



Effect of natural dissolved organic carbon on phosphate removal by ferric chloride and aluminum sulfate treatment of wetland waters

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[1] The use of wetlands for the removal of excess N and P has become widespread. Some sensitive P-limited ecosystems, however, may require additional reductions in the concentration of P entering the system. It has been proposed that the treatment of wetlands through addition of ferric chloride or aluminum sulfate can augment the natural P removal mechanisms. However, high concentrations of natural dissolved organic matter may interfere with the removal of P by metal addition. We evaluated the doses of ferric chloride and aluminum sulfate necessary to reduce total P concentrations below $0.32 \mu\text{M}$ ($10 \mu\text{g/L}$) in water from the Northern Everglades, and we determined the effect of various concentrations (21, 38, and 60 mg/L) of natural dissolved organic carbon (DOC) on the removal of PO_4 and total P. High concentrations of natural DOC inhibited both the short-term removal of PO_4 and the longer-term removal of total P from the water column. Similar results were observed using $15 \mu\text{M}$ citric acid in an experiment to determine whether citric acid could effectively mimic the inhibition of phosphorus removal associated with natural DOC. Stoichiometry of these experiments indicates that the mechanism of natural DOC interference was not complexation of the metal ions by the DOC; we hypothesize that it could be adsorption to the terminal hydroxyl groups on a polynuclear Fe or Al colloid, effectively blocking the adsorption sites from a phosphate molecule. Also, the ability of citric acid to mimic the inhibitory effects also suggests that the results of the study are broadly applicable to wetland and other waters with high natural organic acid concentrations.

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1. Introduction

[2] Iron chloride and aluminum sulfate have been used for many years in wastewater treatment processes to reduce phosphorus concentrations in effluent waters, often with high removal efficiencies [Hammer and Hammer, 2007]. High concentrations of these chemical coagulants must be used to reduce phosphorus concentrations in the effluent to levels mandated by regulatory agencies and also to speed the settling process. It is beyond the scope of most wastewater treatment applications, however, to reduce phosphorus concentrations through the use of low concentrations of chemical coagulants, since the necessary settling time would be prohibitively long.

[3] The addition of aluminum sulfate and ferric chloride directly to lakes and reservoirs to regulate phosphorus availability has been widely used since the 1970s to control eutrophication [Cooke *et al.*, 2005]. Iron and aluminum

compounds have been added directly to lakes to suppress internal cycling [Cooke *et al.*, 2005], or to river or storm water entering the lake to suppress further inputs [Harper, 1994; Lewandowski *et al.*, 2003], both resulting in marked reductions in phosphorus levels. It has been proposed that ferric chloride or aluminum sulfate addition to agricultural runoff entering the Northern Everglades could provide a means for enhancing natural mechanisms of phosphorus removal from the wetland [South Florida Water Management District, 2000; South Florida Water Management District, personal communication, 2008]. Addition of aluminum sulfate to water circulating through a wetland has also been proposed to remove P from a hypereutrophic lake [DB Environmental, Inc., 2006] and, in another case, to enhance P removal from a municipal wastewater treatment wetland [Malecki-Brown *et al.*, 2007].

[4] Historically, the water and peat soils of the wetlands in the Florida Everglades have contained very low levels of phosphorus, with native levels in surface water at or below $0.32 \mu\text{M}$ ($10 \mu\text{g/L}$) total phosphorus [Richardson *et al.*, 2007]. The formation of the agricultural area south of Lake Okeechobee has increased phosphorus loading to the Northern Everglades in runoff from the 284,900 ha of farmland [Moustafa, 1999]. Increased phosphorus loading has resulted in widespread ecological changes in the

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Northern Everglades, with the most noted changes occurring in Water Conservation Area 2A. Ecological changes which have been attributed to this phosphorus enrichment include [Richardson *et al.*, 2007] (1) invasion of cattail (*Typha domingensis*) and the displacement of native sawgrass (*Cladium jamaicense*) dominated stands, (2) changes in algal communities, and (3) soil phosphorus enrichment resulting in changes in soil chemistry and water quality. Many of these changes have been observed to occur at total phosphorus concentrations between 0.32 and 0.65 μM (10–20 $\mu\text{g/L}$) in the water column [Richardson *et al.*, 2007].

[5] Concern regarding the effect of phosphorus enrichment has prompted research to establish viable means to reduce phosphorus loading in the Northern Everglades. Recent restoration efforts have focused on the construction of restored marsh Stormwater Treatment Areas as a means of natural phosphorus removal, utilizing aquatic vegetation or periphyton for phosphorus uptake and removal from the system through peat deposition. The South Florida Water Management District built a 1,545 ha demonstration-scale wetland, the Everglades Nutrient Removal Project, as a means of reducing nutrient loading from storm water runoff from the Everglades agricultural area [Moustafa, 1999]. In the first few years of operation, the Everglades Nutrient Removal Project reduced total P concentrations to an average of between 0.65 to 0.97 μM [Kadlec, 2006]. However, still lower concentrations will be necessary to meet future restoration goals [Richardson *et al.*, 2007].

[6] It has been proposed that the addition of ferric chloride or aluminum sulfate to water directly flowing into storm water treatment areas within the Everglades could augment natural phosphorus removal mechanisms by precipitating the remaining dissolved phosphate in the water column, as well as flocculating and coagulating both chemically formed and naturally occurring particulate P [South Florida Water Management District, 2000]. This method of adding chemicals directly to the water flowing in to the treatment areas would utilize the marsh itself as a settling basin, eliminating the need for high-rate settlers, filters or sedimentation basins which are necessary in wastewater treatment processes. Lower doses of ferric chloride or aluminum sulfate might be used because of the long settling time (days) afforded by using the marsh itself as a settling basin.

[7] Treatment of wetlands through the addition of ferric chloride or aluminum sulfate has remained largely untested under natural field conditions, except for mesocosm experiments of Malecki-Brown *et al.* [2007]. The use of low doses of chemicals in combination with the long settling time afforded by use of the marsh as a settling basin is less well researched than the use of high doses of chemicals and filtration and sedimentation basins in wastewater treatment processes. However, high concentrations of natural dissolved organic matter in wetlands may interfere with the removal of phosphorus by metal addition. The humic substances that comprise most of the dissolved natural organic matter contain multiple carboxylic acid groups that are negatively charged at neutral pH [Cabaniss *et al.*, 2005], and they are known to strongly complex with trivalent ions such as Fe^{3+} and Al^{3+} [Pullin and Cabaniss, 2003], depending on pH, and to inhibit the crystallization of Fe(III) and Al hydroxides [Huang *et al.*, 2005; Hua *et al.*, 2008]. Dis-

solved humic substances can also be precipitated by the high concentrations of ferric chloride or aluminum sulfate as used in water and wastewater treatment processes [Hammer and Hammer, 2007]. Concentrations of dissolved organic carbon (DOC) are very high in water draining from the Everglades agricultural area in the Hillsboro Canal, approximately 40–90 mg/L [Qualls and Richardson, 2003], well beyond the normal levels of drinking and wastewater applications. This DOC may have many effects: (1) It may consume large quantities of Fe or Al, (2) it may compete with phosphate for binding sites, (3) it may produce large quantities of low-density sludge from flocculation of the humic metal complex, perhaps adversely affecting water quality, or (4) it may inhibit precipitation by inhibiting crystallization [Hua *et al.*, 2008].

[8] The objectives of this study were (1) to determine the effect of various concentrations of natural DOC and initial PO_4 on removal of PO_4 and total P from Everglades water by ferric chloride and aluminum sulfate addition, (2) to examine rates of change in PO_4 and total P concentrations after these additions, (3) to determine whether controlled pH would affect the results of objective 1, (4) to determine whether the P in the precipitate was strongly bound, and (5) to determine whether citric acid, an organic acid with known metal complex stability constants, could mimic the inhibitory effects of DOC on P removal. To accomplish these objectives, we performed a series of experiments examining the effects of varying the ferric chloride or aluminum sulfate dose, DOC concentration, and initial PO_4 concentration on the resulting P concentrations in Everglades' water samples. We also examined the changes in P forms over times of 10 s to 4 days, repeated experiments at two controlled pH levels, subjected a precipitate incubated for 3 months to a sequential PO_4 extraction procedure, and finally, determined the concentrations of citric acid necessary to interfere with the removal of PO_4 by aluminum sulfate.

2. Materials and Methods

2.1. Preparation of DOC Stock and the Effects of DOC and Initial PO_4 on P Removal

[9] Water samples were collected from a site in the southern portion of Water Conservation Area 2A, a 43,281 ha impoundment in the Northern Everglades. In this area, the DOC and PO_4 concentrations were initially low, enabling us to vary the concentrations in our experiments through addition of stock DOC and stock PO_4 solutions.

[10] We prepared a concentrated stock of humic substances so that we could produce 3 levels of DOC in the water samples. The humic substance concentration technique of Thurman and Malcolm [1981] was used to concentrate humic substances from Everglades water and elute them from an XAD-8 chromatography column. However, we used a "hydrophobic-hydrophilic break" (k' , or "column capacity factor") of 25 rather than 100 in order to increase the recovery of the more hydrophilic acid components of the DOC so that the concentrate would be more representative of the entire DOC fraction. This factor is related to the volume of adsorbant resin and the volume of solution passed through it, which was 30 mL XAD-8 resin per liter of Everglades water in this study. The alkaline eluate was

immediately filtered through a cation exchange column, yielding the organic acid fraction of the DOC. The concentrated and isolated organic fraction comprised approximately 83% of the total DOC.

[11] The experimental design was a fixed, factorial design consisting of four treatments, with two replications. Three levels of concentration (0.42 μM (ambient, no PO_4 addition), 1.3 μM and 2.58 μM), three levels of DOC concentration (21 mg/L (ambient, no DOC addition), 38 mg/L and 60 mg/L), six levels of metal dose (0, 0.1, 0.2, 0.4, 0.6 and 1.0 mM), and two types of metal (Al or Fe) were investigated. The above DOC concentrations were chosen because they represent the observed range of naturally occurring DOC in the Everglades Nutrient Removal Project area (South Florida Water Management District data, personal communication, 1998). The Everglades water was adjusted to each of the levels of DOC and PO_4 concentrations through addition of DOC and PO_4 stock. These solutions were stirred and measured out into 200 mL aliquots in 250 mL plastic bottles. While keeping the solutions stirred at a set speed, FeCl_3 or $\text{Al}_2(\text{SO}_4)_3$ stock solutions were added to the middle of the vortex of each bottle. Solutions were allowed to mix for 2 min, stir bars were removed, and the bottles were allowed to sit in the dark. A subsequent slow mixing phase to encourage agglomeration of floc, often used in water and wastewater treatment [Hammer and Hammer, 2007], was not used because of the long residence times available by using the wetland as the sedimentation basin.

[12] Samples were taken at 2 h and at 4 days after metal addition. Without disturbing the precipitate within the bottles, three samples were pipetted from a depth of 1 cm below the surface of the solution, one for PO_4 analysis, one for total P analysis, and one for metal analysis. One sample was immediately filtered through a Gelman GN-6 0.45 μm pore size filter to be analyzed for PO_4 , total dissolved P and DOC. Potassium persulfate was used to digest samples for total P analysis. The PO_4 analysis was performed using the ascorbic acid molybdate blue method with a 5 cm cell [Wetzel and Likens, 1991]. Particulate phosphate concentration was calculated as unfiltered total P – filtered total P. Dissolved organic P (DOP) concentration was calculated as filtered total P – PO_4 .

[13] We also conducted a methodological test to confirm whether phosphorus analyzed as PO_4 might be loosely bound colloidal P that simply behaved as PO_4 during analysis by examining the size of P remaining in solution at high DOC concentrations after Al addition. We added 0.4 mM Al to Everglades water with 60 mg/L DOC and 16.1 μM PO_4 in the same manner as described previously for the main set of experiments. A 10 mL aliquot of the solution was withdrawn 2 h after metal addition and subjected to ultrafiltration through a Millipore (Millipore Corp., Bedford, Massachusetts) 30,000 nominal molecular weight ultrafiltration disc membrane. A 10 mL aliquot of 16.1 μM P as Na_2HPO_4 in deionized water was also subjected to the same procedure. Other ultrafiltration membrane discs of smaller pore size were also tried, but Na_2HPO_4 in deionized water failed to pass through them.

2.2. Rates of Change of P Forms

[14] A subset of the full-scale experiment was performed in order to determine the rate of change of PO_4 , DOP and

particulate P forms after the addition of 0.4 mM Fe or Al. One level of PO_4 (ambient, no addition) and one level of DOC (38 mg/L) was tested. PO_4 and total P samples were taken at 10 s, 2 min, 2 h, and 4 days after metal addition, and analyzed in the same manner as the full-scale experiment.

2.3. Effect of Controlled pH

[15] In the first series of tests, pH was not adjusted after metal addition because (1) it was not considered economical in a full-scale operation, (2) the pH change was relatively small (0.1 to 0.7 pH units) owing to the moderately high alkalinity (8.6 meq/L), and (3) the marsh soils in the Northern Everglades contained substantial concentrations of calcium carbonate marl to buffer pH in the field [Qualls and Richardson, 1995]. However, a set of experiments was done at controlled pH to determine if the effects were similar to those done without pH adjustment. Two sets of experiments were performed, one with an initial pH of 7.8 and one with an initial pH of 7.0. The pH was kept at the set pH using the method of Lijklema [1980] in which predetermined amounts of NaOH or HCl were added simultaneously and shortly after addition of the Fe or Al solutions.

2.4. Phosphate Precipitate Fractionation

[16] To determine how strongly bound the phosphorus was to the metal precipitate, an additional experiment was performed in which the metal oxyhydroxide-phosphate precipitate was subjected to an operationally defined fractionation procedure. Anion exchange membranes are widely used in standard soil P fractionation procedures to determine free PO_4 and PO_4 that would be exchangeable at low ionic strength [Tiessen and Moir, 2008]. Generally this step is followed by extraction with a high ionic strength salt to determine exchangeable PO_4 at high ionic strength and the two fractions are often interpreted as “plant available P.” We added 0.4 mM Al to Everglades water with 21 mg/L DOC and 1.3 μM PO_4 , and allowed the solution to sit for three months. After this time the bottle was centrifuged, and the supernatant was decanted, analyzed, and replaced with an equal volume of deionized water. Then an Empore Anion Exchange-SR disk saturated with OH^- was added to the suspension, which was stirred for 8 h. The disk was removed and extracted with a solution of 1 M K_2SO_4 . Although negatively charged DOC could conceivably compete with PO_4 for sorption on the anion exchange membrane, most DOC and Cl^- was removed by centrifuging and removing the supernatant before adding the anion exchange membrane. Finally, the precipitate itself was extracted with 1 M K_2SO_4 to determine the exchangeable PO_4 at high ionic strength. PO_4 analyses were performed on the extract solutions, and the amount of nonexchangeable bound PO_4 was calculated by difference from the initial concentration.

2.5. Citric Acid as a Model Compound and Evidence for a Hypothetical Mechanism of DOC Interference

[17] In order to test a model compound with known carboxylic acid group content and stability constants, we substituted citric acid for natural DOC in an additional experiment. The objective of this experiment was to determine whether citric acid could effectively mimic the effects of natural DOC on the relationship between aluminum added and the PO_4 concentration remaining in the water

Table 1. Stability Constants for Fe(III) and Al(III) Complexes With Citrate Used in the MINTEQA2 Model^a

Species ^b	Log K ^c
HCitrate ²⁻	6.40
AlCitrate	9.90
AlCitrateH ⁺	6.50
AlCitrate ₂ ³⁻	14.13
FeCitrate	13.13
FeCitrateH ⁺	7.99

^aThe only species listed are those that were added to the MINTEQA2 [Allison *et al.*, 1991] database or that were changed to values listed in the National Institute of Standards and Technology (NIST) database.

^bProduct of the ligand formation reaction.

^cK is the stability constant for the ligand formation reaction, taken from the NIST database. Values corrected to zero ionic strength using the Davies equation to correct for the activities of products and reactants as specified by Serkiz *et al.* [1996].

column. Furthermore, we wanted to examine the stoichiometry and the possibility that metal complex formation might explain the results. This experiment was performed with three levels of citric acid (no citric acid addition, 15 μM and 20 μM), one level of PO_4 (2.16 μM), and 5 levels of Al addition (0.2, 0.4, 0.6, 0.8 and 1 mM). Simulated Everglades water was prepared by adding 5 mM NaHCO_3 as a buffer, adjusting to pH 7.8, and adding 2.16 μM PO_4 . Citric acid stock was added to two of the solutions to equal 15 μM or 20 μM citric acid. Aluminum sulfate stock was added to

200 mL aliquots of three replicates of each treatment combination as described for the previous experiments. Samples were withdrawn 2 h after metal addition and analyzed for PO_4 . The pH was controlled at 7.8 by addition of NaOH or HCl as described previously.

[18] In order to determine how much Al and Fe might be complexed by the citrate and therefore prevented from precipitating, we used the MINTEQA2 [Allison *et al.*, 1991] chemical equilibrium program. In these modeling calculations, stability constants for aluminum-citrate complexes were taken from the National Institute of Standards and Technology (NIST) critical stability database [National Institute of Standards and Technology, 1993] and corrected to zero ionic strength. In addition, the species (AlCitrate, AlCitrateH⁺, and AlCitrate₂³⁻) were added to the MINTEQA2 thermodynamic database (Table 1). For Fe(III) complexes, equilibrium reactions for the formation of FeCitrate and FeCitrateH⁺ complexes were already present in the MINTEQA2 database; however, log K values were also changed to those found in the National Institute of Standards and Technology database.

3. Results and Discussion

3.1. Effect of DOC and Initial PO_4 on P Removal

[19] Two hours after addition of ferric chloride, under low DOC concentrations (21 mg/L), our target PO_4 concentra-

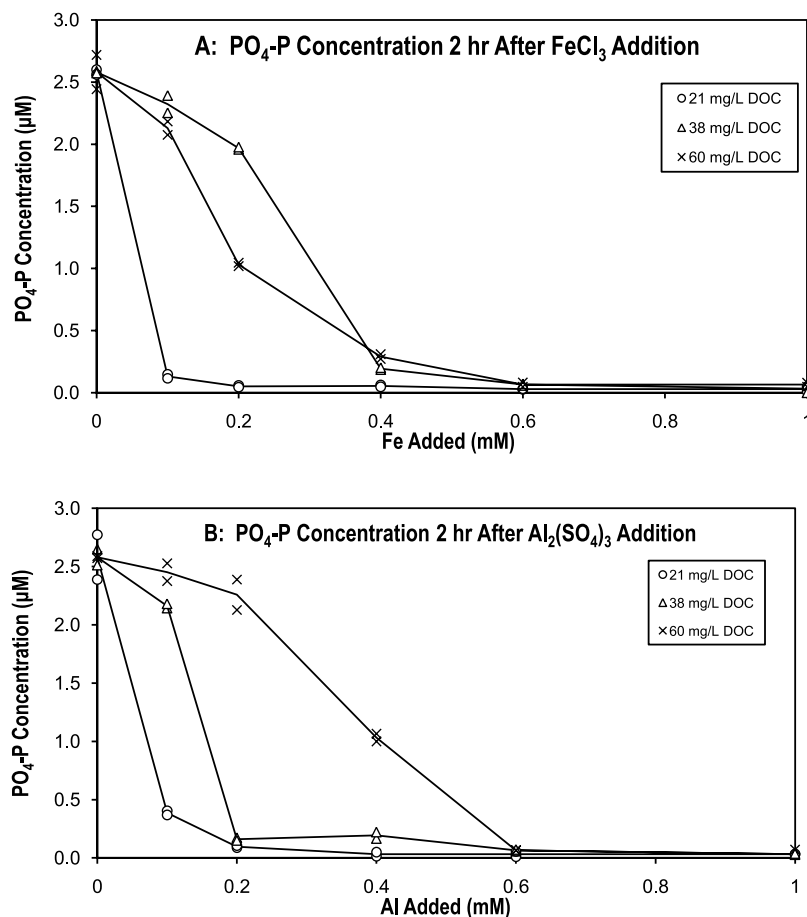


Figure 1. Effect of DOC on PO_4 removal 2 h after (a) ferric chloride addition and (b) aluminum sulfate addition. As DOC concentrations increased, greater metal doses were required to achieve the same reduction in PO_4 .

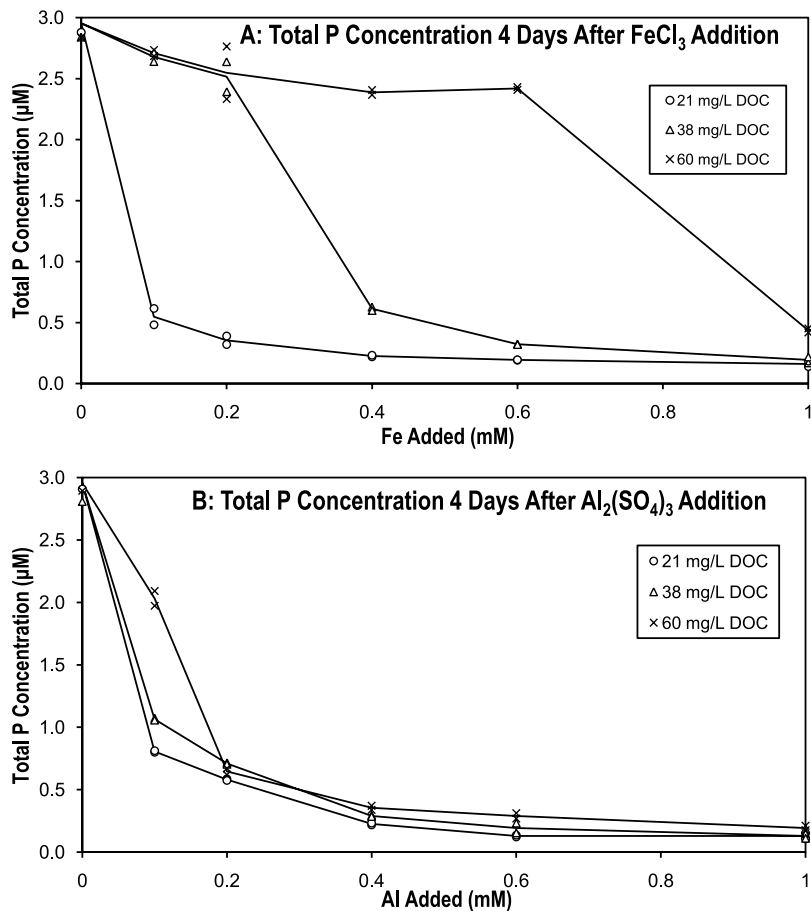


Figure 2. Effect of DOC on total P removal 4 days after (a) ferric chloride addition and (b) aluminum sulfate addition. High concentrations of DOC inhibited the removal of total P from the water column even at the 1 mM ferric chloride dose.

tions of less than $0.32 \mu\text{M}$ could be achieved with only 0.1 mM Fe. However, with high DOC concentrations (60 mg/L), 0.6 mM of Fe was required to achieve very low PO_4 concentrations. An exception to the tendency of higher DOC levels to allow higher PO_4 concentrations to remain in solution for a given dose of Fe occurred at a Fe dose of 0.2 mM , where the 38 mg/L DOC treatment had a higher PO_4 concentration remaining than the 60 mg/L DOC treatment, but both had much higher concentrations of PO_4 remaining than the lowest DOC treatment (Figure 1a).

[20] Addition of aluminum sulfate to reduce PO_4 concentrations under the same conditions showed a similar pattern of inhibition of phosphorus removal at high DOC concentrations (Figure 1b). Low DOC concentrations (21 mg/L) required 0.2 mM Al addition to reduce PO_4 concentrations below $0.32 \mu\text{M}$ at the 2 h interval. Under high DOC concentrations (60 mg/L), 0.6 mM Al was required to achieve equivalent phosphorus concentrations. If we compare the effects of DOC on phosphate removal with Fe versus Al addition, the highest DOC concentration inhibited PO_4 removal with Al addition more than was the case with Fe addition.

[21] Since our analysis indicated that PO_4 remained in solution after addition of Al to water containing high DOC concentrations, we wanted to be certain that the molybdate blue analytical procedure was actually detecting free PO_4 and not perhaps some loosely bound colloidal P that passed

through the $0.45 \mu\text{m}$ membrane filter and then behaved as PO_4 during analysis. The PO_4 in the Al treated supernatant, and NaHPO_4 in deionized water, both passed through the ultrafiltration membrane and matched the results in $0.45 \mu\text{m}$ membrane filtered solution, indicating that the P detected by the molybdate blue procedure in the supernatant was not colloidal.

[22] After allowing precipitates to settle for 4 days, the total P remaining in suspension or solution again showed that high concentrations of DOC inhibited the removal of total P from the water column (Figure 2). Under low DOC concentrations, total phosphorus concentrations of $0.32 \mu\text{M}$ 4 days after metal addition (initial $\text{PO}_4 = 2.58 \mu\text{M}$) could be achieved with a 0.2 mM FeCl_3 dose. However, with DOC concentrations of 60 mg/L , total phosphorus levels were still well above $0.32 \mu\text{M}$ even at the 1.0 mM ferric chloride dose. A significant proportion of PO_4 was found to remain in solution even 4 days after Fe addition, and under high DOC concentrations, 1.0 mM FeCl_3 was required to reduce PO_4 levels below $0.32 \mu\text{M}$ (not shown). Although DOC also affected total P removal using aluminum sulfate additions, the effect was not nearly as great as with ferric chloride additions (Figure 2a versus Figure 2b). Lower total phosphorus concentrations were achieved with even higher DOC levels with Al additions compared to the corresponding ones with Fe additions. At all levels of DOC, 0.2 mM Al^{3+} was capable of lowering TP concen-

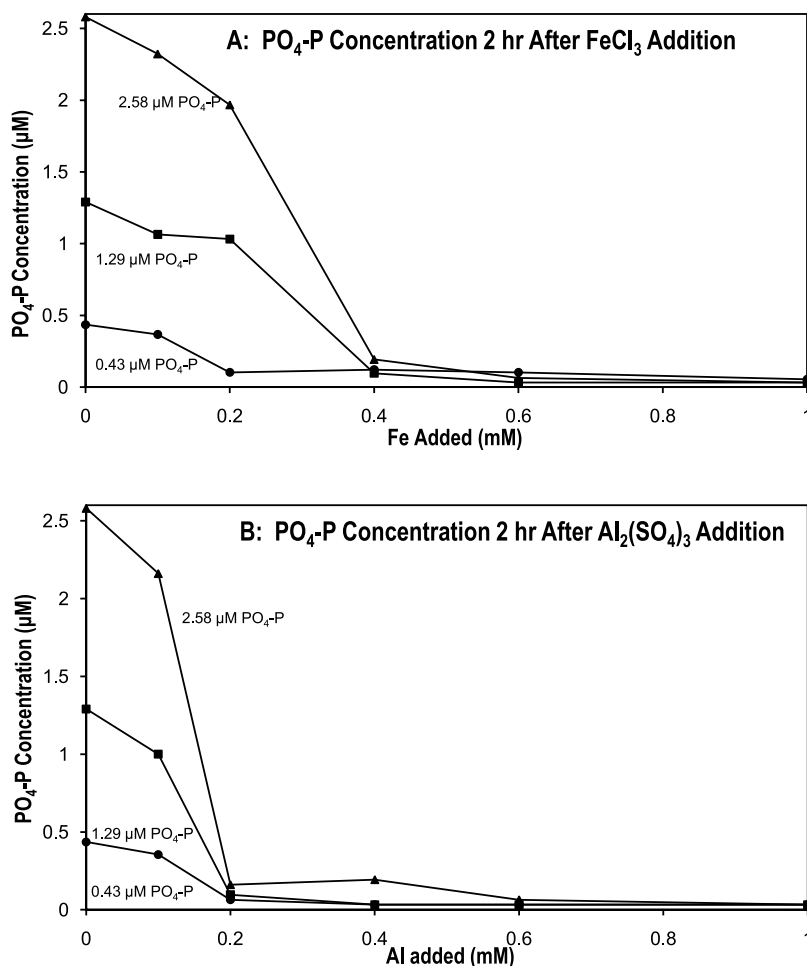


Figure 3. Effect of initial PO₄ concentration on PO₄ removal 2 h after (a) ferric chloride addition and (b) aluminum sulfate addition.

trations below 0.81 µM, but for the highest level of DOC concentration, it still required a dose of 0.6 mM Al to result in total P concentrations of 0.32 µM or lower. Similarly, PO₄ concentrations at 4 days were reduced to below 0.32 µM for low, intermediate, and high DOC levels with only 0.2 mM Al addition (not shown).

[23] Addition of ferric chloride and aluminum sulfate to Everglades water containing low (0.44 µM), intermediate (1.29 µM), and high (2.58 µM) initial PO₄ levels demonstrated that PO₄ removal 2 h after metal addition was approximately proportional to the initial PO₄ concentration present in the water sample. PO₄ removal with 0.1 to 0.2 mM Fe addition was proportional to the initial PO₄ concentration (Figure 3a). However, 0.4 mM or greater concentrations of Fe reduced PO₄ concentrations close to the limit of detection regardless of initial PO₄ concentration. Likewise, removal of PO₄ 2 h after 0.1 to 0.4 mM Al addition was also approximately proportional to the initial PO₄ concentration; however, addition of 0.6 mM or greater concentrations of Al reduced PO₄ to very low concentrations regardless of initial PO₄ concentration (Figure 3b).

3.2. Rates of Change of P Forms

[24] Results of the experiment to determine the rate of change in PO₄, particulate phosphorus and dissolved organic phosphorus forms after addition of 0.4 mM Fe to Everglades

water (0.44 µM PO₄ and 38 mg/L DOC) showed that dissolved species of phosphorus are rapidly removed from the water column after a sufficient addition of ferric chloride (Figure 4). This dose of Fe was chosen because it was the lowest dose that removed most of the PO₄. After approximately 10 s most of the phosphorus in solution was converted to particulate phosphorus. The timing of the sample at 10 s or 0.0028 h was approximate, however the sample was filtered within 2 s of withdrawal. Dissolved organic phosphorus and PO₄ concentrations decreased to less than 0.16 µM approximately 10 s after ferric chloride addition. Most particulate phosphorus remained suspended in the water column during the first 2 h after metal addition. By 96 h, particulate P concentrations declined to below 0.16 µM. PO₄ and DOP concentrations remained below 0.16 µM from 10 s through 4 days after metal addition. Kinetics of the reaction seem to indicate the removal of PO₄ was due to some very fast adsorption or binding while the Fe was still in the colloidal or suspended phase.

[25] While we were concerned mainly with the longer-term settling of total P at 4 days, there was a substantial settling and removal of particulate P within the 2 h period, but only in cases of the highest metal doses, and it was more pronounced in the lower DOC treatments. For Al doses at the lowest level of DOC and an initial total P of 2.97 µM, the average total P remaining in the water column at 2 h was

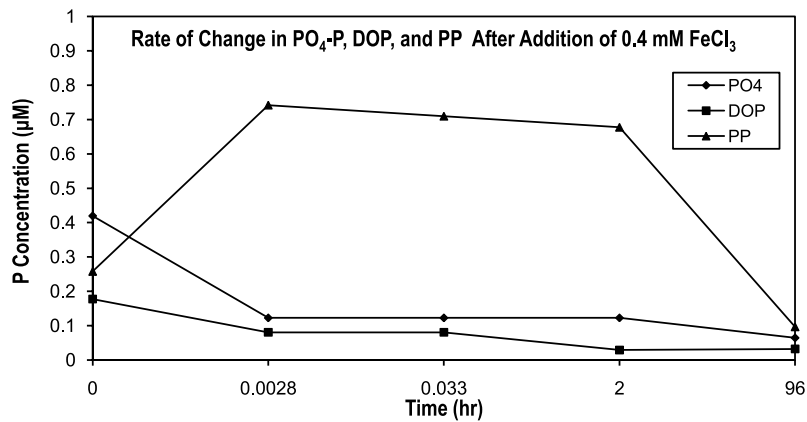


Figure 4. Rate of change in phosphorus forms after addition of 0.4 mM ferric chloride. Dissolved species of phosphorus were rapidly converted to particulate P, which remained suspended in the water column during the first 2 h after metal addition. PP represents particulate P.

2.97, 3.07, 0.84, 0.45, and 0.42 μM , for doses of 0.1, 0.2, 0.4, 0.6, and 1.0 mM Al, respectively (not shown). But for the highest level of DOC (60 mg/L) the corresponding average total P concentrations remaining were 2.91, 2.91, 2.97, 2.81, and 0.58 μM . For Fe doses at the lowest level of DOC and an initial total P of 2.97 μM , the average total P remaining in the water column at 2 h was 2.97, 2.87, 1.68, 0.52, and 0.45 μM , for doses of 0.1, 0.2, 0.4, 0.6, and 1.0 mM Fe, respectively (not shown). But for the highest level of DOC (60 mg/L) the corresponding average total P remaining was 2.97, 2.71, 2.71, 2.65, and 0.61 μM . Thus again, we see an interference of high levels of DOC with the rapidity of removal of total P from the water column.

[26] That DOP was removed by ferric chloride addition is important in the Everglades since a substantial fraction of the total P is dissolved organic phosphorus [Qualls and Richardson, 2003; Cooper *et al.*, 2005]. The DOP fraction is operationally defined by difference, but it never increased after addition of metal and PO₄. So, it did not appear that fine colloid-bound P generated by the metal addition was “falsely” appearing in the DOP fraction (filtered total P – PO₄) that passed through the filter. In addition, dissolved organic P in water from the Everglades Nutrient Removal Project has been characterized by high-resolution P-specific mass spectrometry with ICP ionization for quantitative analyses, and ultrahigh-resolution MS experiments with electrospray ionization for qualitative identification of individual compounds [Cooper *et al.*, 2005].

3.3. Effect of Controlled pH

[27] In the Everglades water experiments without addition of acid or base to control the pH, the pH dropped from 7.8 to 7.7, 7.4, 7.3, and 7.1 with additions of Al of 0.1, 0.2, 0.4, 0.6, and 1.0 mM, respectively, after 4 days owing to hydrolysis and subsequent loss of CO₂. Similar pH levels were found in the corresponding ferric chloride treatments. The pH drop was regulated by the buffering effects of the moderately high alkalinity (8.6 meq/L) of the Everglades water. The experiments using controlled pHs of 7.8 and 7.0 showed similar effects to those of the uncontrolled pH experiments (Table 2 versus Figures 1 and 2). Thus the results were not dependent on the pH changes during the aluminum sulfate or ferric chloride experiments.

3.4. Phosphate Precipitate Fractionation

[28] Our operational fractionation of the metal oxyhydroxide-phosphate precipitate suggested that most of the P was strongly bound to the precipitate. The initial PO₄ level was 1.29 μM prior to addition of 0.4 mM Al. Less than 0.03 μM PO₄ remained free in the supernatant. Analysis of the extract from the anion exchange membrane indicated that only 0.15 μM PO₄ (11.8%) was exchangeable at low ionic strength. Analysis of the 1M K₂SO₄ extract of the remaining precipitate indicated that only an additional 0.04 μM PO₄ was exchangeable at high ionic strength. The remainder (1.1 μM) of the original PO₄ was therefore relatively strongly bound. This seems to indicate that the majority of phosphorus removed by aluminum sulfate addition would remain bound in the aluminum oxyhydroxide-phosphate precipitate rather than being easily exchanged and easily subject to biological uptake.

[29] Ferric chloride might seem an unlikely choice for removing and stabilizing phosphorus in an anaerobic soil, since ferric iron is subject to reduction under anaerobic conditions such as those found just below the soil surface in the Everglades [Qualls *et al.*, 2001]. However, in a parallel study in which radio-labelled PO₄-³²P was precipitated in the same manner as in this study and buried in a peat soil microcosm, less than 1% of radio-labelled PO₄-³²P was remobilized into the water column over 139 days of incubation [Sherwood and Qualls, 2001]. The stabilization of P in the form of vivianite might have contributed the stability of the precipitated P under these conditions [Sherwood and Qualls, 2001].

3.5. Evidence for a Hypothetical Mechanism of DOC Interference

[30] Citric acid was substituted for natural DOC in an experiment to determine whether citric acid could mimic the inhibitory effects of natural DOC on the relationship between aluminum added and the PO₄ concentration remaining in the water column. A concentration of only 15 μM citric acid inhibited PO₄ removal with 200–300 μM Al (Figure 5) in a manner similar to natural DOC (Figure 1b). One hypothesis that might explain this effect is that Al³⁺ was complexed by the citrate, preventing its precipitation and reaction with PO₄. However, the stoichiometry of the experiment indicates that the mechanism cannot be the formation of an Al-citrate complex since there are far too

Table 2. Effect of DOC in Inhibiting PO₄ and Total P Removal at Controlled pH of 7.8 and 7.0^a

Adjusted pH (SU)	Metal Dose (mM)	PO ₄ Concentration at 2 h (μM)		Total P at 96 h (μM)	
		DOC = 21 mg/L	DOC = 60 mg/L	DOC = 21 mg/L	DOC = 60 mg/L
<i>Fe</i>					
7.8	0	2.6 ± 0.03	2.6 ± 0.03	2.9 ± 0.04	2.9 ± 0.06
7.8	0.1	0.13 ± 0.02	2.1 ± 0.03	0.52 ± 0.03	2.7 ± 0.1
7.8	0.2	0.06 ± 0.01	1.0 ± 0.13	0.39 ± 0.02	2.7 ± 0.1
7.8	0.4	0.06 ± 0.01	0.32 ± 0.02	0.2 ± 0.02	2.4 ± 0.09
7.8	0.6	0.03 ± 0.01	0.1 ± 0.02	0.2 ± 0.02	2.4 ± 0.1
7.8	1.0	0.03 ± 0.01	0.1 ± 0.02	0.2 ± 0.03	0.45 ± 0.08
7.0	0	2.6 ± 0.03	2.6 ± 0.03	2.9 ± 0.06	2.9 ± 0.06
7.0	0.1	0.1 ± 0.03	2.1 ± 0.05	0.55 ± 0.04	2.7 ± 0.07
7.0	0.2	0.06 ± 0.03	1.0 ± 0.04	0.4 ± 0.04	2.6 ± 0.08
7.0	0.4	0.06 ± 0.03	0.3 ± 0.06	0.2 ± 0.02	2.4 ± 0.08
7.0	0.6	0.03 ± 0.01	0.06 ± 0.04	0.2 ± 0.03	2.4 ± 0.07
7.0	1.0	0.03 ± 0.01	0.06 ± 0.04	0.2 ± 0.05	0.45 ± 0.1
<i>Al</i>					
7.8	0	2.6 ± 0.05	2.6 ± 0.05	2.9 ± 0.06	2.9 ± 0.06
7.8	0.1	0.4 ± 0.02	2.5 ± 0.03	0.81 ± 0.08	2.1 ± 0.03
7.8	0.2	0.1 ± 0.03	2.2 ± 0.06	0.55 ± 0.04	0.81 ± 0.02
7.8	0.4	0.03 ± 0.02	1.0 ± 0.02	0.2 ± 0.02	0.60 ± 0.02
7.8	0.6	0.03 ± 0.02	0.06 ± 0.02	0.1 ± 0.04	0.3 ± 0.02
7.8	1.0	0.01 ± 0.01	0.06 ± 0.02	0.1 ± 0.02	0.2 ± 0.02
7.0	0	2.6 ± 0.05	2.6 ± 0.05	2.9 ± 0.06	2.9 ± 0.06
7.0	0.1	0.4 ± 0.03	2.5 ± 0.08	0.81 ± 0.06	2.0 ± 0.08
7.0	0.2	0.1 ± 0.02	2.2 ± 0.1	0.52 ± 0.06	0.80 ± 0.07
7.0	0.4	0.03 ± 0.02	0.74 ± 0.06	0.2 ± 0.05	0.52 ± 0.06
7.0	0.6	0.03 ± 0.02	0.06 ± 0.03	0.1 ± 0.03	0.3 ± 0.04
7.0	1.0	0.03 ± 0.01	0.03 ± 0.01	0.1 ± 0.03	0.2 ± 0.05

^aNote that PO₄ concentrations are shown for the 2 h sample and total P is shown for the 96 h sample. Means of three replicates and standard errors are shown.

few μmoles of citric acid to tie up a large proportion of the Al³⁺ as a complex. There were only 15 μM of citric acid available to complex 200–300 μM of Al³⁺. This quantity is too little even if we assumed a greater than one to one Al³⁺ to citric acid ratio for complex formation. In addition, modeling with the MINTEQA2 [Allison *et al.*, 1991] chemical equilibrium program confirmed that only a very small percentage (4.8%) of a 0.4 mM Al addition would be complexed in the form of AlCitrate by 20 μM citrate. Similarly, only 4.7% of a 0.4 mM iron addition would be complexed by a 20 μM citrate addition in the form of FeCitrate.

[31] Young and Comstock [1986] suggested that the complexation between iron and humic acids occurs in such a manner that a humic acid molecule becomes bound to the free hydroxyl groups on the end of a chain of a polynuclear Fe colloid. The hypothetical reaction scheme upon addition of ferric chloride to water is the formation of iron hydroxide complexes, which undergo further hydrolysis to form a colloidal chain. Phosphate ions and the carboxyl groups of humic acids may compete for adsorption sites on the colloidal Fe hydroxide (Figure 6). Since hydrolysis reactions occur rapidly after addition of ferric chloride or aluminum sulfate to water, dissolved organic matter would

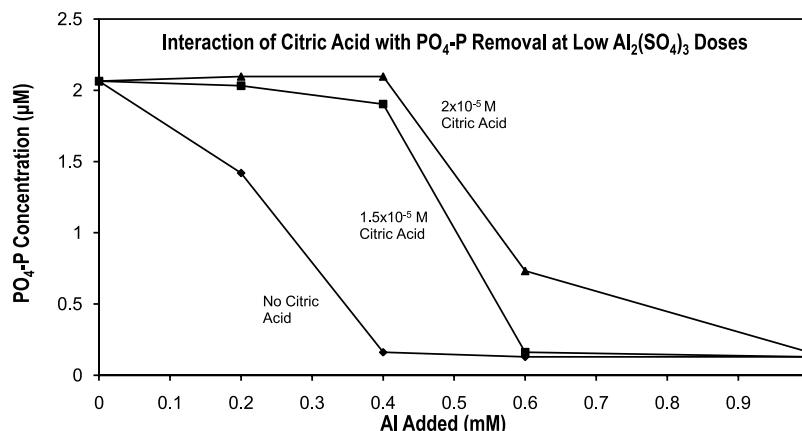


Figure 5. Effect of citric acid on PO₄ removal with aluminum sulfate. A concentration of 15 μM citric acid inhibited PO₄ removal with 200–300 μM aluminum sulfate in a manner similar to natural DOC.

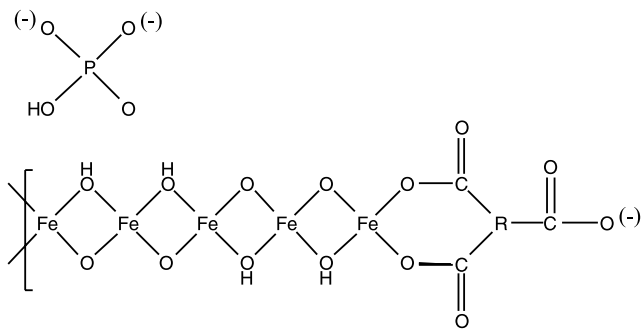


Figure 6. Hypothetical scheme for the inhibition of PO_4 removal under high DOC concentrations. The “R” group and the three adjacent carboxyl groups represent a humic acid molecule bound to two terminal reactive hydroxyl groups of a Fe-hydroxide polynuclear chain, blocking the adsorption sites from a phosphate molecule indicated as remaining in the solution. Modified from *Young and Comstock* [1986].

be essentially out-competed by hydrolysis reactions at first. This proposed mechanism would allow a relatively small number of humic or citric acid molecules to block the reactive hydroxyl groups of a large number of Fe-hydroxide subunits. There may be other hypotheses that explain these results, but this mechanism is consistent with the stoichiometry of the citrate experiments. However, further work and different techniques would be necessary to confirm that this mechanism would indeed explain the observed inhibition of P removal.

4. Broader Applicability of Study

[32] In this study, we found that high concentrations of natural DOC inhibited both the short-term removal of PO_4 , and the longer-term removal of total P from the water column. The ability of citric acid to mimic the inhibitory effects also suggests the results of the study are broadly applicable to wetland and other waters with high natural organic acid concentrations. Citric acid and natural organic matter (e.g., humic substances) have been shown to have analogous effects in the crystallization of aluminum hydroxides [Huang *et al.*, 2005; Hua *et al.*, 2008]. Concentrations in the range of 21–60 mg/L DOC would be unusual in many nonwetland waters, however these concentrations are very characteristic of waters in the Northern Everglades and many other wetland areas. In addition, the carboxylic acid content of natural dissolved organic matter from many sources is not highly variable [Ritchie and Perdue, 2003] so that the more commonly measured DOC levels are proportional to the carboxylic acid content. The pH range used in this study would be representative of neutral to slightly alkaline waters, but most wetlands used to treat eutrophic water tend to be neutral to slightly alkaline [Kadlec and Knight, 1996]. Although these experiments were performed using water from a wetland area, similar effects may also be found to occur in wastewater exhibiting high DOC concentrations.

[33] In these laboratory benchtop studies, the settling of flocculent matter was evaluated in a controlled manner. However, field-scale pilot studies would be needed to

evaluate the stability of the settled floc in beds of wetland vegetation subject to currents and winds. In field-scale applications, a rapid mixing phase, represented in our study by 2 min of rapid mixing, would presumably be achieved in a dosing and mixing basin, as is common in applications in which tributary waters are dosed and mixed before discharge to a lake or wetland [e.g., *Pilgrim and Brezonik*, 2005; *Lewandowski et al.*, 2003]. In water and wastewater plant operations, rapid mixing is commonly followed by several minutes of slow mixing to accelerate to agglomeration of floc so that it can be settled in a matter of hours [Hammer and Hammer, 2007]. It is possible that sedimentation rate of the floc was slowed in our study by the absence of a slow mixing phase, but in the case of a wetland operating as a sedimentation basin with a residence time of days, rapid sedimentation within hours would not be necessary and may not be feasible. Perhaps wind and slow advection around plant stems might also act to encourage large floc formation.

[34] Application to large flows of water such as those entering the Stormwater Treatment Areas of the Everglades would obviously require very large scale quantities of metal salts. Nevertheless, many large-scale applications of metal salts to lakes and other waters have been made over many years [Cooke *et al.*, 2005]. For example, 7000 t of aluminum sulfate (3.8% Al) were applied to Lake Susser See in Germany between 1977 and 1992, and the tributaries have been treated with FeCl_3 since 1993 [Lewandowski *et al.*, 2003]. It has also been proposed to add aluminum sulfate to inflows of the Salton Sea, a large lake in California, where water flows average 1.6 km³/a [Rodriguez *et al.*, 2008]. There may also be other potential ecological effects in wetlands that may need to be investigated using pilot-scale studies, such as inhibition of benthic fauna with heavy accumulations of floc, as has been noted in lakes [Cooke *et al.*, 2005]. An evaluation of other ecological effects during the long experience in additions to lakes is summarized by Cooke *et al.* [2005]. In a study of the addition of aluminum sulfate to wetland mesocosms, Malecki-Brown *et al.* [2007] found that microbial activity was reduced after aluminum addition.

[35] Wetlands are often used for removal of nutrients; however, it may be possible to supplement natural mechanisms with chemical methods to remove phosphorus from the water column of wetlands. Through this study, we have found that phosphate removal from high DOC waters may be feasible, but may require considerably higher doses of ferric chloride or aluminum sulfate than are otherwise needed in waters with lower DOC concentrations.

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