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Spectrophotometric determination of phosphate in matrices from sequential leaching of sediments

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Abstract

The speciation of Phosphorus (P) in geologic materials can inform diverse investigations in such as aquatic biogeochemistry, soil sciences, and paleo-oceanography. Here, we describe in detail a set of sequential extraction schemes to measure P concentrations from several operationally defined phases: pore water, exchangeable or loosely sorbed P, Fe-bound P, P associated with biogenic apatite, P associated with authigenic apatite and carbonate forms, P associated with detrital apatite and inorganic forms, and organic P. The two benefits of the procedures we describe compared to prior methodological studies are: (1) fast, simple and inexpensive colorimetric methods have been developed for P determination in all the different matrices; and (2) the Fe-bound phosphorus pool is separated into two fractions, allowing the P associated with most reactive Fe-fraction to be distinguished from P bound to the remainder of the Fe-oxides. Adaptations of these procedures to optimize the characterization of P pools in a range of material types are also outlined.

Phosphorus (P) is a limiting nutrient for primary productivity, and consequently it is thought to control the atmospheric oxygen level on geological time scales (Van Cappellen and Ingall 1994). Phosphorus-rich fertilizer is used in agriculture, and its use has serious implications for future food security (Cordell et al. 2011). Primary P has an igneous origin and occurs mostly as apatite in bedrock, or in uplifted sedimentary deposits. Once P is released from rocks through weathering, dissolved phosphate ions can be transported in natural waters, become adsorbed onto inorganic particles, or be taken up and incorporated into biomass, where P can participate in a variety of different metabolic reactions and pathways (Ruttenberg 2004).

Degradation of organic matter via various heterotrophic metabolic pathways releases orthophosphate to natural waters, where it is available for biological uptake, sorption to mineral phases (Lijklema 1980; Lajtha and Harrison 1995), or incorporation into authigenic minerals such as carbonate fluorapatite (CFA) (e.g., Berner et al. 1993). The adsorption of phosphate on Fe- and Al-oxide and oxyhydroxide has been extensively studied in soil science because phosphorus is a limiting nutrient in terrestrial ecosystems, and sorptive removal of natural or fertilizer phosphorus impacts the production level of crops and forests (e.g., Barrow 1983; Guzman et al. 1994; Frossard et al. 1995). Fe(III) oxides/oxyhydroxides are subject to reductive dissolution, and consequently redox conditions play an important role in P-bioavailability.

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The main process of phosphorus removal from the aquatic systems is burial within sediments (Broecker and Peng 1982; Ruttenberg 1992). Exchange between sediment and overlying water takes place through benthic biogeochemical processes, including organic-P mineralization, redox-driven Fe–P cycling, and benthic phosphorus efflux from sediments (Mortimer 1941; Krom and Berner 1980; Sundby et al. 1992). A portion of the pore-water phosphate derived from organic matter mineralization may be adsorbed onto detrital or authigenic iron oxyhydroxides in the oxidized zone of the sediment (e.g., Krom and Berner 1980; Sundby et al. 1992; Slomp and Van Raaphorst 1993; Jensen et al. 1995; Slomp et al. 1996a*a*,b). Once advected into the reduced zone of sediments through burial or bioturbation, the most reducible fraction of Fe(III) phases can be solubilized, leading to a release of phosphate (Heggie et al. 1990; Sundby et al. 1992; Anschutz et al. 1998). Eventually, P can be buried over a long period as relict organic-P, P associated with refractory iron oxides, and apatite. Apatite can be formed by igneous, biogenic, and authigenic processes. Authigenic apatite is predominantly CFA that forms sedimentary phosphorite ore and modern phosphorites (Baturin et al. 1972; Jahnke et al. 1983; Froelich 1988; Filippelli 2011). CFA has a greater solubility than igneous apatite, so much so that CFA can be chemically separated from apatite (Ruttenberg 1992). Other authigenic phosphate minerals have been described in marine or lacustrine sediment. For example, vivianite is a ferrous iron phosphate that forms in anoxic or suboxic sediments, where dissolved Fe(II) can

accumulate in pore waters (Martens et al. 1978; Ruttenberg and Goni 1997a,b; Anschutz et al. 1999). Biogenic apatite originates from the bones and teeth of vertebrate organisms.

To be able to describe the burial, diagenesis, bioavailability, and residence time of P in land and aquatic systems, it is necessary to determine P solid speciation. Because total P represents generally less than 0.5% of particle weight, P-bearing phases are difficult to separate from the matrices of most geologic materials. They cannot be detected with mineralogical methods such as X-ray diffraction and scanning electron microscopy, except for in P-rich phosphorites (Schuffert et al. 1990).

Several authors have developed chemical extraction techniques that permit leaching of selectively operationally defined P fractions such as P loosely sorbed to particles, P linked to Fe(III) phases, apatite and particulate organic-P. Loosely sorbed P is the fraction that can be easily desorbed in water in response to a decrease in dissolved P concentration, or an increase in salinity (Fox et al. 1986; Deborde et al. 2007). This exchangeable phosphate pool is determined with rinses of pure water (Psenner et al. 1984), ammonium chloride (Williams et al. 1971), sodium chloride (Li et al. 1973), magnesium chloride (Ruttenberg 1992), or the infinite dilution technique (Aminot and Andrieux 1996).

Several techniques have been developed to extract P bound to Fe(III) phases. The leaching solution consists of a reducing solution buffered at medium pH. The citrate-dithionite buffered (CDB) solution (Aguilera and Jackson 1953; Mehra and Jackson 1960) is one of the most often used solution to measure Fe(III)-bound P (Ruttenberg 1992; Jensen and Thamdrup 1993). CDB solution lacks selectivity because dithionite is a strong reducing compound that reduces amorphous and crystalline Fe(III)-oxides and oxyhydroxides. Selective extraction schemes were developed for the partitioning of Fe(III)-phase according to their reactivity (e.g., Poulton and Canfield 2005). Easily reducible oxides (ferrihydrite and lepidocrocite) are selectively leached with hydroxylamine-HCl (Chester and Hughes 1967) and ammonium oxalate solutions (Philips and Lovley 1987), although the latter also reduces magnetite (Poulton and Canfield 2005). An ascorbate solution buffered at pH 8 also extracts selectively the most reactive fraction of Fe(III) (Kostka and Luther 1994). That is why several studies defined the most reactive fraction of Fe-bound P as the fraction extracted with ascorbate (Anschutz et al. 1998; Caetano and Vale 2002; Rozan et al. 2002; Hyacinthe and Van Cappellen 2004). Phosphorus associated with apatite is generally extracted with a 1 mol L^{-1} HCl solution (Williams et al. 1971; Williams et al. 1976; Hieltjes and Lijklema 1980; Psenner et al. 1988). Because a large fraction of Fe-bound P also can be dissolved in 1 mol L^{-1} HCl, the partitioning of P-bearing phases needs a sequential extraction procedure (Aspila et al. 1976; Lucotte and D'anglejan 1985; Jensen et al. 1995; Deborde et al. 2007; Ruttenberg et al. 2009) in which loosely sorbed P is extracted first, then Fe-bound P is extracted with a reducing solution at near-neutral pH, followed by an acid leaching apatite extraction, and finally organic P forms.

Ruttenberg (1992) and Ruttenberg et al. (2009) made a major improvement by introducing a sodium acetate-extraction step to separate authigenic apatite from detrital apatite in marine sediments. Schenau and De Lange (2000) proposed adding a NH_4Cl extraction step to separately quantify biogenic hydroxyapatite as distinct from authigenic apatite.

Organic-P can be defined either from the difference between total-P and all extracted inorganic-P forms (Aspila et al. 1976), or from a specific leaching solution at the end of a sequential extraction procedure. Total P can be obtained from alkaline fusion followed by acid dissolution or from strong-acid dissolution of the residue of sequential extraction. Once the inorganic forms are removed, organic-P can be extracted directly using HCl after combustion (Lucotte and D'anglejan 1985; Ruttenberg 1992; Jensen et al. 1995). It also can be leached with concentrated sodium hydroxide (Bastula and Krivonosova 1973), with a buffered solution of the surfactant sodium dodecyl sulfate (Vink et al. 1997), or with sulfuric acid (Deborde et al. 2007). NaOH extraction has been used in soil sciences to define mineralizable organic-P because it does not extract the refractory organic-P fraction (Stewart and Tiessen 1987).

Dissolved inorganic phosphate concentrations are commonly determined with the spectrometric method based on the formation of the blue form of reduced phosphomolybdate (molybdenum blue) (Murphy and Riley 1962). The molybdenum blue method is generally used to measure phosphate from particle leaching when the final matrix does not interfere with colored complex formation. The solution that results from 1 mol L^{-1} HCl or H_2SO_4 leaching, and total residue dissolution are acidic. In such solutions, P can be analyzed with the Murphy–Riley method only if the solution is highly diluted. This affects the resolution and the precision of measurements. Solutions that contain a reducing agent, such as the ascorbate and the CDB solutions, produce an interference with reduction of phosphomolybdate to form the blue complex. To measure P with the Murphy–Riley method in the CDB solution, an extraction step is needed (Watanabe and Olsen 1965; Ruttenberg et al. 2009). In this method, the molybdate complex is extracted into isobutanol before reduction with dilute $SnCl_2$. The interferants are left behind in the aqueous phase, having been complexed with $FeCl_3$. This technique works well, but it takes a relatively long time to implement. To simplify the P analysis in CDB extract, Suzumura and Koike (1995) proposed a method based on an extraction technique onto a nonpolar solid phase of extracted phosphate converted into 12-molybdophosphoric acid. The complex adsorbed on the resin is eluted with NaOH and then determined by the standard molybdenum blue method. Huerta-Diaz et al. (2005) proposed to remove P from the CDB solution although alkaline precipitation of $Mg(OH)_2$ by adding NaOH and $MgCl_2$ according to the MAGIC technique (Karl

Table 1. Sequential extraction procedure to characterize P fractions in soils, suspended particles and sediments and associated colorimetric method. RM: reagent mixture; S: Sample or standard solution in the resulting matrix. R1: ammonium molybdate solution; R2: sulfuric acid solution; R3: potassium antimony(III) trihydrate oxytartrate solution; R4: ascorbic acid solution. Ratios correspond to volumetric ratios. Hours represent the duration of each extraction step.

Extracted fraction	Extractant	Chemical reaction	Colorimetric method
Loosely sorbed P	IDE H_2O (+Toluene) 3 × 24 h pH in situ	Desorption without bacterial activity in artificial water	RM (Murphy and Riley 1962): 1/5R1 + 1/2R2 + 1/10R3 + 1/5R4 Cuvette: 1/10 RM + 1 S
Amorphous Fe(III)—bound P	Ascorbate (20 g L^{-1}) 24 h pH 8	Reduction of amorphous Fe(III)-oxides and dissolution of associated P	RM: 4/7R1 + 2/7R2 + 1/7R3 Cuvette: 0.5 mL RM + 1.3 mL S + 130 μL R4
Crystalline Fe(III)—bound P	CDB 4 h pH 7	Dissolution of reducible Fe(III)-oxides and associated P	RM: 4/7R1 + 2/7R2 + 1/7R3 Cuvette: 0.5 mL RM + 1.3 mL S + 200 μL R4
Biogenic hydroxyapatite—bound P	NH_4Cl (2 mol L^{-1}) 16 h pH 7	Dissolution of biogenic hydroxyapatite and associated P	RM: 2/5R1 + 2/5R2 + 1/5R3 Cuvette: 350 μL RM + 2 mL S + 200μL R4
Authigenic CFA—bound P	Na-acetate (1 mol L^{-1}) 16 h pH 4	Dissolution of authigenic CFA and associated P	RM: 2/5R1 + 2/5R2 + 1/5R3 Cuvette: 350 μL RM + 2 mL S + 200μL R4
Detrital apatite and carbonate—bound P	HCl (1 mol L^{-1}) 16 h pH 2.5	Acid dissolution of carbonates and apatite	RM: 2/5R1 + 2/5R2 + 1/5R3 Cuvette: 350 μL RM + 2 mL S + 200μL R4
Organic-P	H_2SO_4 (18 mol L^{-1}) 16 h pH < 0	Acid dissolution of organic matter	RM: 2/3R1 + 1/3R3 Cuvette: 150 μL RM + 5 mL S + 100 μL R4

and Tien 1992). In this, P is quantitatively sorbed on $Mg(OH)_2$, the precipitate is re-dissolved in HCl and analyzed with the Murphy–Riley method. Alternatively, the most widely used techniques for P measurement in complex matrix are noncolorimetric methods such as ICP-AES or ICP-MS (Slomp et al. 1996b; Slomp et al. 1998; Schenau and De Lange 2000; Filippelli 2001; Van Der Zee et al. 2002; Harrell and Wang 2007; Machesky et al. 2010).

The aim of this article is to present in detail a simple and standardized method to determine the speciation of P in geological material. The method can provide information in different areas, such as aquatic biogeochemistry, soil sciences, and paleo-oceanography. Our objective is not to replace the well-established P sequential extraction techniques cited above (e.g., Ruttenberg 1992). Our goal is to focus on two improvements resulting from our work: (1) the separation of iron bound P pool into two specific fractions, the one that can be easily mobilized through redox changes, and the other that may be fossilized; (2) the development of a colorimetric method for each leaching matrix. For that, we describe in detail a sequential procedure in eight steps that can be used partly or entirely to characterize operationally defined P fractions in all types of soil, suspended particles and sediments. The first step is the measurement of P in interstitial water in contact with the solid fraction. The other seven steps are selective leaching procedures of the particulate fraction. The specific colorimetric method determined for each leaching matrix is described. The needed equipment is inexpensive and the work can be done by every laboratory that is able to measure dissolved P colorimetrically.

Materials and procedures

The first stage of the different extraction steps consists of measuring dissolved phosphate in waters that are in contact

with particles. For that we use the common molybdenum blue method. In the following, we present the different selective leaching procedures used in our sequential extraction schemes (Table 1). Loosely sorbed P is extracted using the infinite dilution extrapolation (IDE) after Aminot and Andrieux (1996). Phosphorus associated with Fe(III)-oxides is measured first in an ascorbate solution, which reduces only the amorphous fraction, and second in a CDB solution, which dissolves the remaining crystalline fraction of Fe(III)-oxides. Then, phosphorus associated with calcium is measured in three successive leaching solutions: ammonium chloride, sodium acetate, and HCl (1 mol L^{-1}), which leach P from biogenic hydroxyapatite, authigenic CFA, and detrital apatite, respectively. Lastly, organic P is extracted with sulfuric acid. The chemical analysis of extracted phosphate is described in detail. As we present specific variations of the molybdenum blue method for the different extraction steps (Table 1), the different solutions used are briefly presented below (Murphy and Riley 1962).

Phosphate standard solution and reagents (after Murphy and Riley 1962)

Phosphate standard stock solution (5 mmol L^{-1}): dry KH_2PO_4 in a 100°C oven and cool in desiccator. Weigh out 0.6804 g KH_2PO_4 and transfer to a 1000-mL volumetric flask. Dilute to 1 L with MQ-H_2O. This solution is stable for months if stored refrigerated.

R1: ammonium molybdate: 15 g $(NH_4)_6Mo_7O_{24}$, 4 H_2O in a glass beaker. Add 500 mL MQ-H_2O and stir on magnetic stir plate at 50°C until dissolved. Store in a glass bottle in refrigerator; stable in darkness for months.

R2: 2.5 mol L^{-1} H_2SO_4 solution (in hood): measure out 140 mL concentrated H_2SO_4 in a 250-mL graduated cylinder and 900 mL MQ-H_2O in a 1-L graduated cylinder. Put one half of the MQ-H_2O into a 2-L Pyrex glass beaker with a stir bar. Add the 140 mL H_2SO_4 very slowly while stirring on magnetic stir plate. Add slowly the remaining water. Wait until cooling and transfer to a glass bottle. Stable at room temperature.

R3: weigh 0.68 g $K(SbO)C_4H_4O_6$ and transfer into a glass beaker. Add 500 mL MQ-H_2O and swirl to dissolve. Stable a few months stored refrigerated.

R4: L-ascorbic acid solution: weigh 1.08 g $C_6H_8O_6$ in a vial and add 10 mL MQ-H_2O. This is an unstable solution that must be prepared the same day of P measurement.

Description of the methods

Colorimetric method for determination of phosphate in (interstitial) water

The first step consists of measuring dissolved phosphate in waters that are in contact with particles. Pore waters of soils and interstitial waters of sediments collected in rivers, lakes and seas are separated from the particles through centrifugation. For muddy sediments a 50-mL centrifuge tube may be used, and supernatant waters are filtered through a 0.2-μm cellulose acetate syringe-membrane. Interstitial waters of sandy sediments and sandy soils are extracted using Vivaspin™ 20 centrifugal extractor tube with 0.2-μm PES membrane.

Make standard solutions by diluting the stock solution with MQ-H_2O or phosphate-free seawater. Prepare water-R1R2R3 mixed reagent: in a polypropylene 1-L bottle mix 200 mL R1, 500 mL R2, and 100 mL R3. This solution is stable for months refrigerated in darkness. Mix 10 mL newly prepared R4 with 40 mL water-R1R2R3 solution. Put 500 μL mixed reagent in every 10 mL PP tube stored in a rack. Add 5 mL standard solution or sample to each cuvette. Wait for 5 min and measure at 885 nm on a spectrophotometer using a 50 mm-long glass cuvette. Measure the blank standard first and zero the spectrophotometer. The 1 μmol L^{-1} standard must be at around 0.100 absorbance unit in these glass cuvettes. For interstitial water of muddy sediments, increase the range of standard solution up to 20 μmol L^{-1}, and use 10-mm plastic cuvettes. The water-reagent mixture can be done directly in the cuvette (100 μL reagent first and then 1 mL sample or standard). The standard Murphy and Riley method is suitable to measure P in the different matrix used to extract loosely sorbed P.

Loosely sorbed P

Loosely sorbed phosphorus is measured with the IDE. The method is adapted from Aminot and Andrieux (1996). Add $NaHCO_3$ to 2 L MQ-H_2O to bring the pH to a value close to that of the water in contact with the studied samples (about 8 mg and 80 mg to bring the pH at 7 and 8, respectively). Toluene (1 mL L^{-1}) can be added to inhibit bacterial activity. $HgCl_2$ is frequently used for poisoning, but it makes an interference to the molybdenum blue method. Weigh 50 mg powdered sample in a 50-mL centrifuge tube. Add 50 mL pH-buffered water and set on a rotator or a shaker at room temperature for 24 h. Centrifuge for 15 min at 4000 rpm. Pipette 10 mL supernatant into a 15-mL clean tube for P analysis. Discard gently the remaining water. Repeat the operation three times to extract almost all the loosely sorbed P. Analyse dissolved phosphorus as in water (see above). Standards should be prepared from blank solutions that were treated by the same procedures and with equal timing as samples. The IDE technique can be done on a separate aliquot when the available particulate material is sufficient. In that case, the obtained concentration of loosely sorbed P must be subtracted from the ascorbate-P obtained from a separate aliquot.

Ascorbate leaching (amorphous Fe(III)-oxides)

This technique permits extraction of P from amorphous Fe(III)-oxides (Anschutz et al. 1998). Weigh 100 mg dry sediment or soil samples into 15-mL centrifuge tubes. Weigh 50 g Na-citrate and 50 g $NaHCO_3$ in a 1-L polypropylene bottle. Add 1 L MQ-H_2O and stir on magnetic stir plate. Bubble the solution with N_2 for 5 min. Add gently (because of

strong effervescence) 20 g L-ascorbic acid. This solution must be prepared just before utilization. Prepare a 0.2 mol L^{-1} HCl solution: bring 17.7 mL concentrated HCl (36%) to 1 L with MQ-H_2O. Add 10 mL ascorbate solution to the 15-mL centrifuge tube with tight sealing caps to prevent leakage while shaking, shake overnight (24 h; Anschutz et al. 2005). Also prepare three blank samples. Centrifuge for 15 min at 4000 rpm. Pipette 1 mL supernatant into clean 15-mL tubes. Add 9 mL 0.2 mol L^{-1} HCl solution. P is measured in this (1: 10) diluted solution. Use the blank sample to prepare the matrix for standard solutions: Transfer 25 mL ascorbate solution in a 250-mL bottle. Dilute to 250 mL with 0.2 mol L^{-1} HCl. Prepare this matrix at the same time as the samples.

P analysis in the diluted ascorbate matrix

Make standard solutions (0 to 20 μmol L^{-1}) in the prepared standard matrix. Prepare a mixed reagent by combining 20 mL R1 with 10 mL R2 and 5 mL R3 (Asc-R1R2R3) and prepare R4. Add 500 μL Asc-R1R2R3 to individual 10-mm square cuvette stored in a rack. Then, add 1.3 mL sample or standard solution. Add 130 μL R4. Mix with a small plastic stick. Wait 5 min and measure at 885 nm on a spectrophotometer. Measure the blank standard first and zero the spectrophotometer. The 5 μmol L^{-1} standard must have an absorbance of about 0.075 in a 10-mm square spectrophotometric cuvette.

CDB leaching (reducible Fe(III)-oxides)

This technique permits extraction of P from amorphous and crystalline Fe(III)-oxides (Lucotte and d'Anglejan 1985; Ruttenberg 1992). The CDB technique can be applied to a separate aliquot when the available particulate material is sufficient. The concentration of ascorbate P must be subtracted from the value obtained from the CDB leaching to obtain the crystalline fraction of Fe(III)-oxides. Weigh 100 mg dry sediment or soil samples into 15-mL centrifuge tubes. Weigh 44.119 g Na-citrate and 42.011 g $NaHCO_3$ in a 1-L polypropylene bottle. Add 1 L MQ-H_2O and stir on magnetic stir plate. Bubble the solution with N_2 for 5 min. Add 10 mL of the Na-citrate solution to each 15-mL centrifuge tube. Weigh 0.5625 g dithionite ($Na_2S_2O_4$) in small weighing dishes and add an aliquot to each tube. Also, prepare three blank samples. Bubble with N_2, cap and shake for 4 h. Centrifuge for 15 min at 4000 rpm. Pipette 1 mL supernatant into clean 15-mL tubes. Add 9 mL 0.2 mol L^{-1} HCl solution. P is measured in this (1: 10) diluted solution. Use the blank sample to prepare the matrix for standard solutions: Transfer 25 mL CDB solution in a 250-mL bottle. Dilute to 250 mL with 0.2 mol L^{-1} HCl. Prepare this matrix at the same time as the samples.

P analysis in the diluted CDB matrix

Make standard solutions (0–10 μmol L^{-1}) in the prepared standard matrix. Leave sample and standard tubes for 24 h before analysis to allow decomposition of dithionite. Prepare a mixed reagent by combining 20 mL R1 with 10 mL R2 and 5 mL R3 (CDB-R1R2R3) and prepare R4. Add 500 μL CDB-R1R2R3 to individual 10-mm square cuvette stored in a rack. Then add 1.3 mL sample or standard solution. Add 200 μL R4. Mix with a small plastic stick. Wait 5 min and measure at 885 nm on a spectrophotometer. Measure the blank standard first and zero the spectrophotometer.

For the diluted CDB leaching solution, and for the three next leaching procedures described below, the blank remains stable, and the coloration of the standard and samples that contain P is established within 3 min, but after, the coloration continues to slowly evolve over time. Therefore, it is absolutely necessary to keep the same time interval ($\pm$0.5 min) between the addition of reagents and the reading of absorbance. For this, the easiest way is to make measurements in sequences of five samples at a time. Prepare the mixture with the reagents in a sequence of five cuvettes. Allow to stand 5 min and measure the absorbance. Then, again with five other samples. Insert regularly a sequence of five standard solutions to check the reproducibility.

Ammonium chloride (2 mol L^{-1}) leaching (biogenic hydroxyapatite)

This technique permits extraction of P from biogenic hydroxyapatite after a preliminary CDB leaching (Schenau and De Lange 2000). Weigh 100 mg dry sediment or soil samples into 15-mL centrifuge tubes. Apply the CDB leaching procedure. After centrifugation, discard gently the remaining CDB solution. Rinse tube with 1 mol L^{-1} $MgCl_2$ buffered at pH 8, centrifuge and discard gently the solution. Weigh 106.98 g NH_4Cl in a 2-L polypropylene bottle. Add 1 L MQ-H_2O and stir on magnetic stir plate. Add NaOH to raise the pH to 7. Add 10 mL solution to the 15-mL centrifuge tube. Also, prepare three blank samples. Cap and shake for 16 h. Centrifuge for 15 min at 4000 rpm. Pipette 1 mL supernatant into clean 15-mL tubes. Add 9 mL 0.2 mol L^{-1} HCl solution. P is measured in this (1: 10) diluted solution. Use the blank sample to prepare the matrix for standard solutions: Transfer 25 mL NH_4Cl solution in a 250-mL bottle. Dilute to 250 mL with 0.2 mol L^{-1} HCl. Prepare this matrix at the same time as the samples.

P analysis in the diluted NH_4Cl matrix

Make standard solutions (0–10 μmol L^{-1}) in the prepared standard matrix. Prepare a mixed reagent by combining 20 mL R1 with 20 mL R2 and 10 mL R3 (Ace-R1R2R3) and prepare R4. Add 350 μL Ace-R1R2R3 to individual 10-mm square cuvette stored in a rack. Then, add 2 mL sample or standard solution. Add 200 μL R4. Mix with a small plastic stick. Wait 5 min and measure at 885 nm on a spectrophotometer. Measure the blank standard first and zero the spectrophotometer. The 5 μmol L^{-1} standard must have an absorbance of about 0.105 in a 10-mm square spectrophotometric cuvette. Make mixture and measurement through sequences of five samples or standards at a time.

Sodium acetate (1 mol L^{-1}) leaching (authigenic CFA)

This technique permits extraction of P from authigenic CFA after preliminary CDB and NH_4Cl leaching (Ruttenberg 1992). Weigh 100 mg dry sediment or soil samples into 15-mL centrifuge tubes. Apply the CDB, and then the NH_4Cl leaching procedures. After centrifugation discard gently the remaining NH_4Cl solution. Rinse tube with 1 mol L^{-1} $MgCl_2$ buffered at pH 8, centrifuge and discard gently the solution. Weigh 136.08 g Na-acetate in a 2-L polypropylene bottle. Add 1 L MQ-H_2O and stir on magnetic stir plate. Add acetic acid to lower the pH to 4. Add 10 mL of this solution to the 15-mL centrifuge tube. Also, prepare three blank samples. Cap and shake for 16 h. Centrifuge for 15 min at 4000 rpm. Pipette 1 mL supernatant into clean 15-mL tubes. Add 9 mL 0.2 mol L^{-1} HCl solution. P is measured in this (1: 10) diluted solution. Use the blank sample to prepare the matrix for standard solutions: Transfer 25 mL Na-acetate solution in a 250-mL bottle. Dilute to 250 mL with 0.2 mol L^{-1} HCl. Prepare this matrix at the same time as the samples.

P analysis in the diluted Na-acetate matrix

The procedure is the same as for the NH_4Cl matrix.

HCl (1 mol L^{-1}) leaching (detrital apatite and carbonates)

This technique permits extraction of inorganic P, except for P associated with some crystalline Fe(III) phases (Ruttenberg 1992; Berner et al. 1993; Conley et al. 1995). To measure P from detrital apatite and carbonates, weigh 100 mg dry sediment or soil samples into 15-mL centrifuge tubes. Apply the CDB, the NH_4Cl, and the sodium acetate leaching procedures. After centrifugation discard gently the remaining solution. Rinse tube with 1 mol L^{-1} $MgCl_2$ buffered at pH 8, centrifuge and discard gently the solution. When the HCl leaching is applied after the CDB leaching, we obtain the P pool associated with calcium. Measure 80.7 mL concentrated HCl. Bring to 1 L with MQ-H_2O and stir on magnetic stir plate. Add 10 mL of this solution to the 15-mL centrifuge tubes. Also, prepare three blank samples. Cap tightly and shake for 16 h. Centrifuge for 15 min at 4000 rpm. Pipette 1 mL supernatant into clean 15-mL tubes. Add 9 mL MQ-H_2O. P is measured in this (1: 10) diluted solution. Use the blank sample to prepare the matrix for standard solutions: Transfer 25 mL 1 mol L^{-1} HCl solution in a 250-mL bottle. Dilute to 250 mL with MQ-H_2O. Prepare this matrix at the same time as the samples.

P analysis in the diluted HCl matrix

The procedure is the same as for the NH_4Cl matrix.

H_2SO_4 (18 mol L^{-1}) leaching (organic-P)

This technique permits extraction of P from organic matter after a preliminary CDB and 1 mol L^{-1} HCl leaching (Ruttenberg 1992; Berner et al. 1993; Gleyzes et al. 2002; Deborde et al. 2007). After the 1 mol L^{-1} HCl leaching step, discard gently the remaining HCl solution. Rinse tube with MQ-H_2O, centrifuge and discard gently the solution. Dry in an oven at 50°C. Add carefully 2.5 mL concentrated H_2SO_4 (18 mol L^{-1}, 97–99%) in the 15-mL centrifuge tubes. Also, prepare three blank samples. Cap tightly and shake for 16 h. Uncap gently the tube stored in a rack. Set the rack in a cold water bath and dilute three times by adding slowly 5 mL MQ-H_2O to each tube. Cap and shake gently (because of density difference), and centrifuge for 15 min at 4000 rpm. Pipette 210 μL supernatant into clean 15-mL tubes. Add 10 mL MQ-H_2O. P is measured in this final (1: 144) diluted solution. Use the blank sample to prepare the matrix for standard solutions: Transfer 5.25 mL 6 mol L^{-1} H_2SO_4 solution in a 500-mL bottle. Add 250 mL MQ-H_2O. Prepare this matrix at the same time as the samples.

P analysis in the diluted H_2SO_4 matrix

Make standard solutions (0 to 10 μmol L^{-1}) in the prepared standard matrix. Prepare a mixed reagent by combining 20 mL R1 with 10 mL R3 (H_2SO_4 -R1R3). Pipette 5 mL standard solution or sample in clean 10-mL tubes stored in a rack. In each tube add 150 μL H_2SO_4-R1R3 and 100 μL R4. Swirl to mix and wait 3 h and measure at 885 nm on a spectrophotometer using a 50 mm-long glass cuvette. Measure the blank standard first and zero the spectrophotometer.

Assessment and discussion

All or part of the extraction schemes described above have been used by our research group (e.g., Anschutz et al. 1999, 2007; Deborde et al. 2007, 2008; Canton et al. 2012). These papers provide illustrations of the procedures when applied to riverine, estuarine, and marine suspended particulates and sediments. We believe that our extraction schemes present two major improvements compared with referenced sequential extraction schemes. The first is the separation of the Fe-bound pool into two fractions. The ascorbate solution at pH 8 can only reduce and dissolve the most reducible fraction of Fe(III)-oxides, whereas the CDB solution is much less selective. This has been shown experimentally (Kostka and Luther 1994). Reductive dissolution kinetics of major Fe (oxyhydr)oxides showed two mineral groups (Poulton et al. 2004). Minerals with a lower degree of crystal order (hydrous ferric oxides and lepidocrocite) are reactive on a time-scale of minutes to hours. The more ordered minerals (goethite, magnetite, and hematite) are reactive on a time-scale of tens of days. Dissolution kinetics with CDB solution allows the quantitative dissolution of reducible Fe(III)-oxides.

It can be shown that selectivity of ascorbate is based primarily on reductive power of ascorbate to reduce Fe(III)-oxide phases, i.e., on thermodynamic principles, not kinetics. This can be demonstrated on a thermodynamic equilibrium diagram (Fig. 1). Equilibrium constant (K) of half-redox reactions of Fe(III)-phase reduction for one electron transfer at standard conditions can be compared to ascorbic acid and dithionite oxidation. When the energy yield is favorable, the oxidant species of a redox reaction is that of the half reaction that has

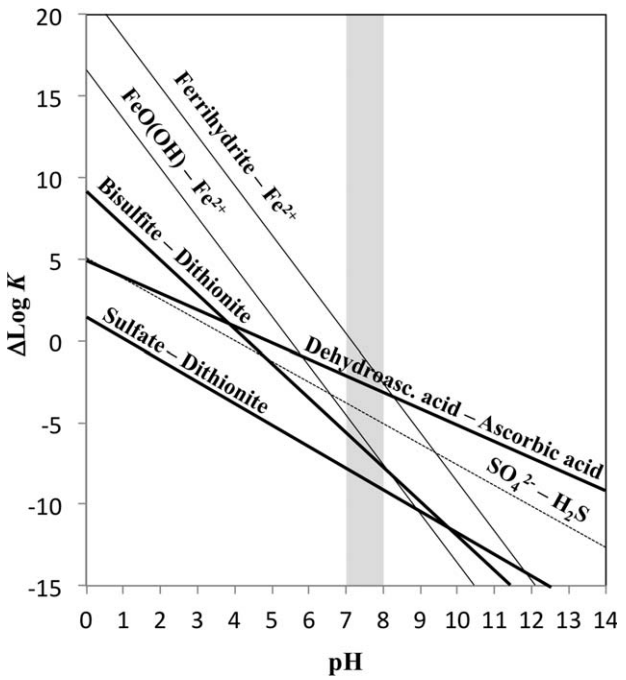

Fig. 1. Variation of Log *K* (ΔLog *K*) vs. pH with an activity of 10^{-3} for Fe^{2+} for redox couples discussed in the text. The grey area shows the range of pH during the ascorbate and the CDB leaching. The energy yield is favorable when the oxidant species of a redox reaction is that of the half reaction that has the highest Log *K*, and the electron donor species is that of the half reaction with the lowest Log *K*. At pH 7–8, ascorbic acid (and H_2S) is a reducing agent for ferrihydrite, but not for goethite, whereas dithionite oxidized to sulfate or bisulfite is a reducing agent for both Fe(III)-phases.

the highest Log *K* (or pE^0), and the electron donor species is that of the half reaction with the lowest Log *K*. The diagram shows (Fig. 1) that ascorbate extracts selectively the most reducible form of Fe(III) phases. This fraction is that which is favorably reduced by sulfide (Fig. 1; Canfield et al. 1992) and by Fe(III) dissimilatory reducing bacteria: the selective extraction of this Fe(III) pool allows quantification of the most reactive form of Fe(III). This fraction also carries much more P than crystalline Fe(III) phases, mostly because of the nanometric size of ascorbate-extractable Fe-oxides and high reactive surface area (Anschutz et al. 1998). Therefore, the ascorbate extraction allows the part of P that can be easily mobilized for redox changes to be distinguished from the part linked to Fe that may be buried in sediment or trapped in soils. Fe extracted with ascorbate and CDB can be measured in the 1: 10 diluted matrix with the ferrozine method (Stookey 1970). There is no matrix effect. Nevertheless, the standard solution must be prepared in the blank solution of each matrix.

The second improvement of our extraction schemes is the development of a colorimetric method for P measurement in every matrix. The proportion of reagents in the different matrices has been defined empirically. For R1, R2, and R3, we prepared grids of 1 μM standard solutions into which different volumes of reagent were added until we obtained a relatively stable molybdenum blue color. Two milliliter standards were pipetted into 64 cuvettes split between four racks in 4 × 4 grids. We put 50, 100, 150, and 200 μL R1 in rack 1, 2, 3, and 4, respectively. Then, we added 50, 100, 150, and 200 μL R2 in each line, and we added the same volume of R3 in each column so that we obtained 64 cuvettes with different proportions of mixed R1R2R3 reagent. Afterward, we added in every cuvette 200 μL R4. A blue color appeared only in a few cuvettes, and a stable coloration was observed only in one or two cuvettes. Then, we optimized the detection and the stability using the mixed R1R2R3 reagent that gave the blue color with different proportions of standard solution or R4 reagent until we obtained the best result. The proportions presented above and in Table 1 are optimal recipes we obtained from our investigations. This work was repeated for every matrix each time a new matrix was used in our laboratory.

Absorbance vs. concentration of standard solutions follows the Beer–Lambert law in the range between 0 μmol L^{-1} and 20 μmol L^{-1}. Calibration relationships are linear in this range of concentration (Fig. 2). This indicates that any problems linked to matrix interferences are insignificant. Colorimetric methods described here do not all have the same reproducibility. The slope of the calibration line remains the same within 10% deviation every time we do the measurements for the ascorbate matrix (concentration = absorbance × 67 ± 3). This estimate is based on more than 30 ascorbate extraction experiments conducted in our lab since 1999. The slope is more operator-dependent for the other matrices. This is because the coloration evolves slowly with time. The different operators need different times. When the measurement is done with sequences of five samples or standard solutions, the timing can be kept constant, which is a necessary constraint. Nevertheless, when the rhythm of measurement is constant, the absorbance vs concentration of standards is remarkably linear, and the replication of colorimetric measurement of the same sample or standard gives a constant absorbance within 1% deviation ($n = 10$). Satisfactory reproducibility is obtained when selective leaching is applied on replicate samples. We obtained maximum relative standard deviation of 10% ($n = 10$). The error is mainly due to the extraction procedure and not to the colorimetric P analysis. During extractions, differences on replicate samples may be due to heterogeneity of a sample, the weighing of aliquots, measuring the volume of leaching solution, or the dilution of solutions.

The sensitivity of the molybdenum blue method for the ascorbate and the CDB matrix is about 30% lower than for the water matrix. The precision is generally satisfactory enough for 100 mg sediment or soil samples. The colorimetric method has a sensitivity close to that of water matrix for the acetate, HCl, and H_2SO_4 matrices. P concentration values in μmol L^{-1} in the final 1: 10 diluted solution in our

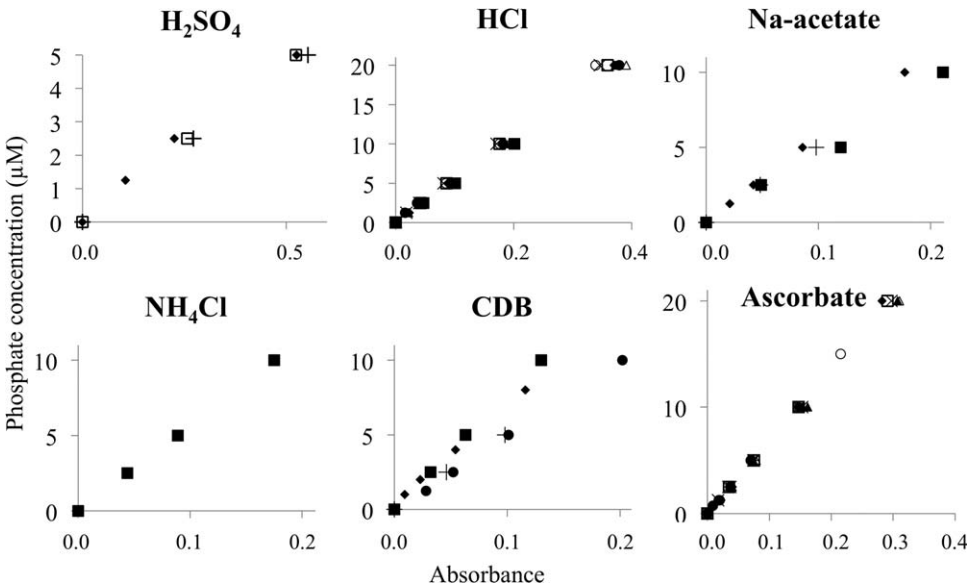

Fig. 2. Phosphate calibration relationships prepared in different matrices of particle leaching. All measurements were made in 10-mm square spectrophotometric cuvettes, except the H_2SO_4 series that were measured in 50-mm glass cuvettes. The different symbols represent results from 10 different operators between the years 2003–2012.

experimental method (100 mg particles in 10 mL leaching solution) correspond directly to concentration values expressed in μmol g^{-1} for particulate P. Samples collected in the oxic layer of marine or lacustrine sediment usually need to be diluted because absorbance is out of range. Such samples are frequently enriched in reactive iron oxides and associated P can be above 20 μmol g^{-1}. We also measured concentrations of P extracted with Na acetate and 1 mol L^{-1} HCl, which were above 50 μmol g^{-1} and 200 μmol g^{-1}, respectively, in surface sediments collected at around 500 m water depth in the oxygen minimum zone of the Peru margin (unpubl. data). For concentrated samples, the extracted solution must be diluted in the appropriate blank solution.

The detection limit (DL) is that of the spectrophotometer. Using a signal to noise ratio of 3.0, the DL is 0.003 absorbance unit. This corresponds to a particulate P DL of 0.1 μmol g^{-1} in the H_2SO_4 matrix and a DL between 0.15 μmol g^{-1} and 0.20 μmol g^{-1} in the other matrices for the conditions described above. The DL can be improved by measuring the absorbance in a 5-cm cuvette. If the material is poor in particulate P concentration, as for instance in sandy sediment, the selective leaching can be performed on 1 g sample rather than 100 mg sample.

The molybdenum blue method is sensitive to pH. When the sample is an acidified water sample for conditioning or a solution from acid extraction of particulate matter, the initial solution must have a pH above 1 (Fig. 3). The lower proportion of R2 in diluted extraction matrices relative to the phosphate analysis in water matrix reflects this pH effect. In the sulfuric acid matrix, R2 is removed from the mixed reagent solution, because sulfuric acid is initially present. The dilution by 144 of the leaching solution permits a final H_2SO_4 concentration similar to that of the reagent-water mixture in the case of the (sea)water matrix.

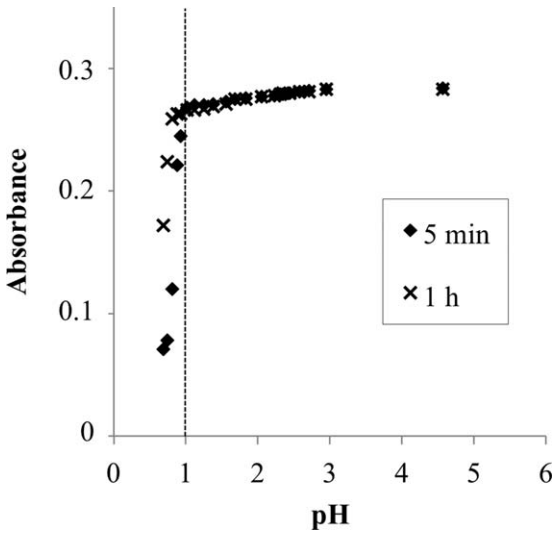

Fig. 3. Molybdenum blue absorbance at 885 nm vs. pH of a seawater sample subsampled after successive additions of nitric acid. Initial phosphate concentration was 3 μmol L^{-1}. Absorbance values were read in a 50-mm glass cuvette 5 min and 1 h after the sample-reagent mixture. Below pH 1 the coloration of the sample is not stable: it continues to develop after 5 min.

Dissolved inorganic P analyses in anoxic pore waters should be done using acidified samples, not in frozen samples, to prevent dissolved Fe(II) oxidative precipitation and subsequent postsampling phosphate sequestration on Fe(III)

phases. Nevertheless, the final pH of the stored sample must be above 1. Pore water sample volume is often small (a few mL) when pore waters are extracted from thin horizontal slices of sediment cores. Consequently, acidification must be done with addition of diluted acid. We usually use 50 μL or 100 μL 1: 10 diluted 37% HCl solution for 10 mL fresh water or seawater, respectively.

The chemical extractions were performed on sediment samples dried by lyophilization. Air-drying or lyophilization of anoxic samples may induce the oxidation of Fe-sulfides present in the sediment, and a subsequent enrichment in poorly crystalline Fe-oxides. This oxidation produces protons that can partly dissolve carbonates and apatite. The released phosphate bound to Ca-phases is subsequently adsorbed on the freshly formed iron oxides. Fe sulfide oxidation, thus, leads to a conversion of acetate, NH_4Cl or HCl-P to Fe-bound P extracted with ascorbate. Such conversion may occur in all reduced samples exposed to oxygen (Kraal et al. 2009). Therefore, for anoxic sediment and soil we recommend to store the samples under inert atmosphere from the sample collection to the sample processing. The extraction schemes can be performed on fresh wet samples to avoid the exposure to air during drying. In that case, the porosity must be measured to calculate the mass of particles that were leached.

Conclusion

Sequential extraction of phosphorus from natural solids is one of the geochemical tools used to study biogeochemical processes. However, sequential extraction and selective leaching of particles to extract P from target phases is labor intensive. Thus, fast and reliable approaches to quantify P in the different matrices issuing from leaching solution are welcome. The first contribution of our work is the implementation of an operationally defined extraction of two different Fe-bound phosphorus pools. The second contribution is the development of different molybdenum blue methods based on matrix-dependent specific mixture of the same reagents used in the colorimetric method to measure dissolved inorganic P in water. The experimental conditions for the analyses have been investigated and optimized. Although an empirical approach was used to obtain optimum recipes, the analytical methodology has the advantages to have an excellent reproducibility, satisfactory DLs, and requires inexpensive equipment. The extraction schemes presented here can be used partly or entirely to characterize operationally defined P fractions in all types of soil, suspended particulates, and sediments.

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