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Physico-chemical characteristics of a colloidal iron phosphate species formed at the oxic-anoxic interface of a eutrophic lake

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Abstract—Natural iron oxyhydroxide colloidal particles have been isolated from the redox transition boundary layer in a eutrophic lake. They have been physically and chemically characterized on a particle specific basis using new technology from the biomedical sciences which enables minimization of artefacts of particle sizing and optimization of the microscopical parameters for chemically quantifying their elemental composition by STEM/EDS. The Fe-rich particles have also been macroscopically analysed using classical techniques. The Fe particle size range lies between 0.4 and 0.04 μm, the latter limit being determined by experimental conditions. Particles larger than 0.4 μm have also been observed but are loose aggregates of smaller Fe-rich particles.

Chemically, the predominant particle type contains Fe, P and Ca exclusively, although occasional associations of the latter elements with Si or Si/Al occur. The former three elements are strongly correlated, and the mean molar ratios in the particles are 0.25, 0.19 and 1.34 for P/Fe, Ca/Fe and P/Ca, respectively. The Fe is approximately 50% Fe(II) and the P is present as phosphate. Although a good correlation exists between Al and Si, neither is significantly correlated to Fe, P or Ca. The chemical composition is relatively constant spatially seasonally and yearly. These results indicate that a well-defined chemical entity is formed at the redox interface in the water column, composed mostly of Fe(III), Fe (II), PO_4 and Ca (and probably OH^-).

SYMBOLS

AAS	Atomic absorption spectrometry
EDS	Energy dispersive spectrometry
NOM	Natural organic matter
STEM	Scanning transmission electron microscopy
TEM	Transmission electron microscopy

I. INTRODUCTION

IRON IS CERTAINLY one of the most common elements in the earth's crust and is furthermore an essential trace element for living species. Not surprisingly in natural aquatic systems, particulate iron oxides/hydroxides, because of their surface properties (SCHINDLER and STUMM, 1987), play a primordial role as a trace elements scavenger (JENNE, 1968; SALOMONS and FÖRSTNER, 1984) and strongly interact with more concentrated dissolved species which may be present, such as phosphorus, organic matter and calcium (BACCINI, 1985; GJESSING, 1976; TIPPING, 1981; SHOLKOVITZ, 1985). In a given water body, the physical and chemical nature of the iron particulates may be expected to be highly variable, depending on both their mode of formation and the concentration of the major dissolved species present, as well as the pH and the local redox environment. The latter factor is of special importance since it plays a role both in the formation of the oxyhydroxides and in their reductive dissolution leading to the release of all the scavenged species.

Since the early work of MORTIMER (1941, 1942) and HUTCHINSON (1957) many studies have been devoted to understanding both the transformations and the physical and chemical nature of naturally occurring particulate Fe species in lakes, soils and marine systems (WHITFIELD and TURNER, 1987; DAVISON, 1985; DAVISON *et al.*, 1981, 1982; STAUFFER and ARMSTRONG, 1986). However, few studies have systematically investigated both the chemical and physical nature of a large number of individual colloidal-sized Fe lacustrine particles on a microscopic scale (TIPPING *et al.*, 1981, 1982). In our work, the limnological conditions governing the formation of the iron particulates have furthermore been well characterized by using a combination of *in situ* probes and species specific analytical methods such as differential pulse polarography and colorimetry used directly in the field (ZALI, 1983; DE VITRE *et al.*, 1988). Complementary parts of the work reported here have already been published elsewhere (LEPPARD *et al.*, 1988; DE VITRE *et al.*, 1988; BUFFLE *et al.*, 1988), and referencing to the latter articles will be made to avoid lengthy repetition.

II. SITE AND PARTICLE DESCRIPTION

Lake Bret is located 10 km east of Lausanne (Switzerland), at an altitude of 673.5 m. It is a dimictic, highly eutrophic lake with a maximum depth of 20 m; its trophic state is due to an excessive phosphorus load of both agricultural and domestic origin. The principal morphological and hydrological characteristics have been described in detail elsewhere (ZALI, 1983; DE VITRE *et al.*, 1988). Its simple morphology enables fast, easy horizontal mixing, whereas estival thermal stratification leads to a depletion of oxygen in the deeper waters and a gradual build up in the concentration of ferrous iron and sulfidic sulfur (ZALI, 1983; DE VITRE, 1986; DE VITRE *et al.*, 1988). Representative concentrations during the estival season of relevant dissolved species in the epilimnion and the hypolimnion, respectively, are as follows: alkalinity (2.6; 4.0 mM), sulfate (75; 30 μM), sulfide (0; 15 μM), *o*-phosphate (1.0; 3 μM), nitrate (4; 0.1 μM), ammonium (10; 90 μM), calcium (0.9; 1.5 mM) and magnesium (0.3; 0.3 mM).

II.1 Origin and seasonal evolution of the studied particles

The development of seasonal or permanent particulate Fe peaks in natural aquatic systems is a well-known phenomenon and has been observed both in freshwater and seawater systems (DAVISON, 1985). Their occurrence must be expected

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in any system which includes a redox interface where the cycling of iron between its reduced soluble ferrous form and its insoluble oxidized ferric form can take place. In Lake Bret, the redox interface moves up from the sediment/water interface into the water column during the summer season. Consequently, the position of the maximum of the particulate Fe species studied here follows the pattern shown in Fig. 1. Note that the hatched zone in Fig. 1 corresponds to a particulate Fe(II) sulfide species, *i.e.* a species different from the Fe particles in the non-hatched zone (Fe(III/II) hydroxophosphate) which are described below. During the months of July, August and September, a quasi-steady-state situation develops as shown in Fig. 2, although from year to year small temporal and spatial differences may occur. The interfacial redox zone is, in fact, chemically and spatially well resolved (DE VITRE *et al.*, 1988), and the vertical distribution of Mn(II)/Mn(IV), Fe(II)/Fe(III) and S(-II)/S(VI) species has been shown to follow their redox properties.

The role of iron oxyhydroxides as a vehicle of *o*-phosphate in lakes (BACCINI, 1985) is well known. Their close association may be seen in the data shown in Fig. 2. Indeed, the analogy in the shape of the ferrous iron and the total *o*-phosphate profiles is striking. In particular, the fact that in both cases the depth, below which their concentration sharply increases, coincides with the peak of particulate iron strongly suggests that the latter is formed by oxidation of Fe(II) diffusing upwards and also acts as a barrier to the upward diffusion of *o*-PO_4.

The rather well defined, chemical and physical quasi-steady state found in Lake Bret at the O_2/H_2S interface led us to attempt to characterize on a particle specific basis, both chemically and physically, the iron particulate formed *in situ*, since it is likely to be quite representative of a widely spread natural process.

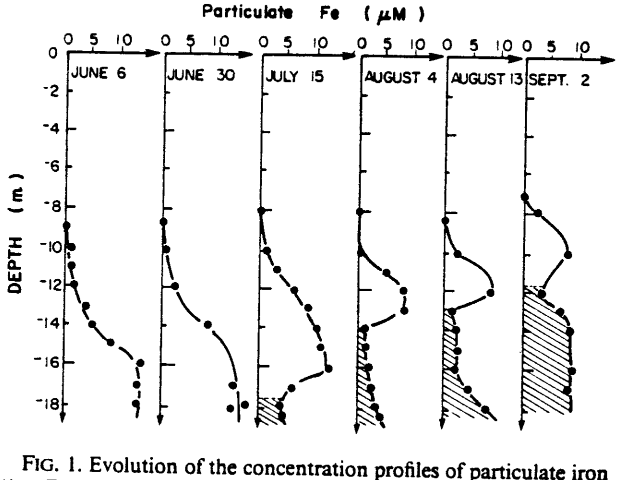

FIG. 1. Evolution of the concentration profiles of particulate iron (*i.e.*, Fe retained on 0.45 μm filters; see section III.2 for discussion) in Lake Bret during the 1981 summer season. Hatched area indicates a particulate ferrous iron sulfide species different from that in the non-hatched zone and which is not discussed in this paper (Data recomputed from ZALI, 1983).

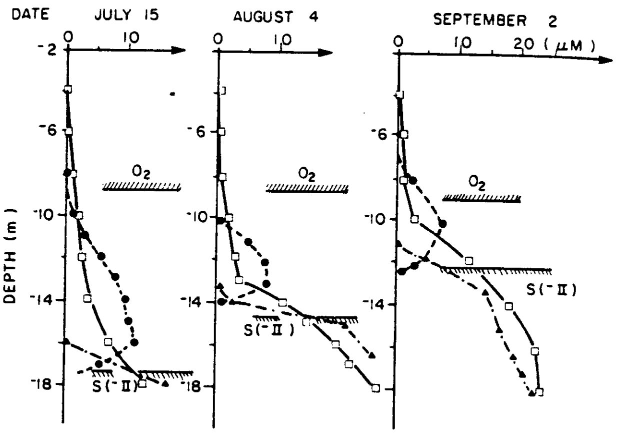

FIG. 2. Concentration depth profiles on three different dates in 1981 for particulate iron (●), reactive ortho-phosphate (□) and electroactive Fe(II) (▲). Oblique bars indicate the higher and lower depths at which O_2 and $S(II)_{tot}$ concentrations are greater than 0.1 μM, respectively.

II.2 Morphological and elemental classification

The morphological and ultrastructural features, as well as some initial chemical information common to the iron-rich fractions isolated from the particulate iron peak in Lake Bret, have already been described in detail elsewhere (LEPPARD *et al.*, 1988). However, for clarity the principal observations made are summarized below:

—the iron-rich colloidal particulates have a globular or roughly spherical shape and a mean diameter less than one-third of a micron, with most being near 0.1 μm;

—morphologically, they could be classified into three basic morphotypes:

a) simple globules with a surface which is not always well demarcated;

b) globules retaining relatively small globulets (7–16 nm) at their surface, as well as small granules of lesser electron opacity having diameters approaching 3 nm;

c) simple globules, as above, attached to and/or bearing a relatively large angular projection (identifiable in some cases as a fragment of diatom frustule) and suspected of being hybrids of two or more colloidal materials;

—chemically, four types of Fe-rich globules were found:

1) globules containing Fe, P and Ca only, 49%
2) globules containing Fe, P, Ca and Si/Al, 23%
3) globules containing Fe, P, Ca and Si, 15%
4) globules containing other combinations of Fe, P, Ca, Si and Al, 13%.

The results presented and discussed here will essentially deal with type (1) globules (*i.e.*, those which contain exclusively Fe, P and Ca). However, as discussed further on, types 2 and 3 are probably associations of type 1 globules with other colloidal lake particles. Types 1, 2 and 3 are hereafter collectively called colloidal iron particles.

III. EXPERIMENTAL

III.1 Methods of analysis

Iron and calcium concentrations were determined by Atomic Absorption Spectrometry (AAS) in both filtered and unfiltered lake water samples acidified upon collection to pH 2. Ferrous iron was also determined in the same samples, colorimetrically using the orthophenanthroline method (APHA, 1980); interference in the determination due to the simultaneous presence of Fe(II) and Fe(III) was checked for and found to be negligible (DE VITRE *et al.*, 1988), provided less than 50 ppm of Fe(III) was present and the analysis is performed within six hours of sampling. Total and dissolved orthophosphate were determined colorimetrically using the molybdenum blue method (APHA, 1980). Transmission electron microscopy (TEM) observations and micrographs were done with a Philips 300 TEM using ultra-thin sections, whereas all energy dispersive spectrometry (EDS) analyses were done on *ca.* 140 nm sections, using a dual stage scanning electron microscope (I.S.I. DE 130) equipped with a scanning transmission electron microscope (STEM) attachment and an energy dispersive X-ray analyser (γ-PGT/System 4). Sample preparation for both TEM and STEM/EDS consisted initially of a sequential double chemical fixation (BURNISON and LEPPARD, 1983), followed by dehydration and embedding in epoxy resin (SPURR, 1969) or by direct embedding (no prior steps) in a water soluble melamine resin (BACHHUBER and FROSCH, 1983). The microtomy was done according to LEPPARD *et al.*, (1977) and all sections were mounted on copper grids. For analyses of the extent of aggregation artifact, a comparative study was done (including EDS analyses) using the acetone fixation procedure of LEPPARD *et al.* (1986) followed by epoxy embedding. A complete description of all the procedures used has already been given elsewhere (LEPPARD *et al.*, 1988).

A Leybold Hereaus LAMMA-500 microprobe (Laser Microprobe Mass Analyser) was also used to obtain qualitative multi-element data on the iron-rich fraction. In this technique, the chemical compounds are fragmented by a laser and the fragments analysed by mass spectrometry. Its interest lies in the fact that qualitative information can be obtained on the nature of the chemical species in which the element of interest is included (DENNEMONT and LANDRY, 1985). Finally, Möessbauer spectroscopy was performed on the particles using a Möessbauer spectrometer of the constant acceleration type equipped with a Canberra 8100 MCA multichannel analyser.

III.2 Sampling

Water samples containing the particulate iron species were removed from the particulate Fe peak using a battery driven peristaltic pump attached to a suitable length of tygon tubing and a counter-weight. All samples were obtained on 25 July, 1985, at a depth of 14 meters. Only samples from this depth and sampling point have been analysed by STEM/EDS on a particle specific basis. However, considering the constant macroscopic chemical composition determined over a three-year period (see section IV.1) they may be considered to be generally representative of the particulate Fe species. Filtered and unfiltered samples were also collected and acidified to pH 2 for AAS analyses. Filtration was performed directly on line with the pump using specially designed Plexiglass filter-holders and 90 mm Schleicher and Schuell cellulose nitrate 0.45 μm filters. It should be stressed that the filtration of natural iron oxyhydroxides can only define operational size fractions, as has been pointed out by a number of workers (MILL, 1980; TIPPING *et al.*, 1982; LAXEN and CHANDLER, 1983; BUFFLE *et al.*, 1988). This proved to be the case for our iron particles, which were found by TEM to be always smaller than one-third of a micron (with at least three-quarters being smaller than 0.13 μm) despite the fact that they were collected on a membrane with a pore size of 0.45 μm. This apparent paradox may be at least partially due to the high flow rates used during "normal" filtration (here *ca.* 300 ml/min) since such rates may induce coagulation due to concentration polarization effects (BUFFLE *et al.*, 1988; BUFFLE, 1988). This effect, however, has no implication for the particle specific results reported here since filtration was used here only as a collection procedure and not to determine a particle size distribution. Both the filtration and particle collection on the filter's surface were performed under N2 in a glove box (LEPPARD *et al.*, 1988).

Sampling for TEM, STEM/EDS, Lamma and Mössbauer analyses was carried out according to the scheme shown in Fig. 3. Details have already been given elsewhere (LEPPARD *et al.*, 1988); however, it should be underlined that several procedures have been used and compared in order to assess the importance of the dehydration step (wet *vs.* freeze-dried samples) and particle fractionation (centrifugation *vs.* filtration) on particle morphology. Both centrifugation and filtration can be used for particle "separation", since at 3000 g, 80% of total Fe is removed from the solution, whereas more than 90% of TOC remains in solution (DE VITRE, 1986). In the case of filtration, previous work had shown that *ca.* 90% of the total organic matter present in Lake Bret waters was smaller than 0.45 μm and therefore passed through such membranes. On the contrary, at the oxic-anoxic interface, most (*ca.* 80–95%) of the Fe is retained.

III.3 STEM/EDS standardization

Peak intensity ratios obtained from good-quality EDS spectra may be considered to be within one order of magnitude of the molar ratios. However, if a more quantitative value is desired, then matrix effects (CHANDLER, 1977) must be accounted for. We have attempted to correct for matrix effects by preparing a series of standards with known P/Fe, P/Ca and Ca/Fe moral ratios. By measuring the corresponding peak intensity ratios, correction factors, *K*, may be computed:

$$K = \text{molar ratio/EDS peak ratio.}$$

EDS measurements were done on 20 individual epoxy embedded particles of each of the four following different standards.

Standard A—was a finely ground anhydrous iron(III) phosphate, pro analysis, from Merck A.G.

Standard B1—was prepared by the hydrolysis of a solution of ferric nitrate (4.5 mM) in Milli-Q water containing $CaCl_2$ (0.18 mM) by a slow addition (200 μl/min) of HCO_3^- to form a soluble polynuclear stable Fe(III) species (NEMBRINI, 1977), followed by the slow addition of a solution of NaH_2PO_4 0.05 M, in order to have a final Fe/H_2PO_4 ratio of 2. Finally, the solution was neutralized to pH = 7 using $NaHCO_3$. A precipitate was formed on the addition of ortho-phosphate and neutralization.

Standard B2—was prepared in a similar way as standard B1 above, except that no calcium chloride was added to the initial solution of ferric nitrate.

Standard C—was prepared by the slow oxidation by air of a solution of ferrous ammonium sulfate (0.002 M) in artificial lake water (prepared using Milli-Q water filtered through a 0.2 μm filter and by adding 0.5 mM NaH_2PO_4, 0.5 mM $CaCl_2$ and 0.5 mM $NaHCO_3$). The ferrous iron was rapidly added to a stirred solution of completely N_2 degassed artificial lake water at pH 7. The flow of N_2 was then stopped and the solution allowed to equilibrate with the atmosphere for 24 hours at constant pH.

The results obtained and the computed values for *K* are given in Table 1. Based on the morphological and dimensional features (see Fig. 4) and the macroscopical chemical composition determined by analysis, standard C is the closest to the natural particles and its *K* values were therefore used to convert peak ratios to molar ratios for the natural samples.

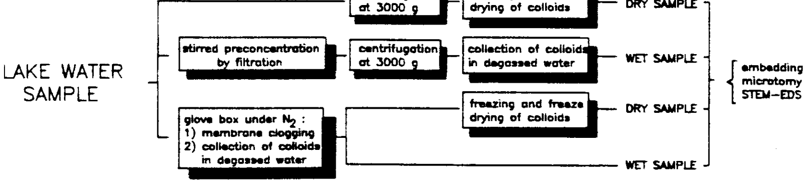

FIG. 3. Sampling procedure used to isolate the iron-rich particles from Lake Bret (adapted from LEPPARD *et al.*, 1988).

TABLE 1. STEM/EDS standardisation data for the conversion of peak height ratios to molar ratios.

Standard type/ mode of preparation (see sec. III.3)	Morphology	P/Fe molar ratio	P/Fe K	P/Ca molar ratio	P/Ca K	Ca/Fe molar ratio	Ca/Fe K
A FePO$_4$ pro analysi	-----	1.0	0.38				
B1 Hydrolysis of ferric nitrate	large particles > 1 micron seen as angular particles with irregular shapes	0.23	0.38	6.46	0.75	0.04	0.29
B2 Hydrolysis of ferric nitrate	-----	0.31	0.41				
C Oxidation of ferrous iron	small particles ca. 0.1 micron seen as crude globules arranged in linear arrays	0.50	0.35	1.89	0.77	0.26	0.46

IV. RESULTS

Below are reported two types of experimental data. The first type, called hereafter "macroscopic data", includes any data obtained by applying a classical analytical method to the water sample containing the studied iron particles. Even if the sampling procedure, as here, is designed to minimize heterogeneity in the collected material (*e.g.*, collection in a well-defined water stratum followed by fractionation before analysis, for instance by filtering through successive membranes with decreasing pore size and retaining a particular fraction), the data obtained in this way are global because the analysed material can never be totally "purified" and may include many types of iron particles differing in their physical and/or chemical composition. The second type of experimental data reported here is called "microscopic data" and refers to measurements made by (1) EDS coupled to STEM and (2) by conventional TEM. In these cases, elemental and size analyses are made on particles which are specifically selected by a TEM image because they possess the same characteristics. This ensures that the microscopic data are collected from particles belonging to a well-defined physico-chemical group. The macroscopic and microscopic data are complementary. Indeed, information on the oxidation state or the species distribution of a particular element cannot be obtained by STEM/EDS, whereas an EM-based technique is the only way to get realistic values of other properties such as a size distribution (BUFFLE *et al.*, 1988; LEPPARD *et al.*, 1988). Furthermore, comparison of macroscopic and microscopic data enables assessment of the "purification" yield of the sampling and fractionation procedures used before analysis. The combination of these two types of data is therefore a useful tool for the interpretation of particle characteristics, and this is the reason why they are reported together here.

IV.1 Macroscopic data

As we shall see from the EDS results, the major elemental components of the iron particles are Fe, P and Ca (probably in addition to OH, not visible by EDS). The macroscopic data below, therefore, report the evolutions of the particulate Fe(II)/Fe(III) and PO$_4$/Fe ratios with depth, time and size. The particulate Ca/Fe ratio could not be measured by classical methods, because the concentration of Ca in iron particles is very low compared to that of dissolved Ca^{2+} or of CaCO$_3$ particles. The magnitude of the errors incurred when trying to measure specifically the Ca of Fe particles, is consequently too great.

IV.1.1 Fe(II)/Fe(III) ratio

The iron particles in Lake Bret, during summer 1985 were found to contain both Fe(II) and Fe(III) (DE VITRE *et al.*, 1988), with a ratio of particulate (Fe(II) ($|Fe^{II}|_p$) over total particulate iron ($|Fe|_p$) close to 50%. Mössbauer spectrometry has shown that Fe(II) is indeed present in the iron-rich samples collected and, furthermore, that these iron particles are amorphous, since no detailed structure in the spectra could be found even at 4°K (MOREAU, unpublished results). Also, no evidence of crystallinity could be found at 1 nm resolution by TEM.

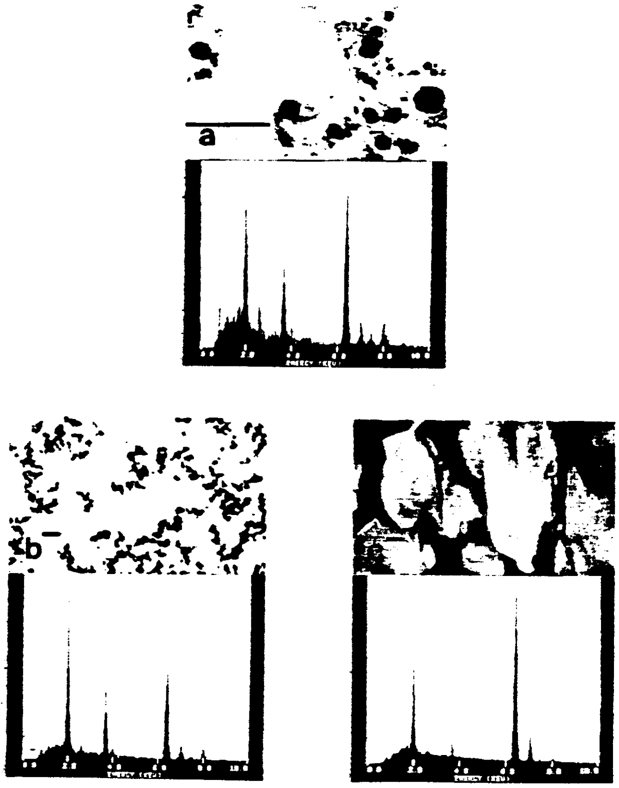

FIG. 4. Electron micrographs and STEM/EDS spectra of lacustrine iron particles and laboratory prepared standards. The scale bars are 0.45 μm. All spectra refer to an individual particle selected from a group. The peaks are: Fe at 6.4 keV; P at 2.0 keV; Ca at 3.7 keV. a) Natural globular colloids of Fe/P/Ca and a representative type 1 spectrum. This micrograph has been enlarged to show the indistinct surface and the size range of the globules. b) Artificial Fe/P/Ca colloids representing standard C. c) Artificial Fe/P/Ca particles representing standard B1. This micrograph is printed with negative contrast to reveal the angular surface of the particles, a feature not found in natural samples of the most common morphotype or in standard C.

An important feature of the particulate Fe(II)/Fe ratio ($|Fe^{II}|_p/|Fe|_p$) is its constancy (DE VITRE *et al.*, 1988). It was, for instance, found to be constant in the various fractions obtained by filtration through membranes with different pore size. Despite all the secondary problems related to filtration (BUFFLE *et al.*, 1988), this suggests that it is independent of particle size. The results of summer 1985 also showed (DE VITRE *et al.*, 1988) that it is independent of depth and of the seasonal evolution of the lake. This is shown in even more detail in Fig. 5, which shows that this ratio remains constant and close to 50% over three years. Note that the points in Fig. 5 are averages of a large number of values measured at various sampling depths. The indicated variabilities correspond to variabilities with depth. The constancy of $|Fe^{II}|_p/|Fe|_p$ in Fig. 5 is particularly striking when it is compared to the fact that the development of anoxic conditions arose later and later in 1985, 1986 and 1987, as shown, for instance, by the longer time necessary to reach a given concentration of dissolved Fe(II) or S(-II) at a particular depth. In this respect the lake evolution was late, in 1987, by about one month compared to 1985 (PERRET, unpublished results). Figure 6 shows the correlation between particulate Fe(II) and total particulate iron, for all depths and dates in 1985, 1986 and 1987. The slope gives a value of 0.49 ± 0.03 for $|Fe^{II}|_p/|Fe|_p$. The remarkable constancy of this ratio, and in particular the fact that it is independent of the redox conditions (since it does not depend either on depth or seasonal evolution), suggests that the iron particles are formed of chemical compounds with a relatively well-defined stoichiometry.

IV.1.2 Iron/phosphate ratio

LAMMA measurements have been performed on particles collected at the optimum depth and in the optimum size fraction (BUFFLE *et al.*, 1988) to get samples as enriched as possible in iron particles compared to other aquatic particles. These data have shown that the particulate phosphorus is ortho-phosphate (the sum of all protonated and non-protonated ortho-phosphate is denoted below by PO_4 without mention of charge). The fact that the iron particles include PO_4 is in agreement with the limnological behaviour of particulate iron and dissolved PO_4 suggested by the shape and evolution of their concentration profiles (Fig. 2).

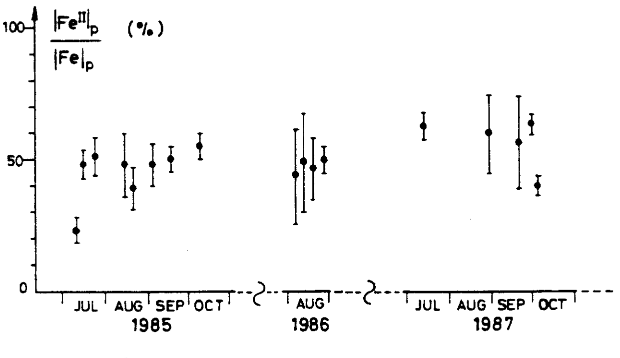

FIG. 5. $|Fe^{II}|_p/|Fe|_p$ ratio determined macroscopically over a three-year period (1985–87). Each point is the average of a number of values measured at various depths. Bars show variability with depth.

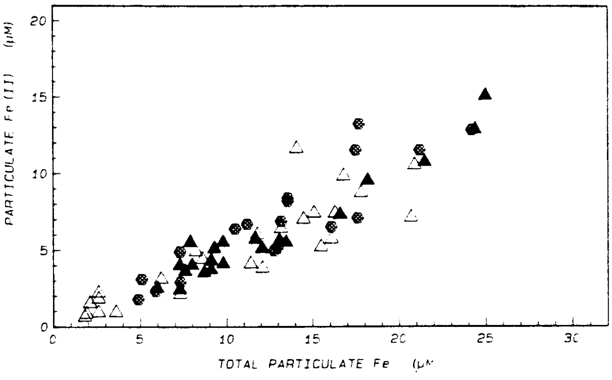

FIG. 6. Particulate Fe(II) ($|Fe^{II}|_p$) as a function of total particulate Fe, $|Fe|_p$. Data from 1985 (△), 1986 (▲) and 1987 (●). From least square regression: slope = 0.49 ± 0.03; ordinate at origin = −0.21 ± 0.4; correlation coefficient = 0.93; number of points = 64.

Figure 7 shows particulate PO_4/Fe ratios ($|PO_4|_p/|Fe|_p$) determined using macroscopic measurements from samples taken from the particulate iron peak, at various dates in 1986 and 1987. The figure clearly shows that this ratio is independent of seasonal changes within experimental error. The data in Figs. 7 and 8 furthermore show that the ratio is independent of depth within the particulate iron peak. This was also confirmed by the data collected in 1987. It should be noted, however, that $|PO_4|_p/|Fe|_p$ varies in the oxic, epilimnetic layer of the lake, but this is probably related to phosphate assimilation by organisms and reflects a completely different process than that studied here.

Our results, therefore, show that $|PO_4|_p/|Fe|_p$ is independent of depth and seasonal evolution. The correlation between the whole of the data set of particulate PO_4 and Fe is shown in Fig. 9. The slope gives the value of 0.22 ± 0.02 for the molar ratio PO_4/Fe in the particles. Interestingly, the same remark made for $|Fe^{II}|_p/|Fe|_p$ can be made for $|PO_4|_p/|Fe|_p$ which is independent of depth and time, despite large changes in concentrations of particulate iron and dissolved phosphate, suggesting that these particles possess a relatively well-defined chemical composition.

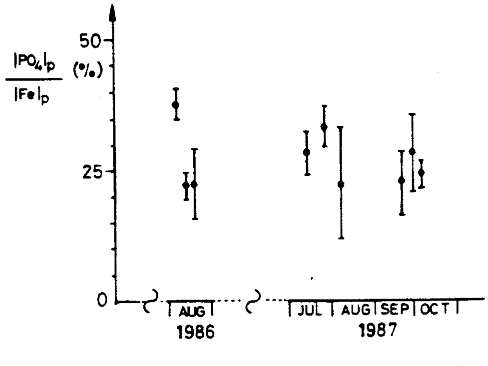

FIG. 7. $|PO_4|_p/|Fe|_p$ ratio in the iron particles determined macroscopically over a two year period (1986–87). Each point is the average of a number of values measured at various depths. Bars show variability with depth.

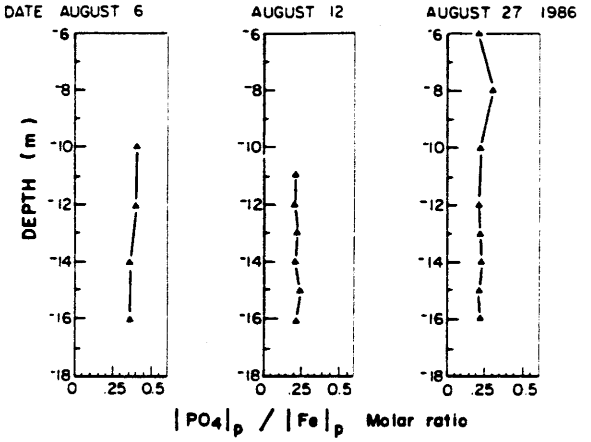

FIG. 8. Depth profiles of $|PO_4|_p/|Fe|_p$ molar ratio determined macroscopically on three different sampling dates in 1986.

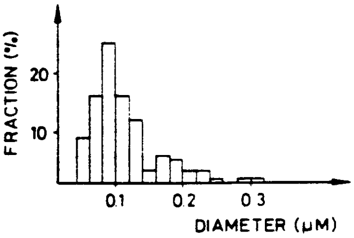

FIG. 10. Size distribution determined by direct measurement on 122 individual iron particles isolated from Lake Bret, on the 25th July 1985 at a depth of 14 meters.

IV.2 Microscopic data (TEM and STEM/EDS)

By means of TEM, the various types of particles can be classified on the basis of their morphology, size and electron opacity. It has been shown (Leppard *et al.*, 1988) that this enables one to identify relatively easily the iron particles formed in the oxic-anoxic boundary layer thanks to their globular shape, their colloidal size and their granular but overall amorphous appearance, as well as their high electron opacity. It is therefore possible to determine, for these specific particles, i) the size distribution by measuring and counting a large number of these particles on TEM photographs, and ii) the elemental composition and the corresponding distributions for a large number of particles, by using EDS coupled to STEM, with a beam of 330 nm diameter focused on single embedded particles within a section of *ca.* 140 nm thickness. This was done for 122 particles and the results are reported below.

IV.2.1 Size distribution

The size distribution of 122 particles is shown in Fig. 10. The severe dropoff in size from the mean at about 0.1 µm may be related to sampling procedure and not to a real feature of the actual distribution curve, a feature amenable to future investigation. Indeed, TEM observations have been made on particles obtained by filtration on membrane filters with a pore size of 0.45 µm. It is therefore likely that very small particles may have been lost during this procedure. Nevertheless it is striking that the diameter of the measured iron particles is never larger than *ca.* one-third of a micron. It has been shown (Buffle *et al.*, 1988; see section III.2) that this discrepancy between TEM and filtration results is largely due to a coagulation process which occurs at the membrane's surface during filtration, leading to retention of particles smaller than the pore size. Due to this problem, the size distribution measured by TEM is closer to the actual distribution than that obtained by cascade filtration on membranes with various pore sizes, provided sufficient care is taken (Leppard *et al.*, 1988) to avoid artefacts which may be produced when preparing the samples for TEM observations. A comparative analysis of the various fixation, dehydration and embedding procedures revealed that, while many of the other colloid types showed a sizing and a morphology dependent on the mode of preparation, the iron-rich globules were always revealed in a similar manner.

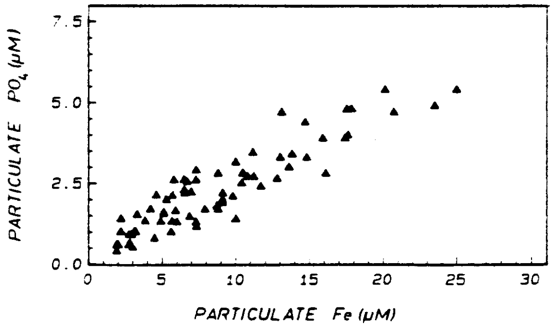

FIG. 9. Particulate PO_4 as a function of particulate Fe, determined macroscopically on all sampling dates and depths in 1986 and 1987. From least square regression: slope = 0.22 ± 0.01; ordinate at origin = 0.42 ± 0.13; correlation coefficient = 0.91; number of points = 71.

When taking into account the above considerations, the most important features of Fig. 10 are that i) the size of at least three-quarters of the iron particles is smaller than 0.13 µm, and that ii) the frequency (%) of a given particle size class is inversely proportional to the particle size. As is discussed in the conclusion, this last point may be related to the nature of the elimination process of the particles and could consequently have implications on the cycling of trace pollutants scavenged by the iron particles.

IV.2.2 Elemental composition

IV.2.2.1 Discriminating between the different types of iron containing particles. It has been shown (Leppard *et al.*, 1988) that the colloidal particles (which are classified as "iron particles" according to morphological criteria) contain, as major elements, Fe, P and Ca, with some of them also containing some Si and Al; and, an exceptionally weak S peak was also found. No other element could be found in significant amounts by EDS (in particular no Mn), the absence of the latter element being also confirmed by LAMMA microprobe analysis (De Vitre, 1986). Based on their elemental composition, it has been proposed (Leppard *et al.*, 1988) that the "iron particles" can be classified qualitatively into the four chemical types given in section II.2.

In order to measure parameters corresponding to a group of particles as homogeneous as possible, it is important to know more precisely whether or not these four chemical classes of particles are really different, and, therefore, whether or not Si and Al are inadvertently associated with the other three elements, as opposed to being an integral component of an Fe/P/Ca particle. Figure 11a–c shows the very good correlations existing between Fe, P and Ca, for type 1 particles with slopes of 1.41 (±0.09), 0.30 (±0.02) and 0.45 (±0.04) for P/Ca, Ca/Fe and P/Fe, respectively. The rather large values of the correlation coefficients in the three cases suggest (as was the case with the results of section IV.1) that the above peak ratios correspond to the composition of a well-defined chemical compound (or association of chemical compounds) for the "iron particle".

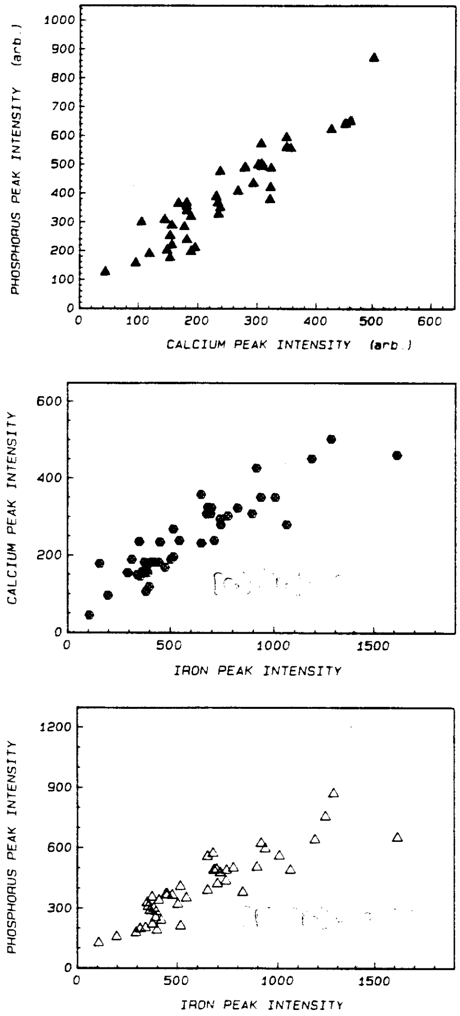

FIG. 11. Correlations between peak intensities of Fe, P and Ca, in iron particles of type 1 (section II.2). All scales in arbitrary units. Slopes (*s*), ordinate at origin (y_0) and correlation coefficients (*r*), computed by least square regressions, are given in each case, with the number of points, *N*. a) Phosphorus *versus* calcium peak intensities. $s = 1.41 \pm 0.09$; $y_0 = 41.9 \pm 24.6$; $r = 0.92$; $N = 41$. b) Calcium *versus* iron peak intensities. $s = 0.30 \pm 0.02$; $y_0 = 65.1 \pm 15.1$; $r = 0.91$; $N = 42$. c) Phosphorus *versus* iron peak intensities. $s = 0.45 \pm 0.04$; $y_0 = 113.8 \pm 27.0$; $r = 0.87$; $N = 43$.

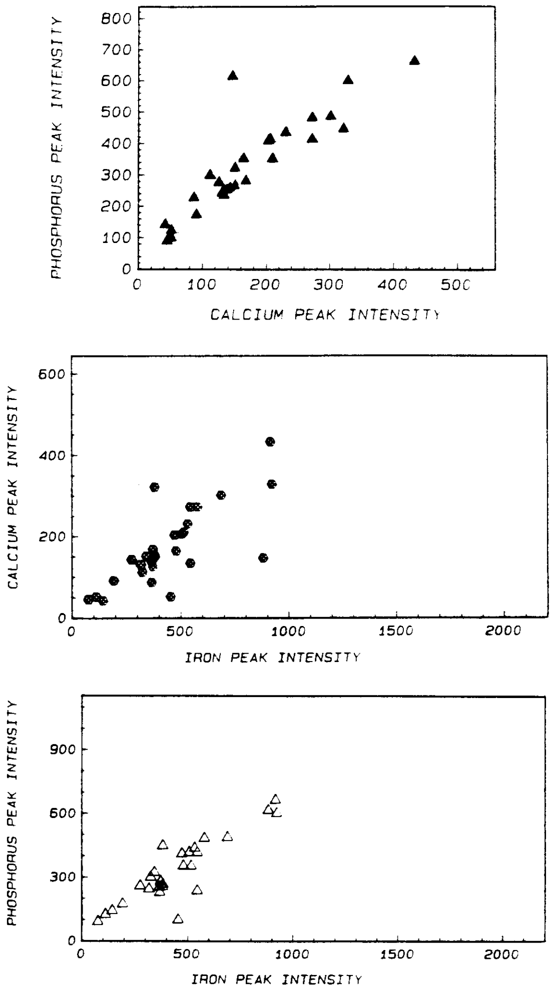

FIG. 12. Correlation between peak intensities of Fe, P and Ca in iron particles of types 2 + 3 (*i.e.*, those containing Si or Si + Al, section II.2). Scales in arbitrary units *s*, y_0, *r* and *N* (see Fig. 11). a) Phosphorus *versus* calcium peak intensities. $s = 1.39 \pm 0.15$; $y_0 = 89.1 \pm 30.7$; $r = 0.87$; $N = 27$. b) Calcium *versus* iron peak intensities. $s = 0.33 \pm 0.06$; $y_0 = 26.4 \pm 29.0$; $r = 0.75$; $N = 27$. c) Phosphorus *versus* iron peak intensities. $s = 0.62 \pm 0.07$; $y_0 = 53.4 \pm 32.3$.

Figure 12a–c shows the same correlations for types 2 and 3 (*i.e.*, those also containing Si and Al). Although the dispersion is larger, similar correlations are observed for the three principal elements. The corresponding slopes give values of 1.39 (±0.08), 0.33 (±0.06) and 0.62 (±0.07) for the ratios P/Ca, Ca/Fe and P/Fe, respectively. If one takes into account the relatively large statistical errors, these values can be considered as being close to those obtained with type 1 particles (Fig. 11).

Figure 13a–c shows the relationships between Fe, Al and Si in particle types 2 and 3. It is seen that Al and Si are correlated together, whereas neither Si nor Al are well correlated to Fe. This suggests that Si and Al may be mostly the constituents of small clay particles which are included in, or are adhering to, the "iron particles" but which do not con-

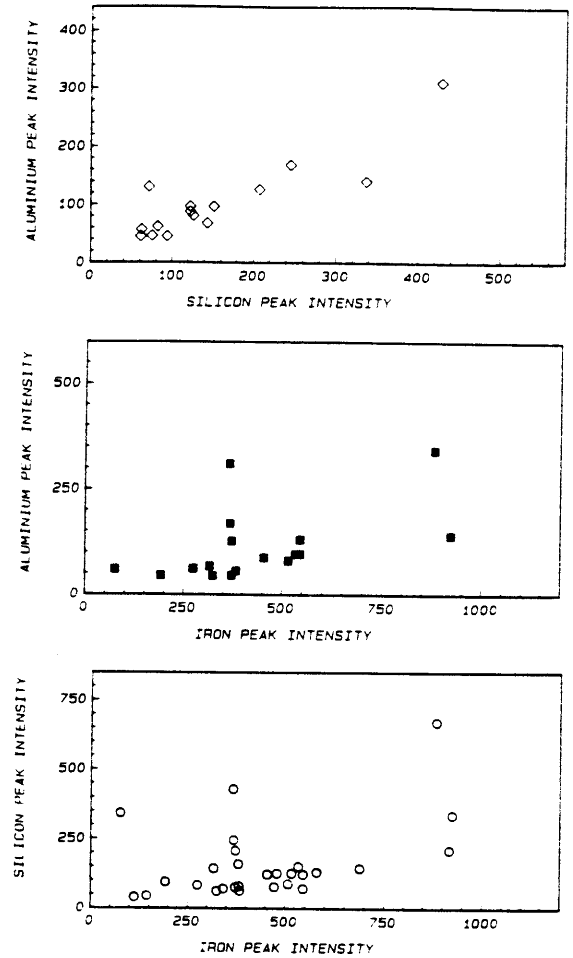

FIG. 13. Correlation between peak intensities of Fe, Al and Si in iron particles of types 2 + 3 (see Fig. 12). a) Aluminum *versus* silicon peak intensities. $s = 0.56 \pm 0.08$; $y_0 = 15.9 \pm 15.6$; $r = 0.88$; $N = 15$. b) Aluminum *versus* iron peak intensities. c) Silicon *versus* iron peak intensities.

tribute to their structure except in an incidental manner. This is confirmed by the peak ratio of Al/Si (0.56 ± 0.08) which corresponds to the order of magnitude of the molar ratio in clays. The absence of correlation between Al and Fe on the one hand, and Si and Fe on the other, suggests that even when the iron particles contain or are associated with such clay inclusions, the proportion of iron due to that clay inclusion is small compared to the total iron in the particle.

Overall, these results suggest that the iron particles have a relatively well-defined composition including Fe, P, Ca and probably OH, but not Si or Al. When these elements are present, they are probably relatively small clay colloids of varying size which adhere to the surface of an iron particle or which may even have served as a nucleus for the formation of an iron particle. Ultra-structural evidence supports both possibilities, but neither the superficial adhering entities nor the included ones documented thus far can be identified with certainty as clay. In the case of iron particles having large Si peaks without Al, there were some examples of a globule associated with a fragment of diatom frustule.

IV.2.2.2 Fe/P/Ca ratios of the iron particles. Because of the last observations, all the results obtained with particle types 1, 2 and 3 were pooled together to compute the mean P/Ca, Ca/Fe and P/Fe peak ratios and their corresponding molar ratios. The computed results are given in Table 2. The frequency distribution of these ratios (Fig. 14a–c) shows that they follow relatively closely Gaussian distributions. Type 4 is not included in this analysis because recent intensive ultrastructural examinations (LEPPARD, unpublished results) indicate that it represents a heterogeneous aggregate of small colloids of varying kinds and proportions.

CONCLUSIONS

Despite the relatively well-characterized composition of the iron particles studied here, more work is required to know the exact nature of the chemical compounds which compose them. The present data show that the chemical composition is Fe(II)/Fe(III)/PO_4/Ca(II) in the following ratio: 2.5/2.5/1.25/1.0. The OH content is at present not known. There is, on occasion, an indication of an S peak, and this could be examined in detail by EDS through the use of extended irradiation times. Carbon-rich anions such as carbonate might also be present, but neither C nor O was detectable with the STEM/EDS system we used. Our results cannot be easily compared with existing data in the literature, since i) results on particle specific analysis are very scarce; ii) many investigations have been done on iron particles originating from sediments and soils and not from the water column; and iii) the particle composition might depend on environmental conditions such as the degree of anoxia, pH or the geological nature of the lake's watershed. A detailed comparison should take all these factors into account; this is not an easy task and beyond the scope of this paper. The following remarks can however be made. Most of the data in the literature, obtained by macroscopic chemical analyses, report a minimum value of approximately 2 for the Fe/P ratio (*e.g.* MANNING *et al.*, 1984; TESSENOV, 1974; MAYER *et al.*, 1982). Our results ((Fe^{II} + Fe^{III})/P = 4) are in general agreement with these findings. TIPPING *et al.* (1981) have published both EDS and macroscopic data on the chemical composition of iron particles isolated from the water column of a seasonally anoxic lake. They found a macroscopic Fe/P ratio ranging from *ca.* 8–18 possibly reflecting differences in the limnological conditions between Lake Bret and Estwaite Water. In this latter case, Ca was also found in the particles, but their shape (oblong) was rather different from ours (close to spherical). As far as the Fe^{II}/Fe^{III} ratio is concerned, our data can only be compared to the Moessbauer results obtained by DAVISON

TABLE 2. Chemical composition of the iron rich colloids based on microscopic particle specific analysis by EDS, N = 71 (S.D. = standard deviation, C.V. = coefficient of variation).

Elemental ratio	P/Fe	Ca/Fe	P/Ca
Mean molar ratio	0.25	0.19	1.34
Sample S.D.	0.06	0.05	0.32
C.V. (%)	24.4	23.7	23.8

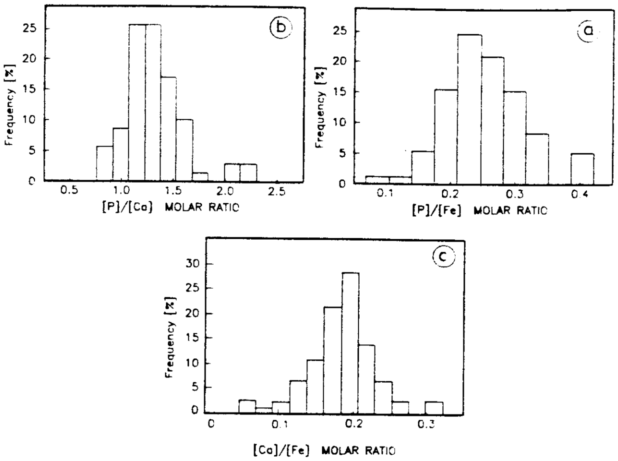

FIG. 14. Frequency histograms for the (a) P/Fe, (b) P/Ca and (c) Ca/Fe peak molar ratios measured on 71 single particles.

and DICKSON (1984) on sediment particles. They found proportions of Fe(II) ranging from 18 to 60% of total Fe. But part of this Fe(II) was probably included as pyrite, which is not the case in our particles.

The presence of PO_4 could be due to vivianite, $FePO_4$, or apatite (although, in view of the values of the molar ratios, Ca alone is not sufficient to explain the content of PO_4 in the iron particles) or to PO_4 adsorbed on a mixed-valency Fe(II)-Fe(III) hydroxide particle. This latter situation is however not very likely because of the probable unstable nature of such a Fe(II)-Fe(III) hydroxide (SCHNEIDER, pers. commun.) and because of the high and remarkably constant value observed for the P/Fe ratio.

In our opinion, the most likely formation process for the iron particles is the very slow oxidation of Fe(II) into Fe(III) resulting in a mixed-valency hydroxophosphate of Fe(II/III), stabilized by the presence of phosphate. At pH 7.5, such a compound could be negatively charged and Ca^{2+} would serve as counter-ions. Note that the iron particles reported here have a granular substructure with a grain size of a few nanometers, with a minimum value as small as *ca.* 1 nm. This can be compared with the findings of SCHNEIDER and SCHWYN (1987) who found that the oxidation of Fe(II) by O_2 at neutral pH leads to iron hydroxides formed as aggregates (5–7 nm in diameter at pH 7–8) of hexameric basic "building blocks" with an individual diameter of 2 nm. In laboratory conditions, the oxidation of Fe(II) by low concentrations of O_2 is generally accelerated by the presence of "dust" particles which can serve as a heterogeneous nuclei (SCHNEIDER, pers. commun.). The clay-like inclusions noted here, as well as some other lacustrine colloids, could play that role. Although the purpose of this paper is not to address the question of the formation mechanism of Fe particles but only their composition, it is interesting to note that particles very similar to lake ones may be synthetized in the laboratory, but in lake conditions, in a solution sterilized by filtration through 0.2 μm, within a few hours. The fact that the oxidation process is rather fast despite a low O_2 concentration (0.1–1.0 mg/l) is not surprising in view of the relatively high pH (7.5) and the fact that Fe(II) oxidation has been reported to be accelerated in presence of phosphate (SARIKAYA, 1979; SUNG and MORGAN, 1980). In any case, this fast chemical oxidation suggests that bacterial mediation is not a necessary requirement for Fe particle formation.

The constancy of the Fe/P/Ca and Fe(II)/Fe(III) ratios with depth and time is a very interesting feature of the studied particles. As mentioned above, this could suggest that these particles are composed of one well-defined chemical entity. One must also realize, however, that these particles being very small, their transport by eddy diffusion in all directions may be much faster than their settling rate. This could result in a "mixing" process, inside the iron particle-rich layer leading to a relatively constant average elemental composition within this layer.

Finally, the size distribution of the iron particles (see Fig. 10) seems to follow the Pareto law (LERMAN, 1979), which might reflect the fact that the major elimination process of these particles is their perikinetic coagulation (BUFFLE *et al.*, 1988). In any case, the large preponderance of small particles compared to large ones suggests that the former may play a major role in the cycling of compounds or elements which are scavenged by the iron particles. Because the smallest particles sediment very slowly, the observed size distribution could mean that the scavenging of trace compounds by the iron particles could help maintain the latter compounds in the water column rather than to carry them away to the sediments. More information, however, is needed for a definite conclusion, in particular about the exact nature of the scavenging process and the mode of particle elimination. The possible association of natural organic matter (NOM) with the iron particles is another important point to be elucidated to understand both the cycling processes of NOM and the behaviour of iron particles. Indeed, it has been shown (*e.g.* TIPPING, 1981; TIPPING and COOKE, 1982) that adsorption of NOM on the Fe particles may strongly influence their surface charge and thereby affect their stability and reactivity. However, under the conditions of Lake Bret (BUFFLE *et al.*, 1982), only a few percent of NOM is adsorbed by Fe particles, which prevents its unambiguous determination from the difference between total and filtered NOM, due to experimental errors. Only particle specific analysis of organic C (as for Fe, P and Ca) would enable unambiguous conclusions to be drawn about Fe particle-NOM association. Unfortunately, the necessary equipment was not available for the present work.

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