopic investigation are combined to classical wet chemical analyses of raw, filtered and ultrafiltered samples.

Lake Lugano is located on the border between Italy and Switzerland and has three distinct basins with steep slopes. Only the northern basin of Lake Lugano will be discussed hereafter. The lake was oligotrophic in the late 1920's (Monti, 1929); since then, a rapid increase in demography and the absence of wastewater treatment plants contributed to the rapid degradation of the water quality, which has been illustrated by algal pigment enumeration (Guilizzoni et al., 1983), a three-fold rise in the total phosphorus concentration between 1960 and 1980, and a important change in the fish population (Barbieri and Mosello, 1992). Since the mid 1970's,

Table 1. Characteristics of the redox interface in the northern basin of Lake Lugano (adapted from Hofmann (1996))

	70 m	80 m	100 m	120 m
T [°C]	5.6	5.5	5.4	5.4
pH	7.6	7.5	7.4	7.3
$[O_2]$ [mg/l]	5.2	0.6	0	0
Conductivity [µS/cm]	250	255	260	267
Turbidity [FTU]	0.45	0.7	0.3	0.2
$[Fe]_{tot}$ [µM]	0.1	0.14	0.6	1.8
$[o\text{-}PO_4]_{tot}$ [µM]	3.15	3.3	4.9	6.0
$[Mn]_{tot}$ [µM]	0.6	1.5	4.2	5.6
$\Sigma\, HS^-$ [µM]	0	0	1.7	3.5

wastewater treatment plants have been built, but 25% of the total wastewater charge is still diverted to the lake without treatment. Nowadays, the northern basin of Lake Lugano is characterized as eutrophic, with the upper part of the hypolimnion (above 80–90 m) being oxic to sub-oxic, and the deeper part (90–280 m) being totally anoxic throughout the year; the basin is meromictic, as the permanent density gradient prevents vertical mixing below ca. 90 m depth. Further details about the chemistry of the lake are found in Table 1 and in Hofmann (1996).

Material and methods

Sampling and analyses

The water column of the northern basin of Lake Lugano (TI, CH) was sampled monthly between December 1995 and June 1996 at the station "Gandria" (46°00′N, 9°00′E). Temperature, pH, [O_2], turbidity and conductivity were measured *in situ* using a Züllig-Hydropolytester probe. The sampling and analytical approach adopted for this study (Fig. 1) consisted of three distinct procedures:

• **Wet chemical analyses:** Lake water was collected by peristaltic pumping (Watson Marlow 701 S/R; 3.4–4.2 l/min) and filtered in line through 0.2 µm membranes (Schleicher-Schüll; cellulose esters) and through a 10 kD ultrafiltration cell (Millipore; polysulfone). Raw, filtered and ultrafiltered samples were directly retrieved in pre-acidified tubes (HCl 10^{-2} M for field colorimetry; HNO_3 3% for ICP-AES) to determine the concentrations of operationally defined particulate, colloidal and dissolved species by difference (Fig. 1).

Wet chemical analyses of these fractions were processed within 4–24 h from sampling without further treatment. Membranes were recovered and particulate material retained on their surface was mineralized (HCl 10^{-1} M for colorimetry, HNO_3 3% + $H_2NOH \cdot HCl$ 10^{-1} M for ICP-AES; 24 h stirring at T_{room}) to determine the concentrations of Fe, Ca, Mn and PO_4 in retained particulate material. Reactive forms of Fe^{2+} (λ_{abs} = 562 nm; Stookey, 1970), o-PO_4 (λ_{abs} = 710 nm; APHA, 1976), and $\sum HS^-$ (λ_{abs} = 665 nm; Williams, 1979) in raw, filtered, ultrafiltered acidified samples and mineralized membrane fractions were measured by colorimetry (Hach DR-2000); Fe (λ_{em} = 259.940 nm and 238.204 nm), Ca (λ_{em} = 393.366 nm and 315.357 nm) and Mn (λ_{em} = 257.610 nm) were measured by ICP-AES (Perkin-Elmer Plasma 1000).

• **Analytical electron microscopic analyses:** Raw waters were collected with Go-Flow bottles at the depths of interest (60–120 m), transferred to bottles in a N_2-purged glovebag (I^2R model X-27-27) and brought to the laboratory within 1 h from sampling. Transmission electron microscopy (TEM) specimens were prepared by a non-disturbing procedure (Perret et al., 1991; Lienemann et al., 1998): A turbidity-dependent volume (4–30 ml) of the sample was ultracentrifuged (Beckman L-7 with swing-out rotor Kontron TST 28.38; 28'000 rpm, RCF = 131'500 g, 60–120 min) above TEM copper grids (collodion coated, carbon covered); these conditions allow for recovery of particles with size >30 nm and density >1.2 g/cm^3. After ultra-

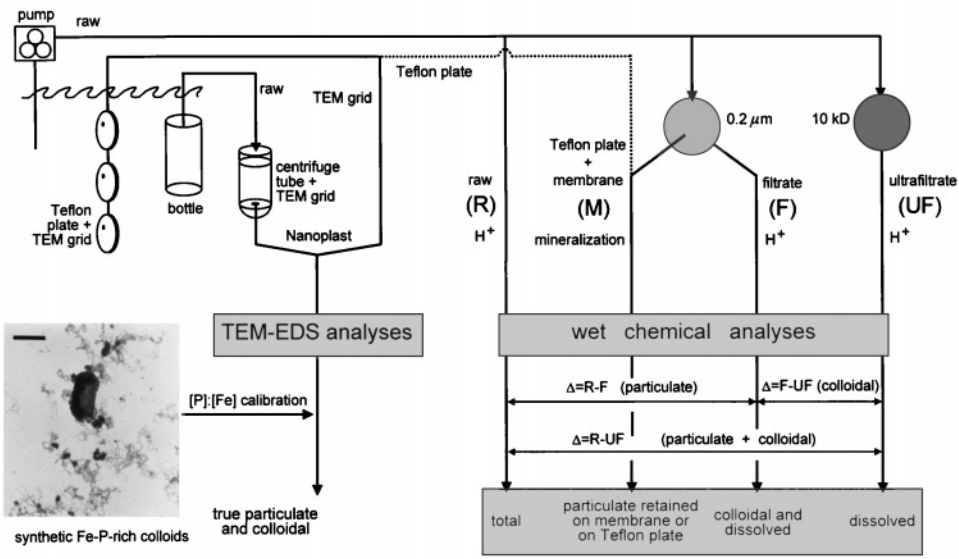

Figure 1. Sampling and analytical scheme used for this study; details of the procedure are given in the text. The micrograph shows synthetic Fe-PO_4-rich colloids (dark spheres) used as TEM-EDS calibration standards; these colloids are attached to a network of fibrillar polysaccharides (xanthan) exsudated by a bacterium (*Xanthomonas campestris*); scale bar = 500 nm

centrifugation, the supernatant was carefully withdrawn under N_2 atmosphere and wet grids were protected with a thin film of a hydrophilic resin (Nanoplast FB101; Bachhuber and Frösch, 1983). After polymerization of the resin, grids were visualized by TEM (Zeiss EM10, 80 kV; nominal magnification: 10'000–80'000) to identify the dominant particles at each depth.

Elemental analyses of individual entities were processed by TEM-EDS (Philips CM12 equiped with an EDAX-STUV detector cooled with liquid N_2; 80 kV, nominal magnification: 19'500, 30° tilt, probe size: 40–400 nm, counting time = 30–300 sec). TEM-EDS spectra of individual particles were recorded in the 0–10 keV region. K_α X-ray emission lines of the following elements were integrated and quantified: Al (1.486 keV), Si (1.739 keV), P (2.013 keV), S (2.307 keV), Cl (2.621 keV), Ca (3.690 keV), Mn (5.894 keV) and Fe (6.398 keV).

In order to determine with a high accuracy the molar ratio $[PO_4]_{part}$:$[Fe]_{part}$ in individual iron-rich particles, hydrous iron oxide colloids containing phosphates were synthetised under chemical conditions similar to the lake and used as EDS calibration standards: Fe-P-rich colloids were obtained by slow (ca. 12 h) oxidation of a deoxygenated ($[O_2]_{t0} < 0.5$ mg/l) solution containing $[Fe^{2+}]$ 10 µM in $[Ca(HCO_3)_2]$ 1.5 mM, $[H_2PO_4^-]$ 1.25–5 µM and polysaccharide fibrils (xanthan 1 mg/l produced by *Xanthomonas campestris*; Lienemann et al., 1997) at pH 7 and 10 °C. $[O_2]$ and pH were controlled during the syntheses by bubbling N_2 and CO_2. At the issue of the syntheses, $[PO_4]_{part}$ (colorimetry) and $[Fe]_{part}$ (ICP-AES) were determined on the particulate material isolated by filtration.

• **Direct *in situ* collection of particulate phases:** Machined Teflon plates ($\phi = 8$ cm) holding TEM gold grids (collodion coated, carbon covered) were vertically inserted in the 60–120 m layer of the water column for occasional periods varying between one and two months; this procedure allowed for the direct *in situ* recovery of freshly formed and reactive non-sedimenting particles for microscopic (TEM grids) and bulk chemical (Teflon plates) analyses. The plates were retrieved and the particulate material adhering onto their surface was mineralized (HCl 10^{-1} M for colorimetry, HNO_3 3 % + $H_2NOH \cdot HCl$ 10^{-1} M for ICP-AES; 24 h stirring at room temperature) prior to analysis by colorimetry (PO_4) and ICP-AES (Fe, Mn). TEM grids were Nanoplast-protected as stated above.

Results and discussion

Wet chemical analyses

Profiles of iron and phosphate species obtained around the oxicline of the northern basin of Lake Lugano are shown in Figures 2 a–c. While profiles of T, pH, O_2, conductivity and turbidity in surface waters (0–50 m) are subject to drastic change throughout the seasons, their trend below the oxicline is constant over the year (Hofmann, 1996); however, the absolute depth at which the oxicline is located varies daily because of internal seiches (Salvadè et al., 1992) with highly variable frequencies (ca. 2–12 h) and amplitudes (ca. 1–10 m). Concentrations of operationally defined particulate fractions (difference between total and filtered acidified samples; direct analysis of filtration membranes) apparently fit and show peaks of particulate iron and phosphates around 90–95 m. Observations made on similar systems (see references in Table 2) suggest that the chemistries of both species could be linked. The higher concentrations of Fe_{membr} compared to Fe_{part} originate from the analytical procedure (mineralization of particulate species onto membranes *vs.* simple acidification for raw and filtered samples).

Occasional profiles of Fe_{part} and Mn_{part} recovered onto Teflon plates (direct *in situ* collection) qualitatively show trends similar to the profiles obtained by conventional water sampling (discrete peaks of Mn_{part} in the 60–85 m zone and Fe_{part} in the 85–100 m zone). However, a quantitative comparison of the two sampling approaches is difficult to establish because Teflon plates accumulate particulate material over long periods of time (1–2 months) while conventional sampling produces a picture of the instantaneous concentrations of species in the water column. In the case of the northern basin of Lake Lugano, interpretation of results obtained with Teflon is complicated by the presence of the internal seiches at the sampling point; Teflon plates thus integrate these events during their residence in the water column.

Nevertheless, we noticed that the proportions of Ca_{part}, Al_{part} and Si_{part} relative to Fe_{part} or Mn_{part} ($[Ca, Al, Si]_{part}$: $[Fe, Mn]_{part}$) were much lower on Teflon plates than in conventional water samples of the same depths. This suggests that freshly formed Fe_{part} and Mn_{part} are selectively preconcentrated on the Teflon surface, as already observed by Belzile et al. (1989) who used Teflon plates in oxic sediments. In our study, microscopic observations (see below) evidenced the systematic binding of Fe-rich and Mn-rich entities to aquagenic organic material; it is thus postulated

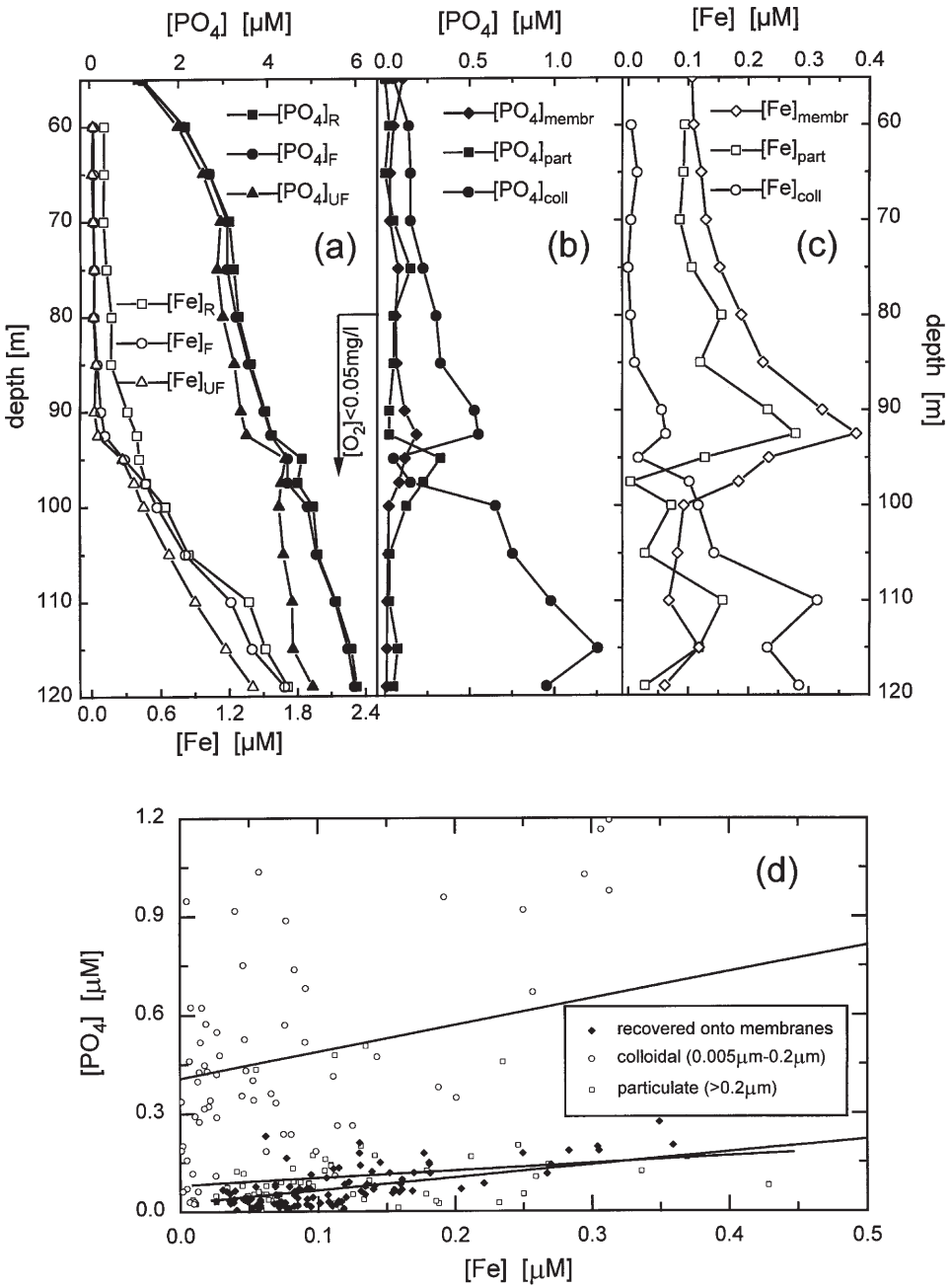

Figure 2. (a) Example of typical concentration profiles of phosphates and iron obtained in the water column of Lake Lugano (March 1996; R = raw samples, F = filtered samples, UF = ultrafiltered samples). (b) and (c): The fractions of particulate ($[X]_{part}$) and colloidal ($[X]_{coll}$) species are obtained by difference between raw and filtered samples (R–F), respectively between filtered and ultrafiltered samples (F–UF), all samples being acidified; $[X]_{membr}$ denotes the fractions of

that the affinity of these organo-mineral particles for the surface of Teflon is mainly controled by hydrophobic interactions.

In order to determine the influence of the iron chemistry on the behaviour of phosphates in Lake Lugano, we attempted to correlate the concentrations of these species under their operationally defined colloidal and particulate forms. Figure 2d presents the results obtained around the oxicline for all sampling campaigns. Data for the colloidal species (filtered – ultrafiltered; $[Fe]_{coll}$: ICP-AES, $[PO_4]_{coll}$: colorimetry) are strongly spread ($[PO_4]_{coll}$: $[Fe]_{coll} = 24 \pm 51$). Although Fe_{coll} is expected to exhibit a large specific surface area (Crosby et al., 1983) which would favour adsorption of phosphates in large quantities, the ratio $[PO_4]_{coll}$: $[Fe]_{coll}$ has obviously no stoichiometric significance in this case. $[PO_4]_{part}$: $[Fe]_{part}$ obtained by difference (raw – filtrate; 1.20 ± 1.29) and $[PO_4]_{membr}$: $[Fe]_{membr}$ obtained by analysis of material recovered onto filtration membranes (0.68 ± 0.52) show much lower values, but these ratios are hampered by relative standard deviations close to 100%. Any attempt to obtain temporal or spatial trends from data of Figure 2d failed.

However, a closer examination of profiles in Figures 2b–c shows that Fe and PO_4 do not necessarily co-exist within the same entities (compare $[PO_4]_{part}$ *vs.* $[PO_4]_{membr}$ and $[PO_4]_{membr}$ *vs.* $[Fe]_{membr}$). Between 60 and 85 m, the concentration of dissolved iron is close to zero and Fe is almost exclusively found as particulate (raw – filtrate $\gg$ filtrate – ultrafiltrate). The concentration of this particulate fraction increases between 85 and 100 m, giving rise to a sharp peak of Fe_{part} and some increase of Fe_{coll}. Below that region, iron is mostly in the form of dissolved Fe^{2+}, with a non negligible contribution of colloidal form (filtrate – ultrafiltrate > raw – filtrate).

On the other hand, dissolved ortho-phosphates are dominant along the water column, but a colloidal fraction of phosphates already appears around 70 m, its concentration increasing with depth except in the 95–100 m region (just below the maximum of the Fe_{part} peak), where it is present as $PO_{4\,part}$.

Interpretation of the possible relationship between $PO_{4\,coll}$ and Fe_{coll} from bulk chemical analyses presents some major difficulties. Above the suboxic zone, Fe_{coll} is undetectable or at very low concentration; analytical electron microscopy shows that non-dissolved iron in this zone is only present as coatings on large particles or as detrital Fe-rich particles settling from the upper layers of the lake. Fe_{coll} becomes significant only in the region of iron precipitation (suboxic zone), and it exceeds Fe_{part} where dissolution of freshly precipitated hydrous iron oxides takes place (anoxic waters). Below the oxicline, $PO_{4\,coll}$ shows a trend which is similar to the

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particulate species retained by filtration membranes and mineralized. Standard deviations are in the order of symbol sizes. Sampling date: March 1996. (d): Relation between non-dissolved phosphate and iron species, obtained by wet chemical analyses of raw, filtered and ultrafiltered samples, and by analyses of particulate material recovered onto filtration membranes; all depths and sampling campaigns were considered for this figure. The correlations obtained for the different fractions are: (i) $[PO_4]_{membr} = 0.026 + 0.388\,[Fe]_{membr}$ ($r = 0.549$), $[PO_4]_{membr}$: $[Fe]_{membr} = 0.68 \pm 0.52$ ($n = 106$ analyses); (ii) $[PO_4]_{part} = 0.080 + 0.223\,[Fe]_{part}$ ($r = 0.179$), $[PO_4]_{part}$: $[Fe]_{part} = 1.20 \pm 1.29$ ($n = 59$ analyses), (iii) $[PO_4]_{coll} = 0.407 + 0.811\,[Fe]_{coll}$ ($r = 0.561$), $[PO_4]_{coll}$: $[Fe]_{coll} = 23.9 \pm 51.4$ ($n = 69$ analyses)

increase of Fe_{coll} (Fig. 2 a). However, the unreasonably high $[PO_4]_{coll}$: $[Fe]_{coll}$ ratio suggests that colloidal phosphates are clearly associated to other carriers than colloidal iron.

We noticed that $[PO_4]_{coll}$ is weakly correlated to $[Ca]_{coll}$; the ratio $[Ca]_{coll}$: $[PO_4]_{coll}$ (150.7 ± 51.5) is within the range obtained by Rossknecht (1980) for the elimination of phosphate by calcite precipitation. In our case, $[Ca]_{coll}$ could originate from calcite dissolution with depth (Hofmann, 1996) or from artifacts during ultrafiltration (release of CO_2 from deep waters brought to atmospheric pressure); however, $[Ca]_{coll}$ is non negligible compared to $[Fe]_{coll}$ and small amounts of phosphates associated to Ca_{coll} may thus induce large errors on the determination of the $[PO_4]_{coll}$: $[Fe]_{coll}$ ratio.

As a consequence, the use of filtration and ultrafiltration, which discriminate particles according to operationally defined size classes, is not suitable for the determination of the true proportion of phosphates in freshly precipitated hydrous iron oxides. In addition, both the chemical heterogeneity of the suspended particles, filtration artifacts (Buffle et al., 1992; Perret, 1989) and biases induced by analysis-dependent preparation of samples (acidification with HNO_3: $H_2NOH \cdot HCl$ for ICP-AES of Fe, and with HCl for colorimetry of PO_4) account for uncertainties in the observed $[PO_4]$: [Fe] ratios obtained by bulk chemical analyses. On the other hand, analytical electron microscopy allows the characterization of particles without requiring fractionation of suspensions into different size intervals, thus minimizing artifacts during sample preparation.

Microscopic analyses

A TEM survey of particulate material recovered throughout the water column by ultracentrifugation of samples onto TEM grids and by insertion of TEM grids at depth (direct *in situ* collection) highlighted interesting features. Micrographs of ultracentrifuged specimens show a high heterogeneity of the particle sizes and types. In the sub-oxic layer of the lake, the iron-rich entities (Fig. 3 a) dominate the pool of particles, although clay leaflets, diatoms, bacteria and organic debris are common. The predominance of iron-rich colloids in the 85–105 m water layer strengthens the hypothesis that the peak of Fe_{part} contributes significantly to the increased turbidity observed at these depths (Hofmann, 1996; Hofmann and Dominik, 1995).

On the other hand, micrographs of specimens obtained by direct *in situ* collection exhibit a much narrower range of particle types. Organic-rich entities (fibrils, ill-defined electron-transparent material, bacteria), and freshly precipitated Mn-rich and Fe-rich entities (Fig. 3 a), are preferentially collected onto TEM grids. The morphology of these colloids and particles is similar for both specimen preparation schemes. As for Teflon plates, the affinity of organic-rich and freshly formed organic-bound material (Mn-rich and Fe-rich entities) for the surface of TEM grids may be due to the hydrophobicity of the collodion-carbon film on the grids.

Iron-rich entities identified in Lake Lugano have a very distinct morphology (Figs. 3 a–b). They consist of electron-dense nano-granules with sizes smaller than 70 nm, which are exclusively attached to loose networks of thin (2–10 nm) fibrils.

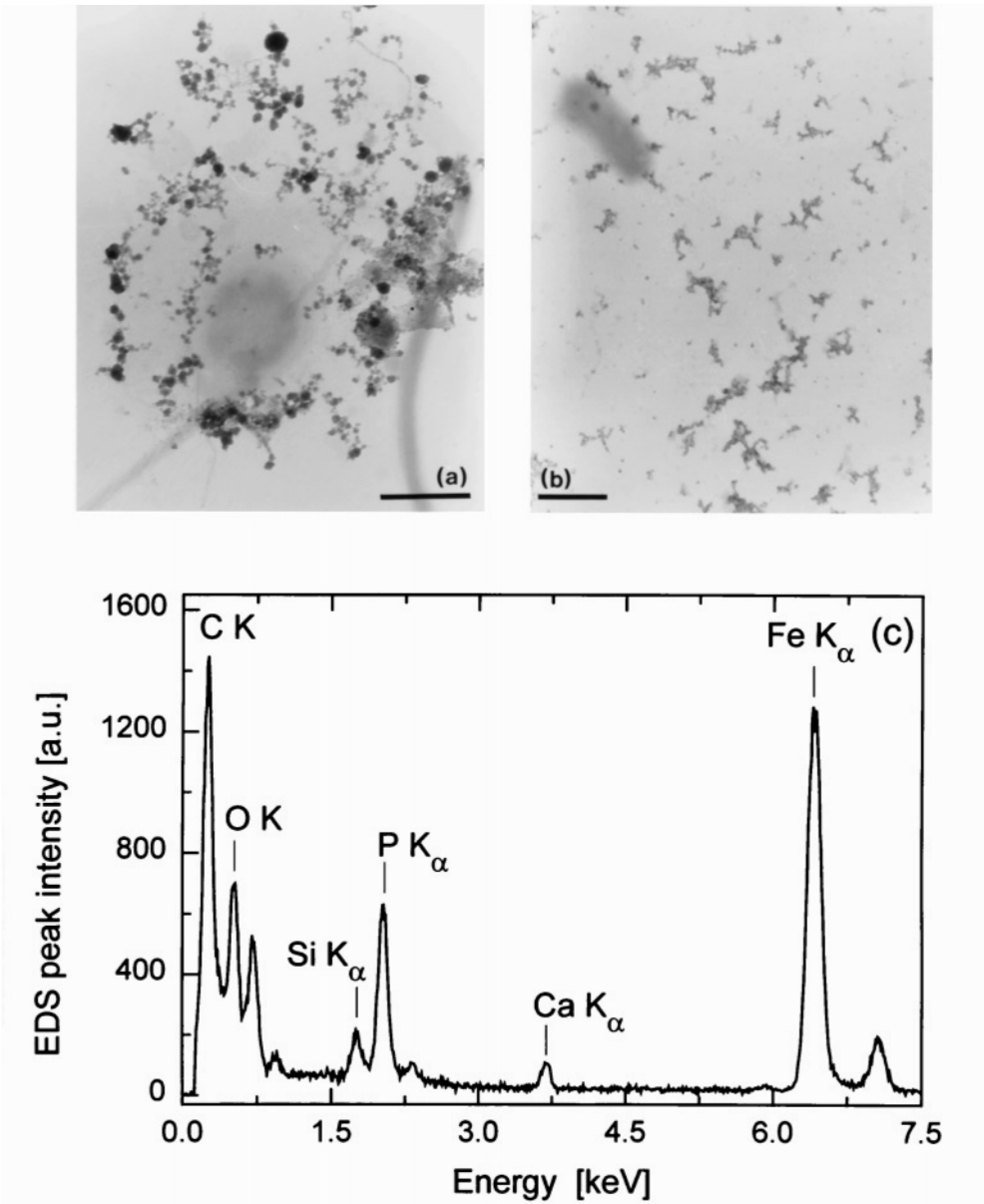

Figure 3. (a) Micrography of typical iron-rich granules identified in the peak of particulate iron (96.5 m); the granules are attached to a network of fibrils connected to a bacterium. This specimen was obtained by direct insertion of TEM grids at depth. (b): Micrograph of typical iron-rich granules identified below the peak of particulate iron (120 m); the morphology of granules is looser, probably because of partial reduction. This specimen was obtained by ultracentrifugation of the sampled suspension onto TEM grids. Scale bars = 500 nm. (c): Typical TEM-EDS spectrum of a grape of Fe-PO_4-rich granules identified in the peak of particulate iron (96.5 m); except for [P] : [Fe], the ratios of EDS peak intensities vary from analysis to analysis

The electron-density of these fibrils suggests that they are covered by a thin layer of mineral material (Degens and Ittekkot, 1982). Fibrils are systematically connected to the outer membrane of quasispherical bacteria and are produced by the latter. The overall size of these complex bacteria-fibrils-granules entities is in the range 1–5 µm. The morphology of iron-rich entities identified in the deep layers of the lake (Fig. 3b) differs from the ones present in the peak of Fe_{part} (Fig. 3a). Below 105 m, granules are smaller (30–50 nm) and the bacteria-fibrils-granules decompose and disperse into looser entities.

Recently, morphologically similar fibrils present in a small meromictic lake (Paul Lake; MI, USA) containing high amounts of iron ($[Fe]_{part} \sim 100$ µM) and organic matter (TOC ~ 6 mg/l) were unequivocally identified at the microscopic level as polysaccharides (Lienemann, 1997; Taillefert et al., 1999) by a silver-based selective staining procedure (Thiéry, 1967; Chenu, 1993) adapted in our laboratory. We believe that the fibrils attached to their parent bacteria in Lake Lugano are also polysaccharidic in nature and that the chemically-driven genesis of iron colloids (Davison and DeVitre, 1992) could be catalysed by the surface of such polysaccharide fibrils, which act as privileged condensation nuclei (Manns, 1988).

In order to accurately determine the elemental composition of Fe-rich entities, 1096 TEM-EDS spectra were recorded on specimens from all depths and sampling campaigns. It was first checked on 50 randomly selected entities that EDS peak intensity ratios obtained on individual granules (40 nm probe) and on grapes of 5–10 granules (400 nm probe) did not differ significantly. [Ca]:[Fe], [Al]:[Fe] and [Si]:[Fe] peak intensity ratios were different from granule to granule, while [P]:[Fe] ratios were identical for narrow and large probes (see insert in Fig. 4).

Although a 400 nm probe averages the elemental composition over the volume of the whole analysed grape, the use of a 50 nm probe requires long acquisition times (ca. 200–300 s) which may cause beam damage to the irradiated colloid and induce specimen drift during analysis. It was consequently decided to record TEM-EDS spectra with a 400 nm probe (counting time = 30 s) to allow for a fast and statistically valid characterization of Fe-rich entities.

Figure 3 c exhibits the typical EDS spectrum of an iron-rich entity. The spectrum indicates that granules adhering to polysaccharide fibrils cannot be considered as pure hydrous iron oxide phases. These iron colloids contain important amounts of silicon, phosphorus and calcium, while minute amounts of aluminum are occasionally present; EDS peak intensities of Al, Si and Ca are highly variable from colloid to colloid. The peak of carbon can not be used for the quantification of organic material within the colloids, because specimens are prepared on a carbon-collodion support film.

The relation between EDS peak intensities of phosphorus and iron is shown in Figure 4 for the 1096 analysed entities. As opposed to the bulk chemical analyses (Fig. 2d) of the particulate and colloidal phases, Figure 4 evidences the strong relationship which exists between P and Fe at the *per particle* level. Conversion of [P]:[Fe] ratio from TEM-EDS peak intensities into $[PO_4]_{part}:[Fe]_{part}$ molar ratio for each individual aquatic entity was achieved with the use of a calibration factor K obtained from the analysis of our synthetic hydrous iron oxide standards; K is the ratio of $([PO_4]_{part}:[Fe]_{part})_{standard}$ analysed by wet chemistry over the ratio of $([P]:[Fe])_{standard}$ analysed by TEM-EDS (Buffle et al., 1989). TEM visualization of

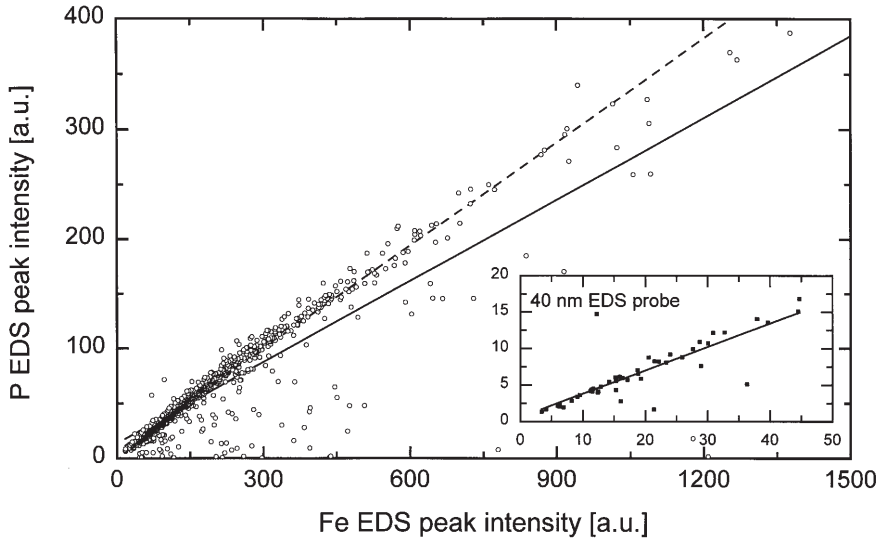

Figure 4. Relation between the EDS peak intensities of phosphorus and iron, obtained by TEM-EDS analyses of 1096 grapes of Fe-rich colloids with a large EDS probe (400 nm; counting time = 30 s). For the whole set of analyses (n = 1096 measurements; solid line), the correlation is: $[P]_{EDS}$ = 13.515 + 0.247$[Fe]_{EDS}$ (r = 0.867); after correction with the K factor, $[PO_4]_{part}$:$[Fe]_{part}$ = 0.50 ± 0.04 (n = 974 particles). The insert in the figure exhibits the results of the analysis of 50 individual colloids with a small EDS probe (40 nm; counting time = 200 s); although the correlation is weaker than for the large probe ($[P]_{EDS}$ = 0.611 + 0.321$[Fe]_{EDS}$; r = 0.847), the molar ratio $[PO_4]_{part}$:$[Fe]_{part}$ = 0.50 ± 0.15 is in agreement with the results obtained on 1096 gapes of colloids

our synthetic hydrous iron oxide colloids containing phosphates revealed slightly aggregated colloids attached to polysaccharide fibrils with morphologies and sizes similar to the ones identified in Lake Lugano (compare insert in Figure 1 and Figure 3 a).

For three syntheses with $[PO_4]_{part}$:$[Fe]_{part}$ in the range 0.34–0.49, the TEM-EDS analysis of 80 individual standard colloids yielded a calibration factor K = 1.39 ± 0.18. For all depths and sampling campaigns, the conversion of [P]:[Fe] with the K factor produces $[PO_4]_{part}$:$[Fe]_{part}$ = 0.48 ± 0.11 (n = 1096). Figure 4 also reveals that ca. 90% of Fe-rich entities have a molar ratio $[PO_4]_{part}$:$[Fe]_{part}$ = 0.50 ± 0.04 (n = 974); noteworthy, 80% of the remaining Fe-rich entities have a molar ratio *below* this value.

Table 2 lists some values of $[PO_4]_{part}$:$[Fe]_{part}$ obtained for the analysis of synthetic and natural Fe-rich particles. Considering the correlation obtained in Figure 4 and the values listed in Table 2, the molar ratio $[PO_4]_{part}$:$[Fe]_{part}$ is usually equal to or less than 0.5, even when the total available concentrations of ortho-phosphates and iron in solution exceed $[PO_4]_{aq}$:$[Fe]_{aq}$ = 0.5 (e.g., Leppard et al., 1988; this study). Values higher than 0.5 have rarely been reported in the literature for Fe-rich particles in natural waters (Sholkovitz and Copland, 1982; Tessenow, 1974).

A ratio of 0.5 can be explained by the formation of a bidentate structure between two Fe^{3+} and one PO_4^{3-} (Anderson et al., 1985; Parfitt et al., 1975; McBride, 1982)

Table 2. Values of $[PO_4]_{part}$:$[Fe]_{part}$ obtained for natural and synthetic Fe-rich particles

$[PO_4]$:[Fe] molar ratio	Sample	Analysis	Reference
0.54 ± 0.58	Lake Lugano	wet chemistry; material collected onto filters; n = 35	this study
0.48 ± 0.11	Lake Lugano	TEM-EDS of individual colloids; n = 1096	this study
0.50 ± 0.04	Lake Lugano	90% of TEM-EDS analyses	this study
0.22 ± 0.01	Lake Bret	wet chemistry; n = 71 samples	Leppard et al. (1988)
0.25 ± 0.06	Lake Bret	TEM-EDS; n = 71 analyses	Buffle et al. (1989)
0.23	hydrolysis of $Fe_2(SO_4)_3$	wet chemistry	Buffle et al. (1989)
0.31	hydrolysis of $Fe(NO_3)_3$	wet chemistry	Buffle et al. (1989)
0.50	oxidation of Fe^{2+}	wet chemistry	Buffle et al. (1989)
0.23–0.32	waste water + $FeCl_3$ with/without poly-electrolyte; pH 4.75	wet chemistry	Narasiah et al. (1994)
≥0.15	soils	TEM-EDS	Rodier and Robert (1995)
0.14 ± 0.03	Lakes Erie + Ontario; oxic sediments	wet chemistry	Manning et al. (1984)
0.28	marine sediments	wet chemistry	Berner (1973)
0.06 ± 0.02 0.08 ± 0.04	river sediments	wet chemistry (10 size fractions)	Stone and Mudroch (1989)
0.76 ~ 0.11	Esthwaite Lake	XRF; bulk sample wet chemistry	Sholkovitz and Copland (1982)
0.24 – 0.46	Lake Sebasticook	wet chemistry	Mayer et al. (1982)
0.02 – 1.65	calcareous lakes	wet chemistry	Stauffer (1987)
0.65 – 0.66	Lake Urse	wet chemistry	Tessenow (1974)
0.5 – 0.56	hydrolysis of Fe^{III}; pH 7	wet chemistry	Tessenow (1974)
0.14 – 0.19 0.17 – 0.18	Shagawa Lake, surface sediments	wet chemistry	Stauffer and Armstrong (1986)
0.18	limnocorrals in Lake St Georges	wet chemistry	McQueen et al. (1986)
0.12 – 0.20	hydrolysis of Fe^{III}, pH 8–5	wet chemistry	Lijklema (1980)
0.18 – 0.20	hydrothermal particles	EM	Wheat et al. (1996)
0.235 ± 0.0004	iron aggregates	SEM-EDS	Caulkett and Ellis-Evans (1996)
0.01 – 0.09	lacustrine diagenetic iron oxyhydroxides	wet chemistry	Fortin et al. (1993)

and by linkages between PO_4^{3-} tetrahedrons and Fe(III) octahedrons (Rose et al., 1997). Analytical electron microscopy shows that, in the present study, less than 2 % of Fe-PO_4-rich colloids contain more than one phosphorus atom for two iron atoms. Similar results were obtained by Gupta et al. (1979), showing that no residual phosphates remain in solution during the synthesis of Fe-PO_4 particles when the initial $[PO_4]_{tot}:[Fe]_{tot}$ ratio was smaller than 0.5.

The high value and the low variability of the $[PO_4]_{part}:[Fe]_{part}$ ratio measured by TEM-EDS for the majority of Fe-rich colloids in Lake Lugano (0.50 ± 0.04 for 90 % of Fe-rich entities) indicates that a stoichiometric phase is formed at the oxicline with a tentative composition $Fe_2[OOH]_{1-x}[(OH)_3]_x[PO_4]$. It is indeed unlikely that phosphate interaction with hydrous iron oxide granules proceeds by adsorption after particle formation, because this would lead to a lower $[PO_4]_{part}:[Fe]_{part}$ ratio (adsorption of phosphates on the outer surface of granules) with a high variability.

The hypothesis of the formation of a stoichiometric phase is further strengthened by the fact that the $[PO_4]_{part}:[Fe]_{part}$ ratio in the smaller granules from anoxic waters is identical to the one found in sub-oxic layers. The existence of a stoichiometric phase in Lake Lugano is in agreement with results of Rose et al. (1996, 1997) obtained on synthetic Fe-rich particles, which show the presence of phosphorus atoms in the second coordination sphere of iron during the initial formation of dimers, and the dependence of the growth mechanism of Fe-PO_4 colloids on the initial $[PO_4]_{tot}:[Fe]_{tot}$ ratio.

Beside phosphorus, TEM-EDS analyses showed that non negligible amounts of Si, Ca and eventually Al are associated to iron-rich colloids. However, they are present in highly variable proportions ($[Si]:[Fe] = 0.42 \pm 0.43$, $[Ca]:[Fe] = 0.23 \pm 0.40$, $[Al]:[Fe] = 0.04 \pm 0.12$; EDS peak intensity ratios), which are independent of depth or season. These elements are mostly present as aggregated species (frustule fragments, silica, clays, Ca-rich entities) onto the bacteria-fibrils-granules entities.

Above the peak of Fe_{part} (60–85 m), bacterially produced Mn-rich particles (Lienemann, 1997) were also shown to contain phosphorus. Manganese oxides have already been described as phosphate scavengers (Yao and Millero, 1996). However in the case of Lake Lugano, [P]:[Mn] is highly variable from particle to particle (0.153 ± 0.109; EDS peak intensity ratio), while phosphorus in these particles is systematically linked to the presence of iron. ($[PO_4]_{part}:[Fe]_{part} = 0.54 \pm 0.06$ within Mn_{part}). This evidences the possibility of partial aggregation of Fe-PO_4-rich colloids onto manganese oxides. Other particle types analysed by TEM-EDS occasionally revealed the presence of phosphorus; no correlation was however found between phosphorus and other elements identified in these particles.

Conclusions

Phosphates in Lake Lugano have been shown by electron microscopic analyses to form a stoichiometric phase with iron ($[PO_4]_{part}:[Fe]_{part} = 0.5$) in the form of nanogranules at the oxic-anoxic interface. This result highlights the possibility of selective incorporation of phosphates into hydrous iron oxide colloids by a mechanism different from adsorption. The iron-phosphorus-rich granules are associated to bacterially-produced polysaccharide fibrils; they contain variable amounts of silic-

on, calcium and aluminum, and are thus far from pure hydrous iron oxides. We think that the networking of granules onto fibrillar material could spatially stabilize them against further coagulation into larger particles with increased sedimentation rates.

The question arises to what extent the incorporation of phosphorus into iron colloids influences the vertical transport of phosphates from deep anoxic layers of the lake to surficial waters, where they are utilized for photosynthetic activity. Although the $[PO_4]_{part}$:$[Fe]_{part}$ molar ratio reaches a maximum limit of 0.5, the overall concentration of iron in the studied water layer may be too small to scavenge an important fraction of available phosphates. However, the scavenging efficiency depends on the relative fluxes of dissolved iron and ortho-phosphate; the measurement of these fluxes at the oxicline of the northern basin of Lake Lugano and the influence of Fe precipitates on the possible auto-purification of the waters is discussed in details in Monnerat et al. (1998, 1999).

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