

# The influence of colloidal organic matter on iron and iron-phosphorus cycling in an acid bog lake<sup>1</sup>

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## Abstract

The relationship between iron and phosphorus and the relationship of these elements to colloidal organic matter (COM) was studied in a meromictic acid bog lake by chemical characterization, filtration and in situ dialysis, and in situ experiments with labeled components.

Bathophenanthroline (BPN) reactive ferrous iron existed in true solution (dialyzable) in constantly anaerobic monimolimnetic water; in the aerobic epilimnion reactive ferric iron was of colloidal size. Intermediate depths, oxygenated only during spring, contained two forms of ferrous iron reactive to BPN: dialyzable ferrous iron ( $\text{Fe}^{2+}$ ) and colloidal  $\text{Fe}(\text{II})$  which may have originated through in situ reduction of colloidal reactive ferric iron and may be present as a  $\text{COM-Fe}^{2+}$  complex.

The amount of COM influenced the fate of dialyzable  $\text{Fe}^{2+}$  (92% of total iron) and dialyzable  $\text{PO}_4\text{-P}$  (85% of total phosphorus) present in anaerobic strata before aeration. Aeration with soluble organic matter present (COM absent) resulted in the formation of colloidal nonreactive ferric iron (84% of total iron) and 65% colloidal  $\text{PO}_4\text{-P}$ , while aeration with 26 mg liter<sup>-1</sup> COM resulted in only 36% colloidal, nonreactive ferric iron and 19% colloidal  $\text{PO}_4\text{-P}$ . COM apparently masks the cationic properties of colloidal ferric iron and retards the formation of nonreactive  $\text{Fe}(\text{III})$ , allowing most of the  $\text{PO}_4\text{-P}$  to remain free in solution and biologically available.

The organic acids found in natural waters may alter the cycle of biologically active metals by means of various complexation reactions (covalent or electrostatic). Iron and phosphorus both accumulate in the anaerobic zones of stratified lakes and are recycled in the trophic waters during overturns. During cycling they may undergo a variety of changes in physical and chemical states so as to become associated with each other as complexes or precipitates (Hutchinson 1957) and both may become associated in one way or another with colloidal as well as larger organic particles.

Precipitation of inorganic phosphate by iron has been reported frequently (Stumm and Morgan 1970) and is believed to limit the availability of phosphorus in trophogenic water, thereby reducing primary productivity (Hutchinson 1941; Mortimer 1941). However, the formation of complexes between iron and organic matter in

natural waters has been suggested as the way iron is maintained in solution within aerated zones (Shapiro 1964) and thereby as a means of sustaining primary production (Schelske et al. 1962). Schindler et al. (1972) presented evidence indicating the absence of ionic iron even in anaerobic lake sediments. Thus, through complexation reactions, organic matter may play an important role in lake productivity by masking the cationic properties of iron, thereby reducing the sorption and precipitation of phosphate.

Here we describe the influence of colloidal yellow organic matter (COM) on the association between iron and phosphorus in an acid bog lake and point out the indirect consequences of this interaction on productivity.

To test the hypothesis that COM may influence productivity through its effect on the interaction between iron, phosphorus, and organic matter and to clarify the coordination states between these elements under varying conditions of aeration, we have studied North Gate Lake, a highly acid bog lake in Gogebic County, Michigan (T45N, R42W), by a combination of in situ

<sup>1</sup> Supported by U.S. Atomic Energy Commission contract AT(11-1)-1771.

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and laboratory techniques. Other limnological features of the lake and details of the phosphorus cycles have been described elsewhere (Hooper unpublished progress rep.; Hooper and Imes 1972; Koenings and Hooper 1973).

We thank R. E. Gordon for providing facilities and use of the Notre Dame tract. Many graduate students at the University of Michigan assisted with this work, including E. Brady and K. Kurko. We also thank P. Kilham and J. Elder for critically reading the manuscript.

### Methods

Particulate and colloidal forms of iron and phosphorus were mechanically separated from water by filtration through a 0.45- $\mu$  membrane and by dialysis, giving three size fractions: materials held by a 0.45- $\mu$  membrane (here referred to as particulate); suspended materials passing through a membrane but not through 4.8-nm dialysis tubing (termed colloidal); and dialyzable substances (termed dissolved). Iron particles range from <0.01  $\mu$  to >15  $\mu$ , and average 1–2  $\mu$ . Hence filtration through a 0.45- $\mu$  membrane does not separate dissolved from suspended iron, but this separation can be made by dialysis. The physical classification scheme we use is similar to that given by Salutsky (1959).

In situ dialysis of anaerobic lake water was accomplished by suspending dialysis bags filled with anaerobic distilled water in the lake for 5 days. The cellulose dialysis bags used had a pore size of 4.8 nm or a molecular weight cutoff (for proteins) of 12,000. They were soaked in dilute HCl acid for 24 h and then rinsed with distilled water to remove contaminants. To define suitable conditions for quantitative dialysis of dissolved iron, 400  $\mu\text{g liter}^{-1}$  of  $\text{FeCl}_3$  was added to a series of dialysis bags containing oxygen-free water and the bags were suspended in laboratory containers in light at 20°C and in the dark at 5°C. After 5 days, iron concentrations inside and outside the bags were not significantly different and 98% or more of the added iron was recovered. Solutions containing known

amounts of orthophosphate ( $\text{H}_3\text{PO}_4$ ),  $^{59}\text{Fe}$  ( $\text{FeCl}_3$ ), and  $^{32}\text{P}$  ( $\text{H}_2\text{PO}_4^-$ ) were treated in the same manner with identical results. From these tests, 5 days was judged to be adequate for quantitative dialysis.

Dowex 1-8X, 100-50 mesh anion exchange resin in combination with either formic acid-ammonium formate or acetic acid-sodium acetate gradient elution systems was used both to separate and identify orthophosphate from other phosphorus-containing compounds and to determine recoveries of phosphate from the experimental waters. The gradient elution system consisted of 0.0 M to 3.0 M acid + 3 M salt (2:1 v/v). The resin was changed from its supplied chloride form to the desired form by washing the column with 1 N NaOH and neutralizing with the appropriate 1 N acid. Columns were 0.9  $\times$  7 cm and were eluted at 1.0 ml  $\text{min}^{-1}$  with 15-ml fractions collected on an automatic fraction collector. Reactive and total phosphorus measurements were made in duplicate on each fraction after Strickland and Parsons (1972).

With this system phosphate could be separated from other forms of phosphorus, and, by comparison to the position on the elution curve of known orthophosphate, the relationship between soluble reactive and orthophosphate in the water could be determined. Since the colloidal organic acids adsorbed irreversibly to the column (with any sorbed iron), the effect of this material on phosphate recovery could also be determined.

Bathophenanthroline (BPN) was used to measure ferrous iron. BPN reacts only with  $\text{Fe}^{2+}$  to give a colored complex that can be extracted by *n*-hexyl alcohol at pH 4 (Smith et al. 1952). Koenings (1976) described the iron characterization in detail.

An operational classification, based on the reactivity of standard iron solutions, was used to compare the predominant type of iron found in natural and experimentally treated waters. In summary, the classification of natural lake iron was as follows. Reactive ferric iron present in the colloidal state is primarily (not exclusively) as a

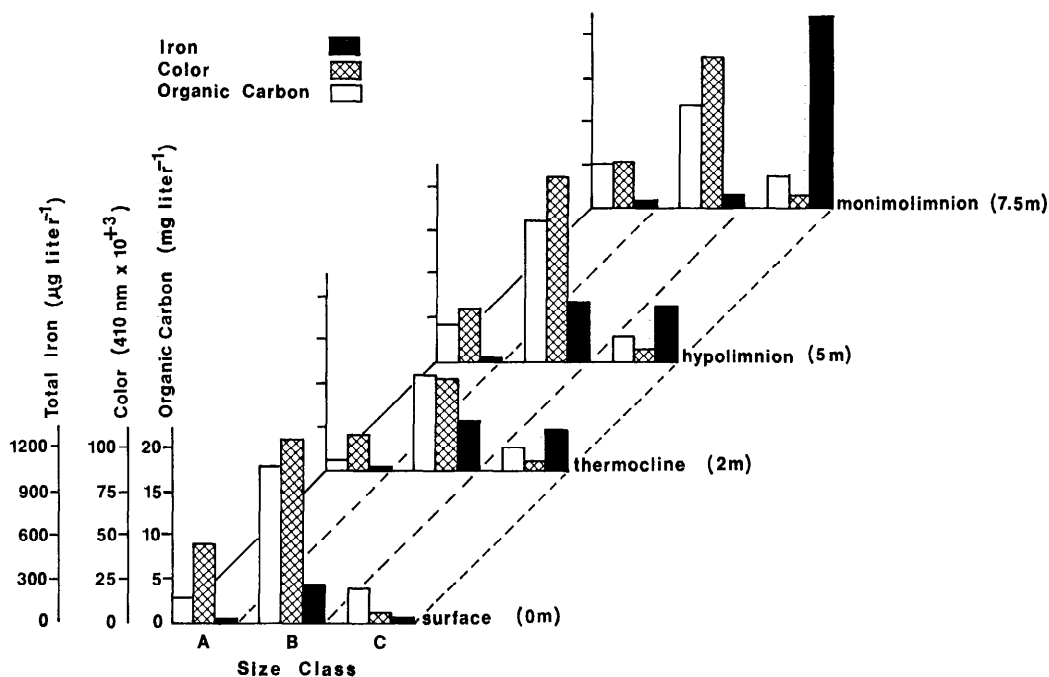


Fig. 1. Distribution of particulate (A), colloidal (B), and dissolved (C) organic matter, color, and iron by strata in North Gate Lake, demonstrating the static colloidal size class of color and organic matter in contrast to the variable size classes of iron.

colloidal  $\text{Fe}^{3+}$  complex. Nonreactive ferric iron present in the colloidal state is primarily an inorganic complex (e.g. ferric oxyhydroxide). In addition to three physical categories of iron (Fig. 1), the iron at four depths was characterized as  $\text{Fe(III)}$  (reactive and nonreactive), colloidal reactive  $\text{Fe}^{2+}$ , and dissolved (ionic)  $\text{Fe}^{2+}$ .

Water samples for chemical analysis were collected with a modified Hale sampler using acid-washed 300-ml BOD bottles. Duplicate samples were analyzed in the laboratory after minimum exposure to sunlight. Since membrane filtration drastically alters the oxidation state of iron, ferrous iron was determined on unfiltered samples, and only the total iron analyses were performed on aerobically filtered samples. Total iron was determined with bathophenanthroline (BPN) by the method of Golterman and Clymo (1969) and ferrous iron by the acid-free BPN method of Koenings (1976). Absorbance of the ferrous-BPN complex was determined at 540

nm on a Klett-Summerson colorimeter with a 4-cm light path. We determined total organic carbon by the method of Maciolek (1962), using a dextrose standard curve prepared according to Strickland and Parsons (1972). Color was determined by absorbance of the sample at 410 nm on a Spectronic 20 with a 1.0-cm light path. Stable chemistry of phosphorus, dissolved oxygen, and hydrogen sulfide was determined according to Strickland and Parsons (1972). All determinations were made against distilled water blanks treated identically to the water samples. Activity of samples was measured by drying triplicate 5- and 10-ml subsamples on planchets and counting on a Nuclear-Chicago low background beta counting system.

### Results

*Distribution of color, organic matter, and iron in natural lake waters*—North Gate Lake mixes during the spring and fall over-

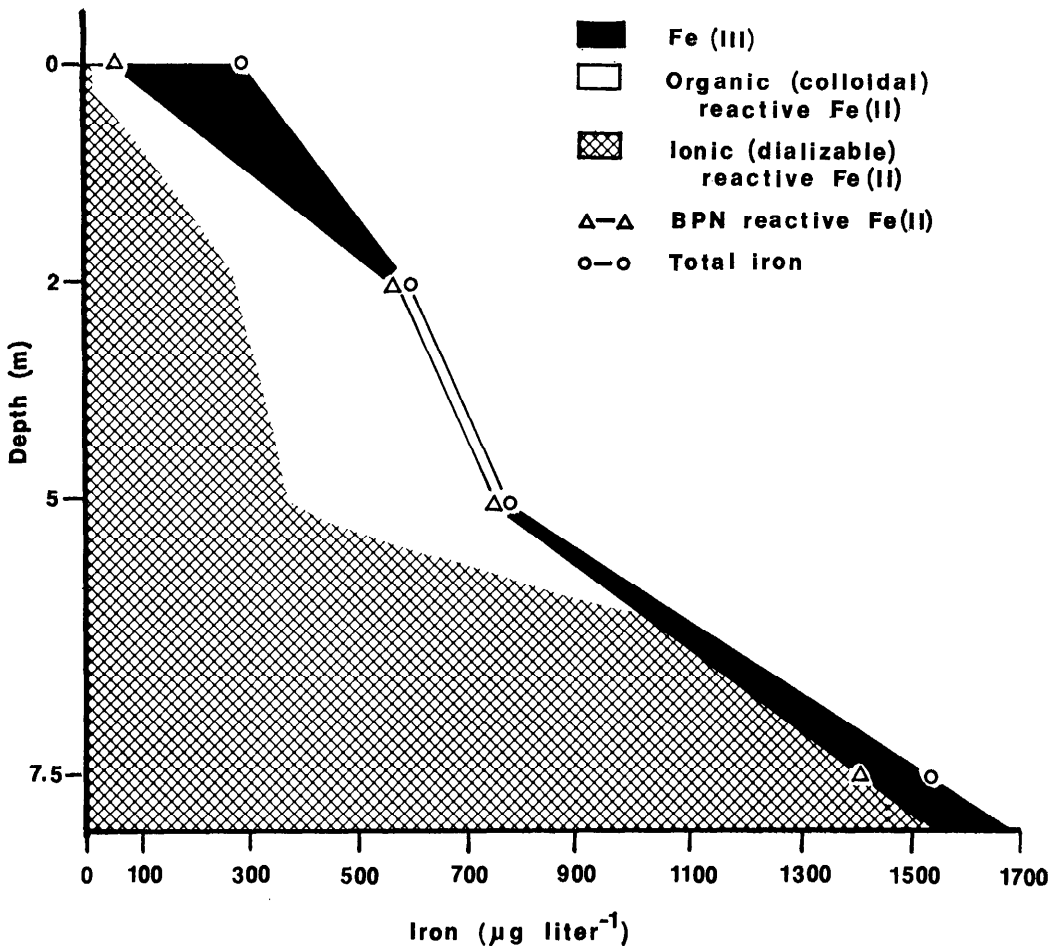


Fig. 2. Distribution of ferrous iron (organic and ionic) and ferric iron by strata in North Gate Lake.

turns to a depth of 5–6 m, leaving an anaerobic monimolimnion below 6.5 to 7 m. The 5-m depth is usually anaerobic and contains  $\text{H}_2\text{S}$  while the 2-m depth alternates between anaerobic conditions and very low oxygen concentrations ( $0.5 \text{ mg liter}^{-1}$ ). The surface layer is oxygenated at all times ( $3\text{--}5 \text{ mg liter}^{-1}$ ) but is only 50–70% saturated. Hence conditions grade from a continuously anaerobic (7–8 m) to a constantly aerobic (surface) state.

Organic carbon ranged from 13,791 to 25,704  $\mu\text{g liter}^{-1}$ . Only 12–18% of the organic carbon of North Gate Lake was in true solution (dialyzable) and 60–85% was colloidal. Colloidal carbon was predomi-

nant at all depths (Fig. 1). Similarly, very little of the lake's color was in solution (3–6%) and the predominant contribution came from colloidal-sized particles (68–74%). Thus most of the lake's organic carbon was colloidal; this fraction had most of the yellow coloring matter. The changes of color and colloidal organic carbon with depth were correlated and both showed minimum concentration in the thermocline (Fig. 1). However, changes in colloidal iron concentrations were not correlated with either color or organic carbon (e.g. monimolimnion), so that the size distribution and color of the COM is independent of iron complexation.

Table 1. Changes in (A) chemical reactivity of iron (percent of total iron in parentheses) and (B) ferrous iron (percent of BPN reactive ferrous iron in parentheses) in North Gate Lake.

Depth (m)	Iron ( $\mu\text{g liter}^{-1}$ )					B	
	A			Dialyzable		Colloidal Fe(II)*	Ionic Fe(II)*
	Particulate	Non-particulate ferric	BPN reactive ferrous*	BPN reactive ferrous*	Total dialyzable		
0 (surface)	10(4%)	226(80%)	46(16%)	6	23	40(87%)	6(13%)
2 (thermocline)	7(1%)	0(0%)	590(99%)	262	266	328(56%)	262(44%)
5 (hypolimnion)	24(3%)	0(0%)	757(97%)	355	358	402(53%)	355(47%)
7.5 (monimolimnion)	50(3%)	99(6%)	1,394(91%)	1,394	1,394	0(0%)	1,394(100%)

\*Iron reacting with bathophenanthroline without prior acidification of the sample.

Iron forms in the water column can also be identified on the basis of oxidation state and reactivity (Fig. 2). In the anaerobic monimolimnion, 1,394  $\mu\text{g liter}^{-1}$  of the iron is present as BPN reactive Fe(II), all dialyzable. This iron is considered to be ionic. Consequently, it appears that reduced iron does not form either a stable inorganic (ferrous gel) colloid or an organic complex with COM in the constantly anaerobic part of the water column above the sediments. However, in the oxygenated surface waters almost all of the iron (226  $\mu\text{g liter}^{-1}$  or 80% of the total iron) is Fe(III) and 209  $\mu\text{g liter}^{-1}$  or 92% of it is colloidal. At 2 and 5 m, 97–99% of the iron is present as reactive Fe(II): 53–56% as reactive colloidal Fe(II), and 44–47% dialyzable and hence present as dissolved  $\text{Fe}^{2+}$  (Table 1, Fig. 2). These depths are oxygenated during spring overturn enabling reduced ionic iron to form colloidal Fe(III) as in the epilimnion. In this low pH, highly colored lake, Fe(II) is not of colloidal size until the iron is oxidized. The colloidal Fe(III) thus formed is subsequently reduced under anaerobic conditions in the presence of COM and other reducing substances (e.g.  $\text{H}_2\text{S}$ ) to form colloidal Fe(II). Since this colloidal Fe(II) is also reactive to BPN, it may exist either as a weak inorganic colloid or as a colloidal organic acid complex less stable than the BPN- $\text{Fe}^{2+}$  complex (Fig. 2).

Mortimer (1941) suggested that  $\text{Fe}^{2+}$ -organic complexes exist in natural waters, but evidence is sparse. We have demonstrated

the presence of BPN reactive ferrous iron that is not dialyzable in recently anaerobic waters. However,  $\text{Fe}^{2+}$  does not form complexes with COM in perpetually anaerobic waters. The formation of this nondialyzable  $\text{Fe}^{2+}$  appears to come from colloidal reactive Fe(III). That is, it is residual colloidal Fe(III) that has been reduced, and, like the EDTA- $\text{Fe}^{2+}$  complex (formed from  $\text{Fe}^{3+}$ -EDTA), which has a moderate stability if not exposed to oxygen (Jones and Long 1952), it may exist in anaerobic to oxygen-deficient acid waters. Coolidge (1932) recognized dipyrindyl reactive iron that was complexed to a protein, so analogs have been demonstrated. We feel that  $\text{Fe}^{2+}$  does not form complexes with COM, but that the complexes are present as residual reduced products of colloidal Fe(III).

Jones and Long (1952) have also shown that organic complexed iron (e.g. ferric citrate, ferric oxalate, and ferric EDTA) is readily reduced by light into a ferrous complex that may exist indefinitely if not exposed to oxidizing conditions. This complexed ferric iron is present as reactive ferric iron (fully dissociated  $\text{Fe}^{3+}$ ). As we will demonstrate, COM reduces the formation of ferric oxyhydroxide (slowly photo-reduced) with more ferric iron present as reactive colloidal  $\text{Fe}^{3+}$ . In this manner, COM may facilitate the photoreduction of iron, which then may exist as a ferrous-organic complex in the presence of a low level of oxygen. The existence of this complex may be prolonged by an acid pH, since at low pH the oxidation of iron be-

Table 2. Particulate (A), colloidal (B), and dialyzable (C) fractions of natural iron and phosphorus of 7.5-m water of North Gate Lake before experimentation (control). The effects of aeration (exp I vs. II) and of the presence of COM (exp II vs. III) are shown for both the stable elements and for the known  $^{32}\text{P-PO}_4$  and  $^{59}\text{Fe-Fe(III)}$  inorganic tracers.

Experiment		Iron			Phosphorus		
		A	B	C	A	B	C
Control (natural anaerobic lake water)	( $\mu\text{g liter}^{-1}$ )	80 (7%)	7 (1%)	1,055 (92%)	106 (15%)	0 (0%)	605 (85%)
I (anaerobic lake water)	( $\mu\text{g liter}^{-1}$ )	70 (7%)	86 (9%)	849 (84%)	74 (21%)	0 (0%)	266 (79%)
	(cpm ml $^{-1}$ )	25 (12%)	25 (12%)	165 (76%)	4 (3%)	2 (1%)	143 (96%)
II (aerobic lake water)	( $\mu\text{g liter}^{-1}$ )	90 (9%)	949 (90%)	15 (1%)	112 (32%)	107 (30%)	132 (38%)
	(cpm ml $^{-1}$ )	41 (16%)	209 (82%)	5 (2%)	45 (30%)	43 (29%)	59 (40%)
III (aerobic dialysate of lake water)	( $\mu\text{g liter}^{-1}$ )	42 (4%)	1,102 (93%)	48 (4%)	0 (0%)	417 (67%)	204 (33%)
	(cpm ml $^{-1}$ )	14 (4%)	346 (93%)	15 (4%)	10 (5%)	116 (61%)	63 (33%)

comes independent of oxygen concentration (Stumm and Lee 1961).

Dissolved  $\text{Fe}^{2+}$  is either formed by solution of the ferrous colloid (e.g. removed by  $\text{H}_2\text{S}$ ) or may originate from forms of iron other than colloidal reactive  $\text{Fe(III)}$ . In an experiment described below,  $^{59}\text{Fe-FeCl}_3$  added to anaerobic lake water equilibrated completely with the colloidal fraction but was later recovered as dialyzable  $^{59}\text{Fe}^{2+}$ , so apparently ionic ferrous iron can be formed from colloidal ferric iron in the presence of organic colloids.

*Experimental analysis of iron-organic matter-phosphorus relationships*—To check the origin of the iron cycle proposed above, and to explore the possible formation of iron-organic acid complexes and their relationship to phosphorus, we undertook the following experiments.

In experiment I (anaerobic containers), water samples were collected from the 7.5-m zone with a 2-liter Kemmerer sampler and transferred rapidly and with minimum aeration to four acid-washed plastic bags. Each bag contained duplicate sealed sections of dialysis tubing filled with anaerobic distilled water. The water in one bag was then labeled with 1–2  $\mu\text{Ci}$  of  $^{59}\text{Fe-FeCl}_3$ , a second was labeled with 1–2  $\mu\text{Ci}$  of  $^{32}\text{P-H}_3\text{PO}_4$ . After labeling, bags were sealed and the contents mixed. The re-

maining two plastic bags, prepared in identical manner but without tracer, were used for stable chemical analyses. All four bags were suspended in the lake at the depth of collection (7.5 m) for 5 days.

For experiment II (aerobic containers), water was collected from 7.5 m as in exp I, but was returned to the laboratory and poured into four acid-washed plastic containers supplied with duplicate dialysis sacs filled with anaerobic distilled water, as before. Two were labeled and two were retained for stable chemical analysis, as in exp I. All four containers were then incubated in a dark, constant temperature room for 5 days at 5°C; during this period all were aerated by bubbling air from the same source to maintain a constant pH and dissolved oxygen concentration.

In experiment III (aerobic dialysate), we first prepared anaerobic dialysate of 7.5-m water by suspending a series of sealed sections of dialysis tubing filled with anaerobic distilled water at 7.5 m for 5 days. This yielded water equivalent to natural 7.5-m water in all respects except that yellow colloidal organic acids were excluded. This water was transported to the laboratory, treated, and labeled in a manner identical to samples in exp II so that differences in results between exp II and III can be attributed to the influence of organic acids.

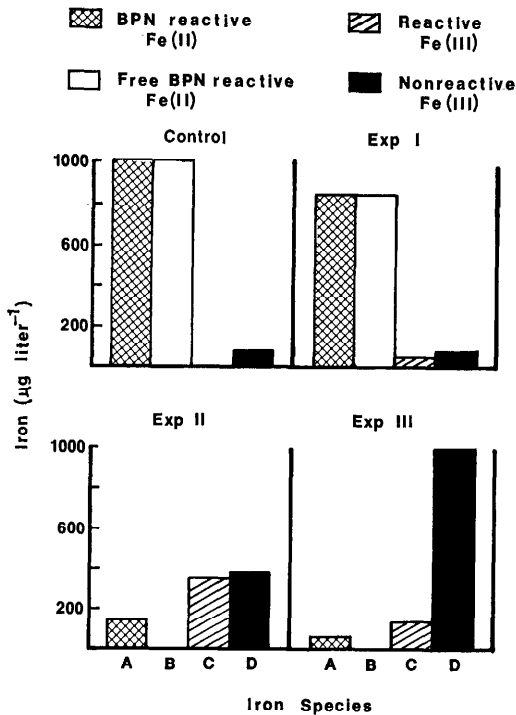


Fig. 3. Change in iron species present in anaerobic lake water [control and exp I (COM present)] and aerobic lake water [exp II (COM present) and exp III (COM absent)] showing the effect of the presence or absence of oxygen and COM.

Four containers were used in each of the three experiments and each experiment was duplicated. One container in each experiment was used to follow iron labeled with  $^{59}\text{Fe}$ , one to follow the  $^{32}\text{P}$  label, and two for stable chemical analysis of iron and phosphorus fractions after equilibration.

*Anaerobic iron and phosphorus distributions in lake water containing COM*—Control conditions for these experiments were established by collecting a series of samples from the 7.5-m depth and analyzing them with minimum exposure to the atmosphere. Initial conditions in controls were typical of the monimolimnion and the coordination of iron was similar to that reported above. Only 8% of the  $\text{Fe}(\text{II})$  was nondialyzable (Table 2); the remainder was BPN reactive ionic ferrous iron. The concentration of BPN reactive  $\text{Fe}(\text{II})$  was

the same in dialyzed samples as in unfiltered samples (Fig. 3). The small particulate fraction was nonreactive  $\text{Fe}(\text{III})$ . Phosphorus, like iron, was nearly entirely dialyzable reactive phosphate (85%), the remainder particulate unreactive phosphorus (Table 2).

After 5 days incubation in the lake, the anaerobic container samples (exp I) apparently equilibrated. The concentration of BPN reactive ferrous iron was nearly the same in the dialysis bags as in the unfiltered samples (Fig. 3). Activity in the dialysis bags indicated that 76% of the added activity was dialyzable. Clearly, the ferrous iron was in a dissolved  $\text{Fe}^{2+}$  state, not as an inorganic colloid or as a COM complex. The fractions of colloidal and particulate activity were similar to those of stable colloidal and particulate iron.

The relationship of phosphorus fractions was the same in the anaerobic bag as in the controls. There was no colloidal phosphorus, 21% was particulate, and 79% was dialyzable. The  $^{32}\text{P}$  data indicate that most of the particulate pool of phosphorus was not in exchangeable equilibrium with the tracer. Thus 96% of the added orthophosphate tracer remained in the dialyzable pool, indicating a detrital form of stable particulate phosphorus. The lack of activity in the colloidal fraction supports our belief that by themselves colloidal organic acids have no affinity for phosphorus.

It is clear that manipulations in preparation and incubation of samples permitted some oxidation and resulted in the formation of larger quantities of BPN unreactive  $\text{Fe}(\text{II})$  and reactive  $\text{Fe}(\text{III})$  and in a measurable reduction in the quantity of dialyzable  $\text{Fe}(\text{II})$  in these samples than in the controls (Fig. 3).

*Aeration effects*—Comparing aerated container samples (exp II) with controls and anaerobic bag samples indicates major changes in iron and phosphorus fractions (Table 2, Fig. 4). Colloidal iron increased at the expense of dialyzable iron, which decreased (Table 2). At the same time, dialyzable BPN reactive ferrous iron was eliminated and ferric iron became the

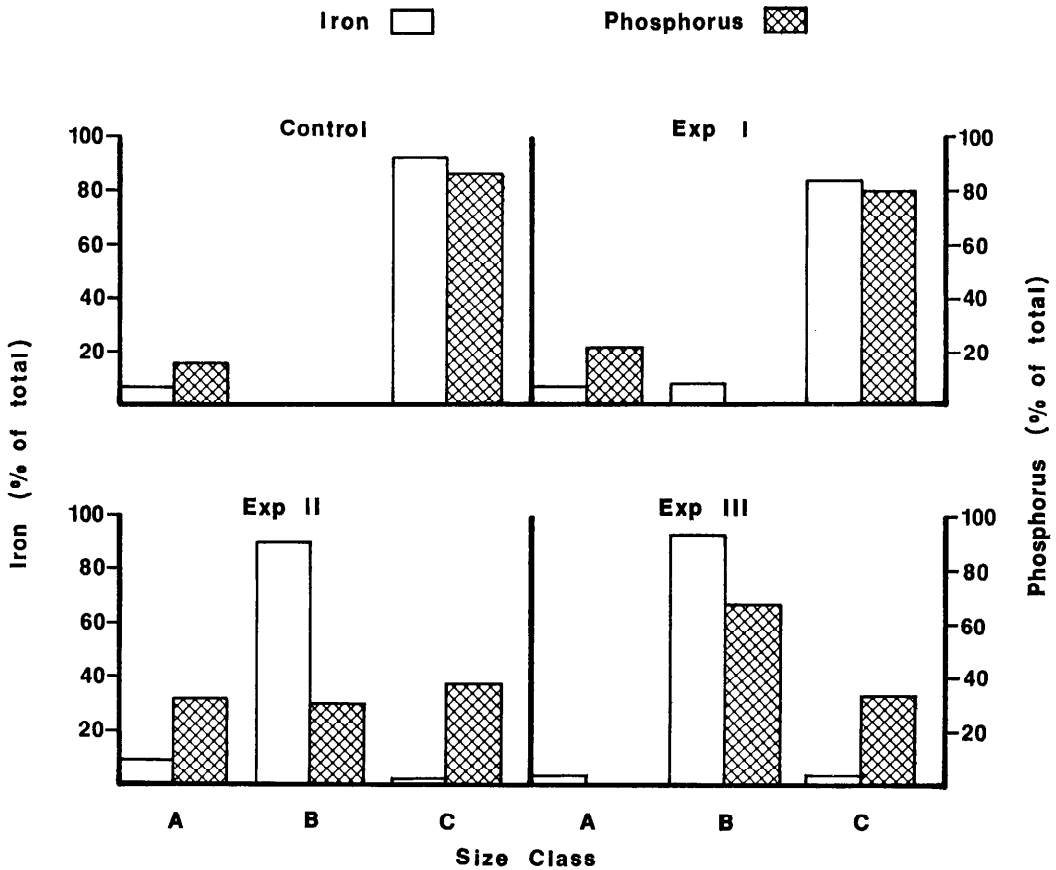


Fig. 4. Changes in stable iron and phosphorus present in the particulate (A), colloidal (B), and dialyzable (C) size classes in anaerobic lake water [control and exp I (COM present)] and aerobic lake water [exp II (COM present) and exp III (COM absent)] showing the effect of the presence or absence of oxygen and COM.

dominant form (Fig. 3). However nonreactive Fe(III) represented only 53% of this, or only 37% of the total iron, while 13% of the total iron was still ferrous. The radiochemical data support these results in that the  $^{59}\text{Fe}$  was in a colloidal state in equilibrium with only a minimal pool of dialyzable iron. More important, colloidal phosphorus increased from 9% in the anaerobic system to 30% of total phosphorus, while dialyzable phosphate was reduced from 79% to 38% (Table 2). The generation of colloidal  $^{32}\text{P}$  from dialyzable  $^{32}\text{P}$ -orthophosphate was very like the change in stable phosphorus and paralleled the change of free ferrous iron to colloidal ferric iron.

The dialysate used in exp III was identical to undialyzed 7.5-m lake water except that it lacked COM, and all of the iron was in the ionic  $\text{Fe}^{2+}$  state before oxygenation. After oxygenation colloidal ferric iron again predominated. However, unlike aerated lake water containing COM, almost all was nonreactive ferric iron while ferrous iron decreased to only 5.5% of the total (Table 2, Fig. 3). Just as noncolloidal inorganic ferrous iron formed colloidal ferric iron when oxidized, colloidal phosphorus was formed from noncolloidal phosphate (Fig. 4). Only 33% of the reactive phosphate remained in true solution and colloidal reactive phosphate increased to



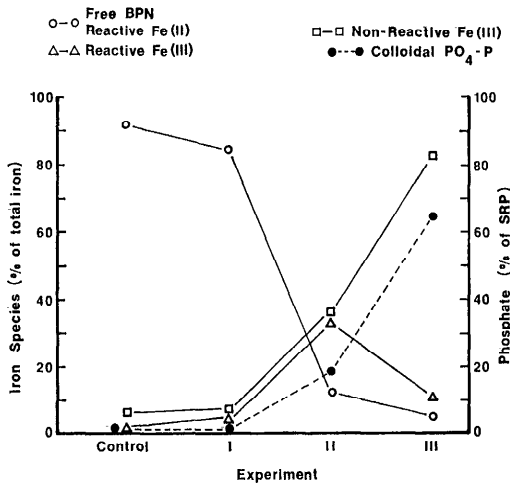


Fig. 5. Change in colloidal phosphate in response to the species of iron present in anaerobic (control and exp I) and aerobic (exp II and III) experimental lake waters.

67%. Thus, like iron, upon oxygenation and in the absence of COM, orthophosphate changed from a dialyzable to a colloidal form.

**COM influence**—Differences in size distribution of iron after aeration between 7.5-m water containing organic colloids and dialyzed 7.5-m water were small. However, the chemical separation showed that significantly greater quantities of nonreactive ferric iron colloids and a lesser amount of ferrous iron were formed than when the COM was present (Fig. 3): the organic matter reduced both the oxygenation reaction of ferrous iron and the formation of nonreactive ferric iron during aeration.

In anaerobic water a colloid of iron, phosphorus, and COM is absent. However, in recently oxygenated water colloid formation of iron and phosphorus takes place both in the presence and absence of the COM. The components of this colloid appear to be related to the amount of COM and form of ferric iron present (Fig. 5). Where 26–38 mg liter<sup>-1</sup> of the COM and reactive ferric iron were present, 19% of the inorganic phosphate was colloidal after aeration. In the absence of COM, but in the presence of about 7 mg liter<sup>-1</sup> of dia-

lyzable organic matter, and nonreactive ferric iron, 65% of the inorganic phosphate was colloidal after aeration. The differences in both iron and phosphate distributions indicate that the COM reduces the ability of colloidal ferric iron to react with the phosphate anion; this is consistent with the proposed formation of the low fraction of iron-organic acid-phosphate complexes. These data demonstrate both the formation of colloidal phosphate from the non-colloidal inorganic pool (Fig. 4) and the effect of COM on colloidal phosphate formation (Table 3). Compared to lakes with low organic matter, more unadsorbed phosphate would therefore remain in the water column of stained acid bog lakes during circulation. Along with the latter major fraction, there is the iron-phosphate-organic colloid, which may be mobile and less subject to sedimentation, but from which the phosphate is less readily desorbed.

Experiment	Phosphorus (µg liter <sup>-1</sup> )		
	SRP (A)	Free reactive† (B)	Colloidal reactive (A-B)
Control (natural anaerobic lake water)	605*	605	0
I (anaerobic lake water)	262*	266	0
II (aerobic lake water)	163 <sup>‡</sup>	132	31
III (aerobic dialysate of lake water)	588 <sup>‡</sup>	204	384

\*unfiltered sample.

†filtered (0.45 µ) sample.

‡dialyzed (4.8 nm) sample.

lyzable organic matter, and nonreactive ferric iron, 65% of the inorganic phosphate was colloidal after aeration. The differences in both iron and phosphate distributions indicate that the COM reduces the ability of colloidal ferric iron to react with the phosphate anion; this is consistent with the proposed formation of the low fraction of iron-organic acid-phosphate complexes. These data demonstrate both the formation of colloidal phosphate from the non-colloidal inorganic pool (Fig. 4) and the effect of COM on colloidal phosphate formation (Table 3). Compared to lakes with low organic matter, more unadsorbed phosphate would therefore remain in the water column of stained acid bog lakes during circulation. Along with the latter major fraction, there is the iron-phosphate-organic colloid, which may be mobile and less subject to sedimentation, but from which the phosphate is less readily desorbed.

Chamberlain and Shapiro (1973) argued that molybdate reactive material present in natural lake waters may be regarded as inorganic phosphate, although not necessarily free phosphate ion. They proposed that water-soluble fulvic acids forming

Table 4. Recovery of soluble reactive phosphorus (A) as orthophosphate (B) from distilled and from experimental lake waters in the presence and absence of colloidal organic matter (exp II and III) using gradient elution anion exchange chromatography.

Experiment	Elution system	Aeration time (h)	Elution volume (range)*	Elution volume (peak)*	SRP (A) <sup>+</sup>	Ortho-P (B) <sup>+</sup>	Recovery*
Control (distilled water)	acetate	6	23-33	26.5	100.0	99.6	99.6
	formate	48	11-19	16.0	100.0	99.4	99.4
II (aerobic lake water)	acetate	6	16-37	27.0	116.0	94.0	81.0
	formate	48	10-24	18.0	47.0	31.5	67.0
III (aerobic dialysate of lake water)	acetate	6	18-45	26.0	151.0	149.8	99.2
	formate	48	11-19	16.0	38.0	37.9	99.7

\*percent.

<sup>+</sup>  $\mu\text{g}$ .

complexes with phosphate through iron coordination may be the source of the observed differences between reactive phosphorus and free phosphate analyses. We have demonstrated the formation of similar but colloidal-sized adsorption complexes in natural lake waters. Even in the presence of a high concentration of COM, reactive colloidal phosphate was formed from ionic orthophosphate. This colloidal inorganic phosphate may be the source of the discrepancies between chemical estimates of soluble reactive phosphate (SRP) and radiobiological assay or ion-exchange estimates of free orthophosphate ion. The mechanism of colloidal phosphate formation described above (by oxygenation of natural lake water) supports the observation of Hutchinson (1941) that acid-soluble phosphorus appears during and for a short time after lake overturn.

*Formation of colloidal phosphorus*—The aerated samples (exp II and III) differed markedly from anaerobic samples in that the reactive phosphorus of the dialysate was less than the reactive phosphorus of the filtered samples (Table 3). This indicates that a new reactive phosphate component of colloidal size was formed. This conversion of stable phosphorus is paralleled by the movement of  $^{32}\text{P}$  into the colloidal fraction, which suggests that the stable phosphorus and the added  $^{32}\text{P-PO}_4\text{-P}$  are chemically similar. Since phosphorus shows little tendency to react with organic

matter when not in the presence of colloidal ferric iron, this suggests iron-phosphate complexation and demonstrates that SRP is not equal to free reactive orthophosphate.

Anion exchange chromatography was used to test whether this colloidal reactive phosphate could account for the difference between reactive phosphate and free phosphate analysis. Of the predominantly colloidal phosphate present in dialyzed lake water (e.g. exp III) after aeration, 99.2–99.7% of the SRP found before the sample was put through the resin column was recovered upon elution (Table 4). Since recoveries were nearly complete, and equal to the standards, it appears that the iron-reactive phosphate colloid was not ferric phosphate or occluded phosphate, but orthophosphate adsorbed on colloidal ferric oxyhydroxide. That this was orthophosphate was demonstrated both by the synchronous elution position of the reactive component in the elution curve with known orthophosphate and by the exact agreement between the total phosphorus and the reactive phosphate analyses of the orthophosphate peak. Therefore, the SRP analysis does measure orthophosphate. However, when water from the same depth but containing COM was analyzed (e.g. exp II), only 67–81% of the SRP was eluted, while all the COM remained on the column. This suggests that the presence of

COM was responsible for the incomplete recovery of SRP as orthophosphate.

SRP was thus not equal to free reactive phosphate when the water contained a portion of colloid (COM) bound reactive phosphate that was not measurable by ion exchange extraction. This colloidal reactive phosphate represented 19% of SRP, while nonrecovery of SRP as orthophosphate equalled 19 and 33%. It is therefore conceivable that the colloid (COM) bound reactive phosphate is responsible for the incomplete recovery by ion exchange of SRP as free orthophosphate.

### Discussion

Before studying the large pool of metabolically produced DOP in North Gate Lake, we had first to determine where and to what extent abiotic DOP (an organic matter-iron-phosphate complex) is formed in the lake. Fortunately, the relative chemical and physical distributions of the three components of such a complex varied between anaerobic and aerobic strata. This allowed us to ascertain if a COM-ferric iron-phosphate colloid could be formed in trophogenic waters. By first defining the existing chemical and physical states of the three components in the different lake strata, and then by experimentally manipulating water from these strata, we found that a small amount of the proposed colloid did indeed form. However, a majority of the  $\text{PO}_4\text{-P}$  remained free in solution.

In natural lake waters, iron in oxygenated and deoxygenated strata differed in both oxidation state and physical size. Constantly anaerobic waters contained ionic ferrous ( $\text{Fe}^{2+}$ ) iron fully filterable and dialyzable, and not adsorbed to COM. Aerobic waters contained filterable but nondialyzable (colloidal) ferric iron, only partially reactive, as ferric oxyhydroxide. Most of the iron is in the colloidal state as are the organic acids, but the COM is not dependent on iron complexation for its size or its color.

In contrast to perpetually anaerobic waters, those that undergo brief aeration during spring overturn contain ionic  $\text{Fe}^{2+}$

along with a reactive form of ferrous iron that is not dialyzable. This colloidal BPN reactive iron was either in a complex with the yellow organic acids as  $\text{Fe}^{2+}$  (which was less stable than the BPN- $\text{Fe}^{2+}$  complex) or existed as a weak residual ferrous iron colloid reactive to BPN. From the circumstances of its formation, it seems to have been derived from colloidal reactive  $\text{Fe(III)}$ , since COM reduces formation of colloidal nonreactive ferric iron (i.e. ferric oxyhydroxide) and thus may be a COM- $\text{Fe}^{2+}$  complex, as was observed by Koenings (1976).

Through the use of experimental chambers, we further learned that the oxidation of iron and the presence of COM influenced the extent of the reaction of iron with phosphorus. Neither dissolved  $\text{Fe}^{2+}$  nor orthophosphate in anaerobic waters had any sorption reaction with the colloidal organic acids. Iron [nonreactive  $\text{Fe(III)}$ ]-phosphate colloid formation occurred readily without COM. The truly soluble dialyzable organic matter did not affect the adsorption or desorption of phosphate from the colloid as did COM. Colloidal ferric iron in the presence of COM contained less nonreactive  $\text{Fe(III)}$  and formed a smaller amount of colloidal reactive phosphate. Colloidal nonreactive ferric iron may be more absorptive for phosphate than other forms of colloidal ferric iron since its concentration parallels phosphate adsorption (Fig. 5). COM may decrease the formation of colloidal phosphate by reducing the cationic properties of the colloidal nonreactive ferric iron, and, more important, by reducing the amount of colloidal nonreactive iron formed, definitely modify the behavior of the dissolved (dialyzable) inorganic components of the lake water.

We have shown for both natural and experimentally manipulated lake waters that iron-organic acid complexation is much less important in anaerobic than in aerobic waters, where colloidal organics did interact with iron during these experiments. COM not only decreases nonreactive iron formation and subsequent orthophosphate adsorption but forms a small fraction of an

iron reactive, phosphate-organic acid colloid. The explanation suggested by Mortimer (1941) for the lack of productivity in acid bog lakes (the formation of phosphate sorbed onto iron-organic complexes) is not supported, since phosphate adsorption to iron decreases in the presence of COM.

With such a mechanism, any existing  $\text{PO}_4\text{-P}$  would be readily available and quickly adsorbed by the phytoplankton. A hypothesis for the lack of productivity in acid bog lakes from the standpoint of phosphorus availability is that there may be only two types of dissolved phosphorus potentially available to the phytoplankton. The first is inorganic phosphate, which is maintained at an undetectable level through a combination of complete biotic removal and only limited resupply either from the surrounding *Sphagnum* mat or from bacterial regeneration. The second form is a large pool of metabolically produced, yet biologically unavailable DOP in the lake which may be generated in large part by the *Sphagnum* mat. Since the *Sphagnum* mat and the open lake water contain high levels of dissolved hydrolytic enzymes (e.g. acid phosphomonoesterase), lake DOP may be preprocessed and thus be refractory and unavailable to the phytoplankton that utilize similar hydrolytic enzymes to cleave  $\text{PO}_4\text{-P}$  from DOP.

Even though present in small amounts, COM bound phosphate may be similar to a class of organic bound phosphorus (humic phosphorus) suggested by Golterman (1973) and may account for the differences between soluble reactive phosphorus (SRP) and orthophosphate measurements as indicated by our ion-exchange experiments. Nonrecovery of SRP equalled 19–33% depending on the length of aeration, which suggests a time dependent phenomenon. However, some of the nonrecovery may be due to phosphate capture by the ion-exchange resin in the presence of colloidal organic acids. The apparent porosity, which determines the size of the ion that can migrate through the gel matrix of the resin to the exchange sites, is about  $40\text{\AA}$ . The colloidal organic acids in our studies can-

not pass through  $48\text{\AA}$  dialysis tubing, but can and do absorb irreversibly to the outside of the resin bead. By this enveloping action these acids may trap migrating orthophosphate ions in the matrix of the resin, resulting in low orthophosphate recoveries.

We propose that the effect of oxygenation on the iron and phosphorus in these experiments may be applicable to those reactions occurring during lake overturn, since all concentrations of the elements, and the elements themselves, were of natural origin. The conditions under which these experiments were performed closely simulated natural conditions, and our results paralleled those we observed in natural bog lake waters.

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*Submitted: 17 June 1974*

*Accepted: 8 March 1976*