

Quantification of individual phosphorus species in sediment: a sequential conversion and extraction method

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Summary

Common sequential phosphorus (P) extraction methods are not specific to particular chemical species and have several limitations. This work presents the first chemical method for quantification of individual mineral and sorbed P species. It was developed by combining a conversion technique with a sequential extraction procedure. Mangrove sediments with different characteristics were incubated in pH-adjusted 0.01 M CaCl₂ with and without reference material additions of octacalcium phosphate (Ca₈H₂(PO₄)₆·5H₂O; OCP), hydroxyapatite (Ca₅(PO₄)₃OH), strengite (FePO₄·2H₂O) or variscite (AlPO₄·2H₂O). The changes in soluble phosphate concentration were measured in the supernatant solution, while pH-induced variations in P composition were determined by subsequent sequential extraction of the sediments. Dissolved phosphate concentration was controlled by adsorption below pH 7.8. Above this pH, soluble phosphate concentration was governed by OCP, which was qualitatively determined by plotting the experimental values of pH + pH₂PO₄ and pH – 0.5 pCa on a solubility diagram including the isotherms of known crystalline phosphate compounds. In contrast to the often-predicted slow dissolution rate of crystalline phosphates in soils or sediments, drastic changes in P composition by dissolution, precipitation and adsorption processes were detected after 7 days. These were mainly not observed indirectly by changes in dissolved phosphate through adsorption effects, but were determined quantitatively by subsequent sequential extraction, thus enabling the quantification of individual species. Evaluation of the method was performed by standard addition experiments. Besides P species quantification, the method provides the means for other applications, such as the determination of P mineral dissolution kinetics in soils and sediments, the prediction of P composition in changing environmental settings and the refinement of theoretical models of phosphate solubility in soil and sedimentary environments.

Introduction

The main challenge for the investigation of P dynamics in soils and sediments can be attributed to adsorption effects. These effects increase the number of involved compounds and impede the quantification of P species. The majority of studies concerning P dynamics in wetlands have attributed enrichment, availability and limitation of P to adsorption effects, reflecting the importance of adsorption effects in P distribution and composition (Fisher & Acreman, 2004). However, because of a lack of reliable discrimination methods, the role of amorphous or mineral P forms in P cycling is likely to have been underestimated.

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Sequential extraction techniques were developed for agricultural soils (e.g. Hedley *et al.*, 1982) and subsequently varied for sedimentary studies (e.g. Ruttenberg, 1992; Jensen & Thamdrup, 1993; Jensen *et al.*, 1998). The sediment extraction (SEDEX) scheme suggested by Ruttenberg (1992) has been widely used in sediment P analysis, probably because of the extensive standardization during method development. Five steps separate the following pools: (i) exchangeable or loosely sorbed P; (ii) Fe-bound P; (iii) authigenic apatite + CaCO₃-bound P + biogenic apatite; (iv) detrital apatite + residual inorganic P; and (v) organic P. Exchangeable P is operationally defined and extracted by a MgCl₂ solution at pH 8. A number of extractions are available for the determination of exchangeable P, e.g. deoxygenated water as used by Paludan & Morris (1999) for the determination of loosely adsorbed P and porewater P of freshwater marsh sediments. Fe-bound P is

measured by citrate dithionite bicarbonate (CDB) extraction. Reagents such as CDB or BD (a bicarbonate-buffered dithionite solution, Psenner & Pucska, 1988) reduce the oxidized species of iron and manganese and thereby liberate the phosphate adsorbed onto oxides/hydroxides of the two metals (Jensen & Thamdrup, 1993). Sodium hydroxide is also often used, as it extracts Fe-bound P, but in addition it extracts a considerable quantity of organic P (Golterman, 2001). Therefore, the replacement by other reagents such as CDB or BD, which may liberate less organic P, has been often recommended. However, the inclusion of organic P determination in fractionation schemes that focus on the determination of inorganic P compounds is questionable. According to Beauchemin *et al.* (2003), it may be necessary to determine organic P within each fraction of the inorganic P pools. Therefore, separate determination of organic P would be advisable, because the conditions for the inorganic P fractionation do not necessarily correspond to those of separable organic matter types, such as humic or fulvic acids. Separate organic P determination is possible on a chemical basis (e.g. Legg & Black, 1955) or by ^{31}P nuclear magnetic resonance spectroscopy (e.g. McDowell & Stewart, 2005). Sodium hydroxide extracts amorphous or mineral Al/Fe-P (Kurmies, 1972) and adsorbed P. As NaOH is replaced by CDB in the SEDEX method for the determination of P sorbed to reducible compounds, data will be misinterpreted in the presence of, for example, Al-sorbed P, variscite or strengite. These compounds were not tested during the development of the SEDEX scheme, are therefore not operationally defined and, if present, obviously cause an overestimation of defined fractions. Moreover, OCP was not implemented in the standardization procedure and may be determined together with the authigenic carbonate fluorapatite (CFAP) pool, extracted by acetate buffer solution adjusted to pH 4. The SEDEX method focuses on the separation of CFAP and detrital apatite of igneous or metamorphic origin. As the high pH values of marine sediments provide conditions promoting OCP precipitation, it is not unreasonable that CFAP, which represents an oceanic sink for reactive P, is often overestimated by this method.

As a consequence of the individual modifications for adjusting the schemes to the matrix and the expected P compounds, comparison of data is difficult and fractionation data become deficient, if unexpected P compounds are present.

Most standard methods for direct identification of mineral phases, e.g. X-ray diffraction (XRD), are not suitable for P analysis in soils and sediments, because of their low P content. More recently, direct determination of P species in soils was performed by using X-ray adsorption near-edge structure spectroscopy (XANES, e.g. Beauchemin *et al.*, 2003; Sato *et al.*, 2005). By this technique, it is possible to determine phosphate sorbed on ferrihydrite, goethite, Al-hydroxide and alumina as well as non-crystalline FePO_4 , hydroxyapatite (HAP) and OCP. However, until now, the promising direct identification by XANES has been restricted by limitations (Beauchemin *et al.*,

2003) and it is only meaningful to use this method if the main aspect of the study deals with P species identification.

Beauchemin *et al.* (2003) argued that such non-destructive techniques are specific to particular chemical species, whereas chemical fractions are macroscopic and operationally defined fractions that cannot be verified as being specific to particular chemical species. However, in the past, solubility criteria were used for indirect qualitative determination of particular minerals in soils. In the present work, we will show that a solubility experiment conducted by Murrmann & Peech (1969a), which induces conversions between mineral and adsorbed P forms, can be used for chemical quantitative determination of particular species, if the solubility experiment is combined with a suitable extraction procedure. Moreover, the application of the conversion-extraction (CONVEX) technique offers insights into conversion processes and explains discrepancies between observed phosphate solubilities and related theoretical models. Beauchemin *et al.* (2003) further pointed out that more than one scenario can be constructed to explain pore-water data, but direct identification of P species is useful for predicting the probability of increased P concentrations in solution under given conditions. On the other hand, knowledge of solid phase processes, which account for changes in phosphate solubility under changing conditions, is scarce and soluble phosphate can therefore hardly be predicted even if all solid components can be measured. In contrast to the large number of studies dealing with soluble P changes after manipulation of soils or sediments (e.g. studies on adsorption kinetics after pH or redox potential changes), few studies perform P fractionations on the same substrate sample that has been subjected to different treatments in order to detect and explain solid phase processes.

The objective of our study was to gain a deeper insight into the dynamics of P speciation in waterlogged substrates. For this purpose we focused on the utilization of the different solubilities and dissolution kinetics of various P minerals for differential dissolution through incubation at different pH values and subsequent P species quantification. In most cases, adsorption effects inhibit the observation of P mineral dissolution in the supernatant solution. We expected to be able to observe the pH-induced changes on the solid phase by incubation of the sediment at various pH values and subsequent fractionation of the sediment into Al/Fe-P (amorphous and mineral Al/Fe-P + adsorbed P) and Ca-P (mineral Ca-P). Additionally acquired fractionation data are more detailed and quantitative with respect to the solid phase and therefore provide the necessary information for species determination.

Materials and methods

Sediment sampling and preparation

Sampling sites were located in reforested mangrove stands of the Saigon River Delta, Vietnam, which had been exhaustively destroyed by the spraying of herbicide mixtures during the

Second Indochina War. In some areas, the change to oxidizing conditions resulted in acid generation from sulphide-rich sediments with the acidity remaining until the present day. The aeration-induced formation of sulphuric acid by the oxidation of iron sulphides is well known from land reclamation projects. The low productivity of the generated 'acid sulphate soils' can be generally attributed to toxicity of alumina and to nutrient deficiencies, especially of phosphate (e.g. Dent, 1986).

Sediments were selected to cover a broad range of pH values and differences in P composition. Therefore, samples were selected from a strongly acidic forest sector (SA), from a slightly acidic forest sector in the hinterland (HL) and from an alkaline mud flat (MF). Replicates of strongly acidic (SA1, SA2), slightly acidic (HL1, HL2) and alkaline (MF1, MF2) sediment samples from different depth intervals (35–40 cm, 40–45 cm) were chosen as controls for similarity in P composition under similar environmental conditions and consequently as indicators for method reproducibility. The differences in the sediment pH and sediment Eh between these depth intervals were relatively small, whereas the discrepancies were higher at the surface layers (J. F. Oxmann, unpublished data). Sediments were sampled immediately after they had been taken with piston-corders, which were provided with inlets for pH measurement prior to sectioning. Sectioned sediments were kept on ice until sample preparation started within a few hours. Preparation of the samples included careful removal of visible roots, drying at 37°C and grinding with a planetary mill. At least 50 g of a dried sample was ground to <300 µm mesh in order to prepare a homogenous

sample. Basic properties of the sediments used in this study and the experimental set-up are presented in Table 1.

Reference materials for standard addition experiments

Hydroxyapatite was purchased from Sigma-Aldrich (St Louis, MO). The following standards were synthesized according to the references cited with slight modifications concerning the initial pH value and/or incubation time. Precipitate for OCP synthesis (Christoffersen *et al.*, 1990) was incubated at 42°C for 5 hours or 7 days and washed with deionized water. The initial pH value for the precipitation of strengite (Ghosh *et al.*, 1996) was 0.8. The precipitate formed after 30 minutes of heating at 90°C on a steam plate. The crystalline precipitate was washed after 6 days of heating on a steam plate at 90°C with 0.1 M NaCl, deionized water and 70% ethanol. For variscite synthesis (Hsu & Sikora, 1993), 20 ml of AlCl₃ solution adjusted to pH 1.2 and 180 ml of Na₂HPO₄ solution adjusted to pH 1.2 were mixed. The final concentration was 0.05 M in Al and 0.15 M in phosphate. The slowly formed precipitate was washed with 0.1 M NaCl, deionized water and 70% ethanol after 6 days of heating on a steam plate at 90°C.

X-ray diffraction was performed after drying and grinding of the synthesized minerals using an XPert Pro multipurpose diffractometer (PANalytical, Almelo, The Netherlands) equipped with a Cu-tube ($\text{K}\alpha$ 1.541, 45 kV, 40 mA), a fixed divergence slit of $\frac{1}{4}^\circ$ 2-Theta, a secondary monochromator and an XCelerator detection system. The minerals were found to be highly

Table 1 Basic sample related properties and sediment related analysis

Sample and origin	Field pH	Vegetation	Inundation ^a	Texture ^b	Al/Fe-P/mg g ⁻¹	Ca-P/mg g ⁻¹	Conversion time and P additions ^c
MF1 (mud flat, 35–40 cm)	7.28	Absent	253.8, 365	13, 82, 5	0.183	0.160	7 days, nP 30 days, nP 7 days, Pi 7 days, OCP 7 days, HAP
MF2 (mud flat, 40–45 cm)	7.47	Absent	253.8, 365	12, 83, 5	0.174	0.166	7 days, nP 7 days, OCP 7 days, HAP
HL1 (hinterland, 35–40 cm)	6.62	Mixed mangrove species	2.5, 22	10, 82, 8	0.306	0.132	7 days, nP 30 days, nP 7 days, Pi
HL2 (hinterland, 40–45 cm)	6.64	Mixed mangrove species	2.5, 22	10, 84, 6	0.269	0.118	7 days, nP 7 days, STR 7 days, VAR
SA1 (acidic site, 35–40 cm)	3.28	Mainly <i>R. apiculata</i>	4.0, 38	5, 74, 21	0.082	0.035	7 days, nP 30 days, nP 7 days, Pi
SA2 (acidic site, 40–45 cm)	3.26	Mainly <i>R. apiculata</i>	4.0, 38	6, 82, 12	0.079	0.032	7 days, nP 7 days, STR

^aIn days per year. Data were calculated from measured heights above mean sea level and local tide table data. First and second values indicate the inundation time in days per year (duration) and the number of days per year at which one or two inundations occurred (frequency), respectively.

^bPercentages by weight of clay, silt and sand are given by the first, second and third values, respectively.

^cExperimental set-up for the performed CONVEX analysis. Experiments with no P additions are indicated by nP. Pi, OCP, HAP, STR and VAR mark addition experiments with additions of K₂HPO₄, OCP, HAP, strengite and variscite, respectively.

crystalline (Figure 1). The XRD pattern of OCP obtained after 5 hours of incubation was comparable to the pattern of HAP, as described by Suvorova & Buffat (2001). The XRD pattern of OCP obtained after 7 days of incubation showed additionally a HAP characteristic reflection, which was not observed after 5 hours of incubation, suggesting the conversion of some OCP to a HAP-like phase. Therefore, the OCP phase obtained after 5 hours was used for the described experiments. The XRD pattern of the purchased HAP was almost identical to a natural HAP from Puerto Rico (Downs, 2006), and the synthesized variscite was almost identical to a natural variscite from Arkansas (Downs, 2006). As the XRD patterns of the variscite phases shown by Hsu & Sikora (1993) were less comparable to the pattern of the natural variscite from Arkansas, the successful crystallization was probably achieved by using a lower initial pH value and therefore by an increased crystallization time.

Development of the CONVEX method

The CONVEX procedure (Figure 2) includes a pH-induced conversion of P compounds and a modified version of the fractionation suggested by Kurmies (1972).

The conversion method (step 1 in Figure 2) was adapted from a solubility experiment conducted by Murrmann & Peech (1969a). The authors studied the effect of varying soil pH, by pH adjustment on the concentration of phosphate in 0.01 M CaCl_2 extract and on the amount of labile P, as determined by isotopic exchange. Both decreasing or increasing the pH of the soils resulted in an increase of phosphate in the soil solution and the amount of labile P in the soil. It was shown that the phosphate concentration in the supernatant solution was governed by the equilibrium of a crystalline compound, namely OCP, only above pH values of 8. It was concluded that the phosphate concentration in solution was controlled by the amount of labile P rather than by the solubility of crystalline phosphate compounds. The importance of this early work concerning adsorption-desorption as well as precipitation-dissolution processes was recognized more recently by Hinsinger (2001), but the results were partly misinterpreted in the next publication of Murrmann & Peech (1969b), because of a lack of information, which we will provide and discuss in our paper. Briefly, as they have shown for OCP, other P mineral compounds also dissolve during the incubation procedure, but related changes of P ion concentration in solution cannot be

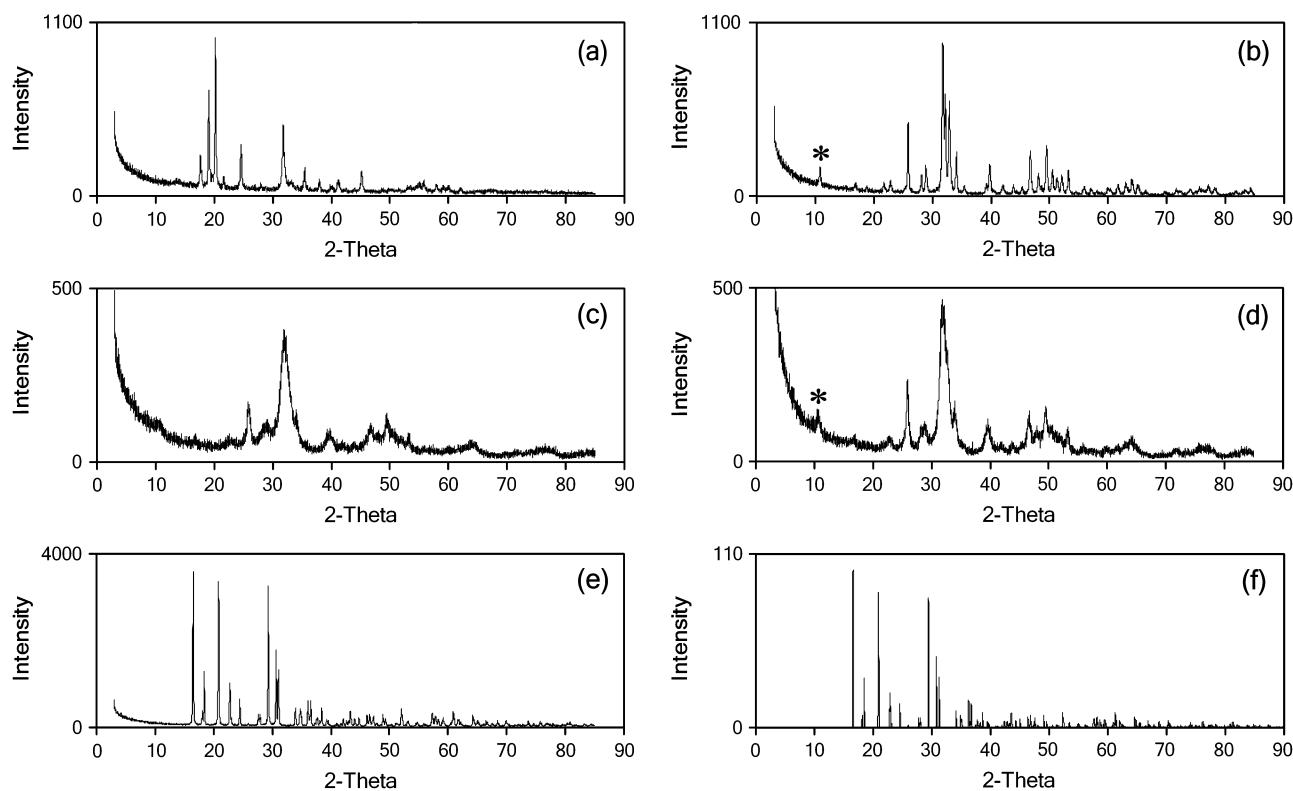


Figure 1 X-ray diffraction patterns (in degrees 2-theta for Cu radiation) for synthesized phases of strengite (a), hydroxyapatite (b), octacalcium phosphate (c) and variscite (e). The X-ray diffraction pattern from a specimen taken after a 7-day incubation of octacalcium phosphate in the pH range 5–5.5 (d) indicates the presence of a hydroxyapatite phase, because a hydroxyapatite characteristic reflection was observed (asterisks, see Suvorova & Buffat, 2001). Successful crystallization is illustrated for variscite by comparison with a natural variscite (f) from Arkansas (f: printed with kind permission from R.T. Downs, RRUFF Project, 2006, RRUFF-1D: R040180; <http://ruff.info/>).

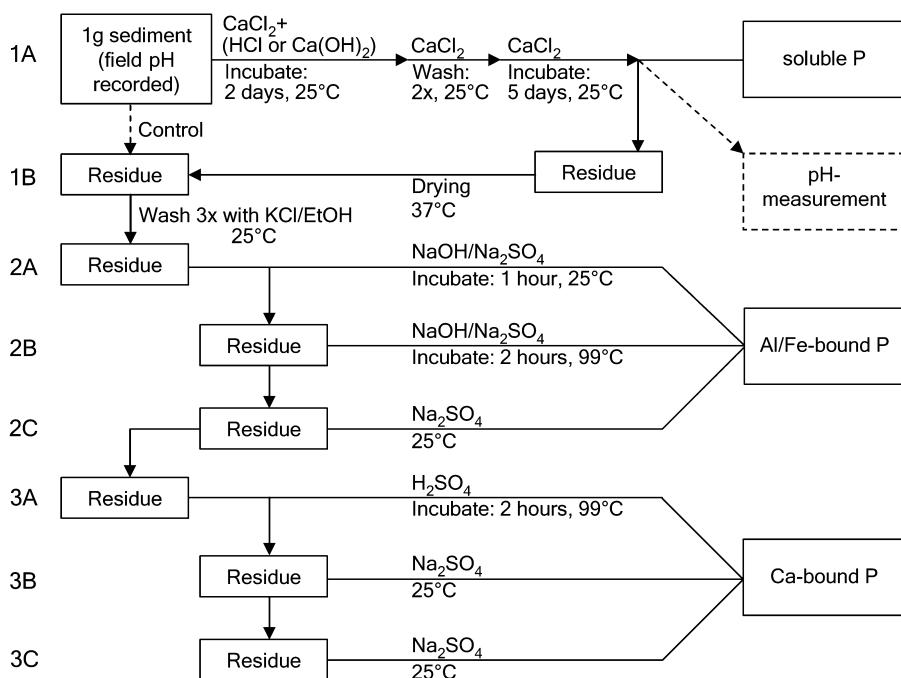


Figure 2 CONVEX scheme for the quantification of phosphorus species in sediment. 1A & 1B, conversion procedure; 2A–3C, extraction procedure. A conversion time of 7 days is recommended for species determination.

detected, because of the immediate adsorption of free phosphate to a solid phase.

To observe the pH-induced variations in P composition, a sequential extraction scheme for the fractionation of inorganic P into Al/Fe-P (by NaOH, 2A/B, Figure 2) and Ca-P (by H₂SO₄, 3A, Figure 2) as suggested by Kurmies (1972) was used, as it presented the following advantages for the development of the CONVEX method: (i) preliminary elimination of Ca²⁺ by KCl/ethanol in order to prevent OCP precipitation; (ii) fractionation of inorganic P into one Ca-P and one Al/Fe-P pool; (iii) desorption of subsequently adsorbed P (by Na₂SO₄, 2B/C, 3B/C); and (iv) short incubation times.

Method evaluation by standard addition experiments

Ruttenberg (1992) performed efficiency and specificity tests with different extractants and a number of phases that closely approximate naturally occurring P-bearing phases. Such a method is extensive and may include systematic errors, as not all P-bearing compounds in soils and sediments are known. Moreover, comparison with soil treatment is difficult by using relatively pure extraction conditions.

Because standard addition experiments provide the opportunity to test the efficiency and specificity of a procedure directly with the involved matrix, they offer the most confident application for standardization. Therefore, sediments were analysed by the CONVEX procedure with and without standard addition of the following XRD-proofed mineral standards: octacalcium

phosphate, HAP, strengite and variscite. Fractionations of the sediments with and without mineral additions but without previous incubation were used as controls.

Analytical methods and procedures

The sediment pH was measured in the field with a SensoLyte SE (WTW GmbH & Co. KG, Weilheim, Germany) electrode inserted directly into the sediment. After centrifugation of the incubated sediments the pH in the clear supernatant solution was measured with an InLab 423 (Mettler Toledo GmbH, Greifensee, Switzerland) electrode. All chemicals used were of analytical grade. Deionized water was used and purified by a Millipore Milli-Q system (Millipore Corp., Billerica, MA).

Sediment incubations in 0.01 M CaCl₂ and calculation of ionic activities were performed as described by Murrmann & Peech (1969a), but several modifications to the procedure were introduced, mainly to enhance sample through-put. Each sample was partitioned into subsamples by weighing series of 1 g into 50 ml polyethylene centrifuge tubes before addition of 10 ml 0.01 M CaCl₂ containing varying amounts of HCl or Ca(OH)₂. For the mineral addition experiments, minerals were weighed into small polyethylene caps, which were added separately to the subsamples before the addition of 10 ml of pH-adjusted 0.01 M CaCl₂ to ensure homogeneity of added mineral P between the subsamples. For experiments with soluble P addition, 10 ml of pH-adjusted 0.01 M CaCl₂ containing 0.155 mg P as K₂HPO₄ was added to the subsamples. After

the tubes were capped and shaken in a water bath at 25°C for 2 days, subsamples were centrifuged for 5 minutes at 1000 g and the supernatant liquid was discarded. Subsequently, the sediment pellets were washed two times with 10 ml of 0.01 M CaCl₂. After addition of 10 ml of 0.01 M CaCl₂ and a few drops of chloroform for precluding bacterial growth, the subsamples were shaken in a water bath at 25°C for 5 days and subsequently centrifuged for 10 minutes. Aliquots of the supernatant solution were taken for the determination of the concentrations of soluble phosphate, iron and calcium and for pH measurement.

After drying the pellets in the same tubes, sequential extraction of the sediments was performed. Subsamples of 1 g without incubation were taken as controls. Sediment fractionation was carried out according to Kurniawati (1972), but again modifications were applied in order to accelerate the determination. The measurement of organic P, as being part of the original scheme, was not performed, because we recommend a separate determination of organic P. The fractionation scheme used in this study is included in Figure 2. The KCl/ethanol solution was prepared by mixing three parts of ethanol with two parts of 1 M KCl. The NaOH/Na₂SO₄ solution contained 0.5 M Na₂SO₄ and 1 M NaOH. The Na₂SO₄ solution contained 4% of Na₂SO₄, and H₂SO₄ solution was 0.5 M. Extractant volume was 15 ml for each step, and the tightly capped centrifuge tubes were shaken (120 r.p.m.) in a water bath at the corresponding temperatures. Centrifugation was performed for 10 min at 1000 g. For removal of humic acids, 0.5 ml of 2.5 M H₂SO₄ was added to 2.5 ml of the solutions obtained by the fractionation pro-

cedure. After incubation for 30 min, 7 ml of deionised water was added and the solutions were centrifuged. The concentration of phosphate in the obtained supernatant solutions, as well as the concentration of soluble phosphate, was measured according to Murphy & Riley (1962) by the use of a Pharmaspec UV-1700 spectrophotometer (Shimadzu Corp., Kyoto, Japan).

Atomic absorption spectrometry (AAS) was performed for the determination of calcium and iron concentrations in solution by the use of a Spectra AA 300 (Varian Australia Pty Ltd., Mulgrave, Victoria, Australia). Particle size was determined by laser diffractometry (LA-300, Horiba Ltd., Kyoto, Japan). For grain size analysis, the wet sediments were dispersed in 1% sodium hexametaphosphate solution after sieving to <500 µm mesh.

Results

CONVEX method: soluble phosphate data

In general, the soluble phosphate data from the selected sediments, incubated at different pH values for 7 or 30 days with and without mineral additions, varied with pH as described by Murrmann & Peech (1969a) for different soils. Results of the incubations from the mud flat sediment MF2 displayed a typical 'u-shaped' curve, when soluble P is plotted versus pH (Figure 3 a). Soluble phosphate concentration was relatively low in the pH range 4–7 and increased rapidly below 4 and above 7. Concentration of phosphate in solution decreased above pH 7.8, as a precipitation-dissolution equilibrium was established. As reviewed

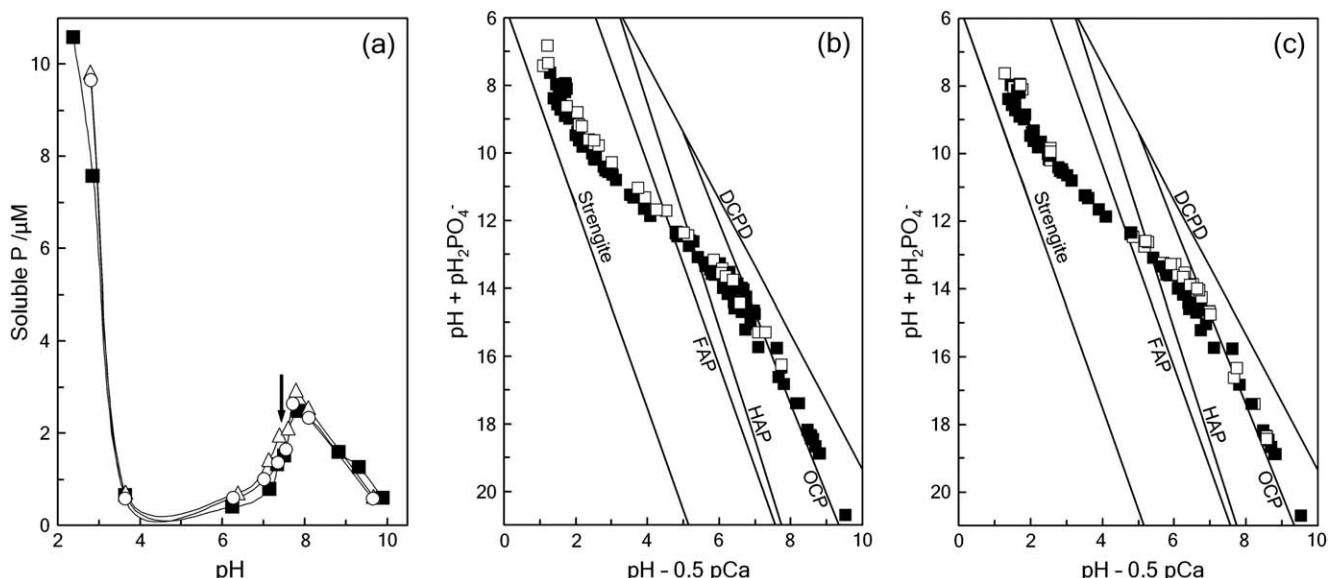


Figure 3 Effect of varying pH of the sediment on the concentration of phosphate in solution and the value of $pH + pH_2PO_4^-$. (a) 7-day incubations of MF2; ■, MF2; ○, MF2 with hydroxyapatite; △, MF2 with octacalcium phosphate. (b) All incubations with and without mineral additions; □, 30 days; ■, 7 days. (c) All 7-day incubations with and without mineral additions; □, MF1 and MF2; ■, HL1, HL2, SA1 and SA2. The arrow marks the original sediment pH. Strengite: strengite at 50 µM of Fe³⁺. The isotherms of octacalcium phosphate, hydroxyapatite, fluorapatite and brushite are marked by OCP, HAP, FAP and DCPD, respectively.

in detail by Hinsinger (2001), the data of Murrmann & Peech (1969a) showed increased solubilization of soil P by varying their original pH. Soils with pH values from 4.48 to 6.30 were used in their study. As we also selected sediments with high original pH values (MF1, MF2), we observed additionally decreased values of soluble phosphate by lowering the pH of these sediments.

The analytical data of soluble phosphate obtained by the conversion experiments were transformed to values of pH + pH₂PO₄ and pH - 0.5 pCa and plotted on solubility diagrams shown in Figure 3(b, c). To develop these diagrams, calculations and log K^o -values of the equilibrium reactions were used as described by Lindsay (1979) but the following changes were made: (i) an equilibrium of Ca²⁺ with calcite at 0.0003 atm of CO₂ at high pH values was not taken into account, because we observed no significant depression in the activity of Ca²⁺ above pH 7.88 after incubation (see Figure 4); and (ii) an additional strengite isotherm was calculated for a fixed value of Fe³⁺ (50 µM), because we expanded the pH scale to lower pH values compared with the diagrams suggested by Lindsay. In general, strengite is more stable at low pH values, because the solubility of Fe³⁺ is controlled by Fe(OH)₃ (amorphous). At extremely low pH values, further liberation of Fe³⁺ is restricted, because most of the amorphous or mineral iron in the soil or sediment is already solubilized. Thus, the amount of strengite in a soil or sediment decreases at pH values <2. As 50 µM is the lowest Fe³⁺ concentration in solution for most soils and sediments, if 1 g of soil or sediment is incubated in 10 ml of extractant under strongly acidic conditions, this isotherm probably indicates the unstable limit of strengite in soils and sediments at low pH values under the given conditions.

All soluble phosphate data of the CONVEX analysis with and without mineral additions listed in Table 1 were plotted in Figure 3(b). In agreement with the data of Murrmann & Peech (1969a), values of pH + pH₂PO₄ increased nearly linearly

with increasing values of pH - 0.5 pCa from pH 4 (pH - 0.5 pCa = 3) until the sediment phosphate isotherm intersected the OCP isotherm and further followed this isotherm. Additionally, an increased slope below pH 4, parallel to the plotted strengite isotherm at a fixed Fe³⁺ concentration of 50 µM is visible, because we expanded the pH range down to 2.0. Discrepancies between the different incubations were visualized by different symbols in Figure 3(b, c). Higher P solubility in the pH range 3–6 was found for the sediments incubated for 30 days (Figure 3b). Figure 3(c) includes all soluble phosphate values obtained by the incubations carried out for 7 days. Sediments with high original pH values (MF1, MF2) intersected the OCP isotherm at lower pH values compared with the other sediments. As will be shown below, MF1 and MF2 initially contained OCP, while in the other sediments OCP precipitated subsequently, when they were adjusted to alkaline pH values.

The concentrations of total Fe and Ca in solution indicated that Ca²⁺ was nearly constant across the pH range, while the logarithm of the Fe concentrations decreased linearly with increase in pH until the AAS detection limit was reached. Figure 4(a–c) shows the logarithm of soluble Ca and Fe versus pH for 7-day incubations of MF2, HL2 and SA2. The measurable concentrations of Fe in solution were much higher at any pH than expected for soluble Fe³⁺ in equilibrium with Fe(OH)₃. Through its relatively high solubility, amorphous Fe(OH)₃ is often used as a reference solid phase controlling the solubility for Fe³⁺ in soils. The activity of Fe³⁺ maintained by Fe(OH)₃ is ca. 10⁻⁶ M at pH 3 and decreases 1000-fold for each unit increase in pH (Lindsay, 1979). Therefore, it is likely that soluble Fe³⁺ was in equilibrium with a mixed valency Fe₃(OH)₈ (ferrosic hydroxide). This compound is thought to cause elevated levels of soluble Fe, if present. Ferrosic hydroxide formation has been known to take place under altering redox conditions, which also occur in the described

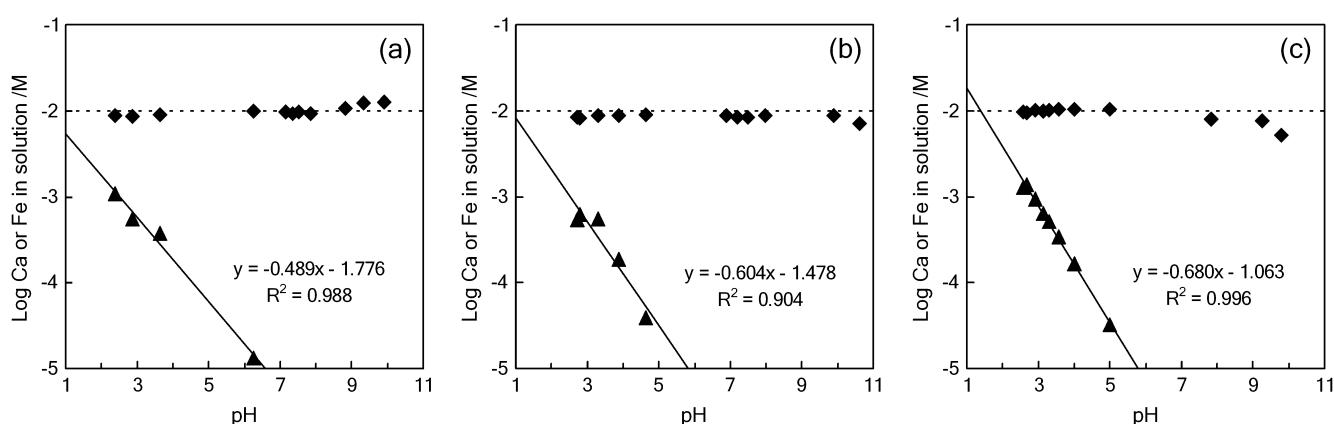


Figure 4 Effect of varying pH of the sediment on the total Fe (▲) and Ca (◆) concentrations in solution as measured by atomic absorption spectrometry for MF2 (a), HL2 (b) and SA2 (c) sediment incubations. Equations and R^2 -values for linear regression of the Fe concentrations are shown. Concentration of Fe in solution was below the detection limit of the atomic absorption spectrometry at higher pH values.

environmental setting, and can apparently persist in soils for extended periods of time (Lindsay, 1991).

CONVEX method: fractionation data

Fractionation data from 7-day incubations of MF1, HL1 and SA1 are illustrated in Figure 5(a–c), respectively. Data from fractionations of untreated sediments are shown as controls by plotting the amounts of Ca-P and Al/Fe-P against the original pH values of the sediments measured in the field. No significant changes in P composition were observed for incubations carried out at the original pH values compared with untreated sediments. Therefore, just the pH-induced changes during the 7-day incubation procedure accounted for changes in P composition and no significant loss of P was caused by the initial extraction procedure used for Ca elimination. The amount of phosphate extracted by the 7-day incubation of the conversion procedure was not large enough to account for significant changes in the amount of Al/Fe-P or Ca-P in the pH range 3.5–8. In contrast, fractionation results obtained after 30 days of incubation showed slightly decreased amounts of P for both pools at any pH. Therefore, fractionation results obtained after the 30-day incubation differed significantly from those of the controls (data not shown).

Acidifying the sediments resulted in a decrease of Ca-P, accompanied by an increase of Al/Fe-P, at which the ratio of $\Delta\text{Ca-P}/\Delta\text{Al/Fe-P}$ was generally close to 1. An inverse effect was caused by increasing the original pH of the sediments. It is evident that pH-induced dissolution processes could not be observed by the determination of total inorganic P after incubation, because losses through soluble phosphate were low due to adsorption effects. Thus, total inorganic P content of the sediments (data not shown) was nearly constant in the pH range

3.5–8. Exceptions to this conversion behaviour occurred at very low and very high pH values. At very low pH values both pools decreased, whereas at high pH values loss of Al/Fe P was greater than the increase in Ca-P. The sum of Ca-P and Al/Fe-P, which represents the total inorganic P pool, decreased because of the negative balance under these conditions. Generally, the variation of total inorganic P with pH was the reverse of the ‘u-shaped’ pH dependency of soluble P (see Figure 3a), showing the lowest total inorganic P levels at the lowest pH values according to the highest soluble P concentrations. Fractionation data further indicate that highest amounts per pH increment were exchanged between Ca-P and Al/Fe-P above pH 7.

Murmann & Peech (1969a) observed an increase in the amount of labile P, when $0.125 \text{ mg P g}^{-1}$ as K_2HPO_4 was added to soils before incubation, but the increase in the amount of labile P was substantially less than the amount of soluble phosphate added. They concluded that in the case of the P addition experiments, some phosphate was removed during the initial extraction procedure even though preliminary experiments had shown that very little phosphate was removed by repeated extraction of the soil samples that had received no soluble phosphate. They referred this discrepancy to different degrees of saturation of phosphate-adsorbing surfaces. However, we observed that very little phosphate was removed even if we added $0.155 \text{ mg P g}^{-1}$ as K_2HPO_4 , because the added soluble phosphate was found nearly quantitatively in the Al/Fe-P fraction. The amount of Ca-P increased just slightly as a consequence of soluble phosphate addition (Figure 5).

The interpretation of the information about solid phase processes given by the fractionation data was verified by standard addition experiments. The fractionation data of the CONVEX procedure with and without mineral additions are illustrated in Figure 6(a–c) for MF2, HL2 and SA2 sediments, respectively.

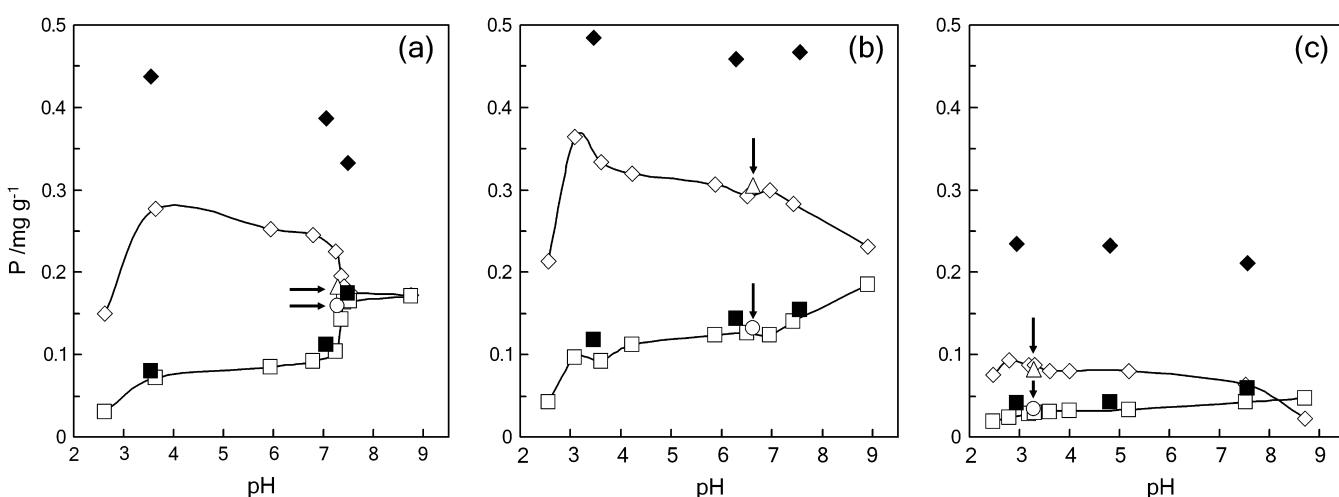


Figure 5 Effect of varying pH of the MF1 (a), HL1 (b) and SA1 (c) sediments on the Ca-P (□) and Al/Fe-P (◇) fraction and the effect of water-soluble phosphate additions on the Ca-P (■) and Al/Fe-P (◆) fraction. Fractionation data of controls without preliminary incubation (arrows) are presented by plotting the amount of Ca-P (○) and Al/Fe-P (Δ) against the original sediment pH.

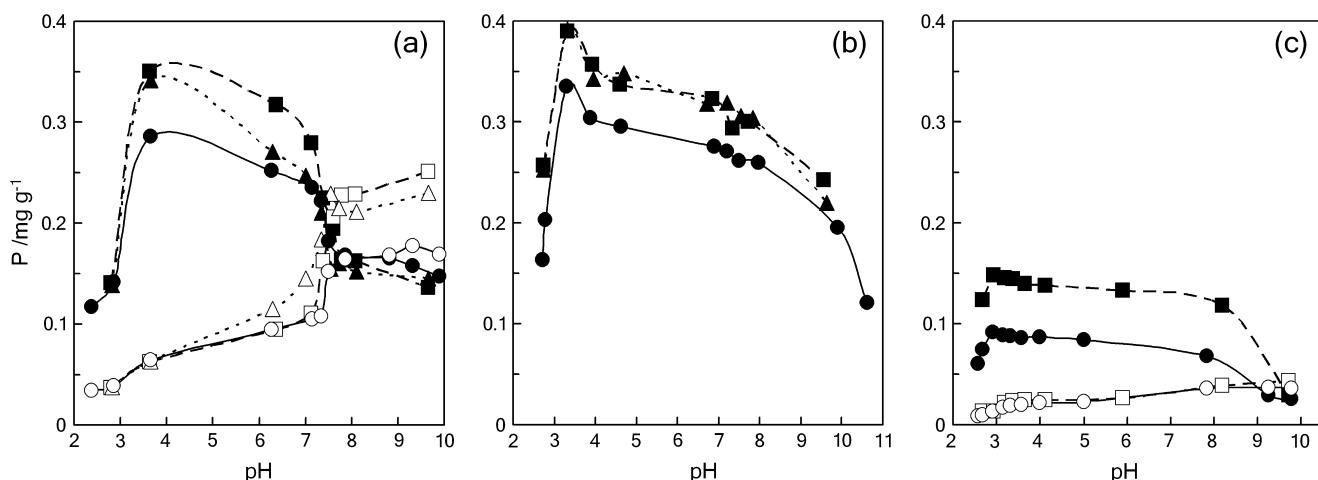


Figure 6 Effect of varying pH of the MF2 (a), HL2 (b) and SA2 (c) sediments with and without mineral additions on the Ca-P (open symbols) and Al/Fe-P (closed symbols) fraction. (a) ○, ●, no minerals added; □, ■, octacalcium phosphate additions; Δ, ▲, hydroxyapatite additions. (b) ●, no minerals added; ■, strengite additions; ▲, variscite additions. (c) ○, ●, no minerals added; □, ■, strengite additions.

Phosphorus transfers through the pH-induced conversions of MF2 incubations without mineral additions are comparable to the P transfers observed for incubations of MF1, while Ca-P was increased by the amount of added HAP or OCP at high pH values for the mineral addition experiments. The soluble phosphate data of MF2 incubations with and without added OCP intersect the OCP isotherm at pH 7.8 (Figure 3c). By comparing these results with the fractionation data, it becomes obvious that this is the pH value at which OCP began to dissolve. The relatively high losses of Ca-P in the pH range 7–7.8 (Figures 5a, 6a) were therefore a result of OCP dissolution. Adsorption of P was enhanced by the liberation of phosphate through dissolution of Ca-P minerals and caused an increase in the amount of Al/Fe-P. The added synthetic OCP and the sediment OCP of MF2 were completely dissolved at pH 7 after 7 days for incubations with and without OCP additions. Therefore, it can be concluded that the decrease in Ca-P of the incubations with added HAP from pH 7.8 to 7 was caused by the natural OCP content of MF2. By setting the pH of the incubations to values lower than 7, HAP also dissolved. Complete HAP dissolution was observed at pH 3.7 after 7 days. It is probable that a thermodynamically more stable compound dissolved below this pH value, until no further dissolution occurred in the pH range 2.4–2.8. Changes in Al/Fe-P contents mirrored the changes in Ca-P contents, except for values below 3.7 at which liberated phosphate was not sorbed completely. Comparison of the data with and without mineral additions further indicated that no adsorption occurred at pH values below 2.8, because the additional dissolved P from OCP and HAP additions was not found within the Al/Fe-P fraction at these values.

Additions of variscite or strengite had no significant effect on the Ca-P pool, as shown for strengite additions of SA2 incubations (Figure 6c). The amount of Al/Fe-P of HL2 and SA2 was increased by the amount of added strengite or variscite at any

pH, except the highest pH values of the variscite addition to HL2 and strengite addition to SA2, where the increase was lower than the amount of added variscite, and no increase was measured by strengite addition.

Calculation of the data for P species quantification

An amount of $0.043 \text{ mg P g}^{-1}$ sediment was added as HAP in the corresponding addition experiments, and $0.042 \text{ mg P g}^{-1}$ was found by the difference in Ca-P content of the controls with and without HAP addition (Figure 7a). The difference between the incubation experiments of MF2 with and without HAP addition at pH 7 corresponds almost quantitatively to the amount of added HAP. Therefore, in contrast to the complete dissolution of OCP, HAP did not significantly dissolve at pH 7 after 7 days of incubation.

The content of a Ca-P species was calculated by the difference in the amounts of Ca-P without dissolution and after complete dissolution of the concerning component. For calculation of the most soluble Ca-P component in a sample, the amount of Ca-P given by the control without mineral addition was used as the amount of Ca-P without dissolution of the most soluble Ca-P component. The amount of amorphous or mineral Al/Fe-P was determined by the amount of Al/Fe-P in the pH range 2.5–2.9 of the 7-day incubation experiments without mineral additions, because no adsorption of liberated P occurred and variscite or strengite did not significantly dissolve after 7 days of incubation in this pH range, as observed by comparison of pure mineral incubations (data not shown) and mineral addition experiments. Less than 1% of strengite or variscite dissolved by incubation of the pure minerals in the pH range 2.5–2.9 in 10 ml of 0.01 M CaCl_2 after 7 days of incubation when using quantities expected in 1 g of sediment. Adsorbed P was calculated by the difference in the amount of Al/Fe-P in controls without mineral

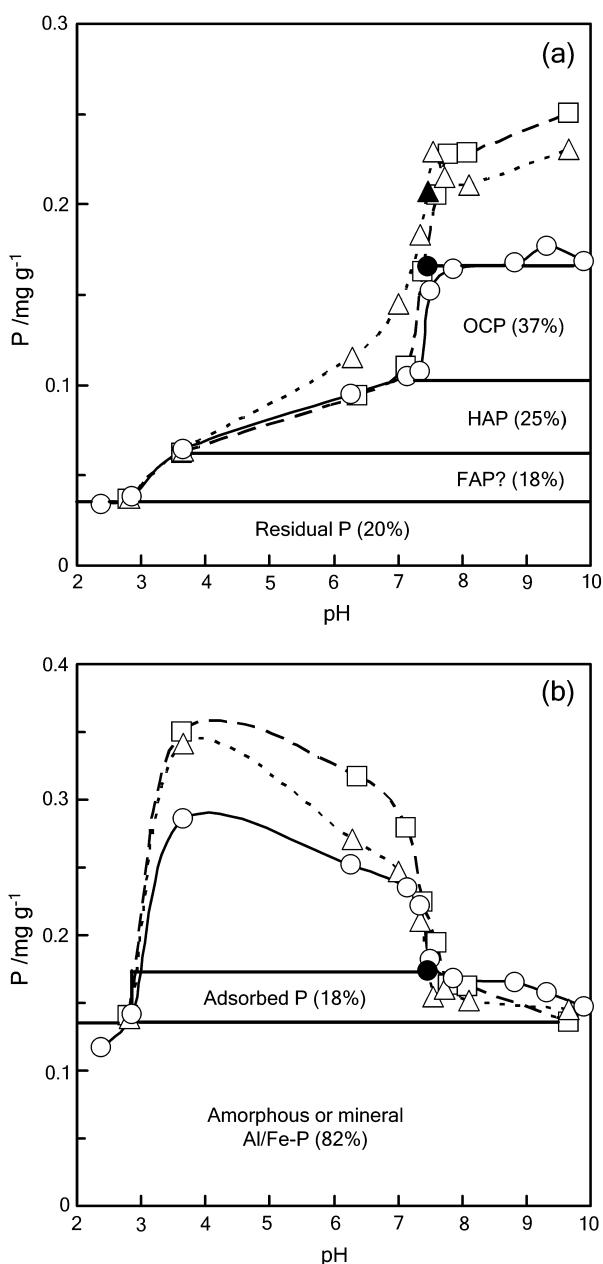


Figure 7 Calculation for P species quantification by the CONVEX scheme as illustrated for Ca-P species (a) and adsorbed P, amorphous or mineral Al/Fe-P (b) of the MF2 sediment. Fluorapatite was not used in standard addition experiments. ○, no minerals added; □, octacalcium phosphate additions; Δ, hydroxyapatite additions; ●, control without preliminary incubation; ▲, control without preliminary incubation and with hydroxyapatite addition. Abbreviations: FAP, fluorapatite; HAP, hydroxyapatite; OCP, octacalcium phosphate.

additions and amorphous or mineral Al/Fe-P content. Calculations are illustrated in Figure 7 for P species of MF2. Figures 8–10 show the enhanced dissolution of octacalcium phosphate in sediments by adsorption effects, the effect of

varying pH on the P composition in % of total inorganic P and phosphorus species composition of the sediments in relation to their sediment type and original, field-measured pH value as determined by the CONVEX method, respectively.

Discussion

The results clearly indicate that a chemical quantification of P species in sediments is possible by the use of the different solubilities of P minerals and the pH-dependent adsorption and desorption of P. As the effect of the sediment pH on soluble phosphate was similar to the effect described for soils and the procedure was tested for different sediment characteristics, the CONVEX method can possibly be applied for different kinds of soils and sediments without adaptation of the method. In contrast to common sequential extraction schemes, the described method provides the discrimination between OCP and apatite-like minerals and enables separate determination of amorphous or crystalline P compounds and adsorbed P. Moreover, pH-induced changes in P composition of the solid phase can be directly linked to changes in soluble phosphate.

By comparison of the Ca-P mineral addition experiments and the variscite or strengite addition experiments with the incubations without mineral additions, it becomes obvious that the liberated phosphate from Ca-P dissolution was not adsorbed, and that there was no measurable dissolution of variscite or strengite in the pH range 2.5–2.9. As the Al/Fe-P pool contains mineral and adsorbed P compounds, the apparent lack of dissolution of variscite or strengite cannot be discriminated from dissolution and subsequent adsorption of phosphate. Dissolution and subsequent adsorption can be excluded at low pH values, because no adsorption was observed under strongly acidic conditions. It was further confirmed by pure mineral incubations that the dissolution of significant amounts of variscite and strengite in the pH range 2–3 is unlikely. Moreover, soluble phosphate increased for all sediments at higher pH values as expected for strengite solubility at a fixed Fe^{3+} concentration of 50 μM (Figure 3b,c). Further, soluble Fe concentration measured by AAS suggests greater stability of strengite than expected by the isotherm at 50 μM of Fe^{3+} (see Figure 8b). Therefore, the increase in soluble phosphate by sediment incubation at low pH values can be attributed to the desorption of P, as also shown by the isotopic exchange experiments conducted by Murrmann & Peech (1969a). Amorphous or mineral Al/Fe-P compounds dissolve significantly by decreasing the pH below 2, as used for acid extractions for total P determination.

The compound with apparently lower solubility than HAP was not identified. The probable presence of the thermodynamically more stable fluorapatite (FAP) in the sediments is a likely explanation for the decrease in Ca-P below pH values, where complete dissolution of HAP occurred. An improvement of the procedure may possibly be achieved by standard addition

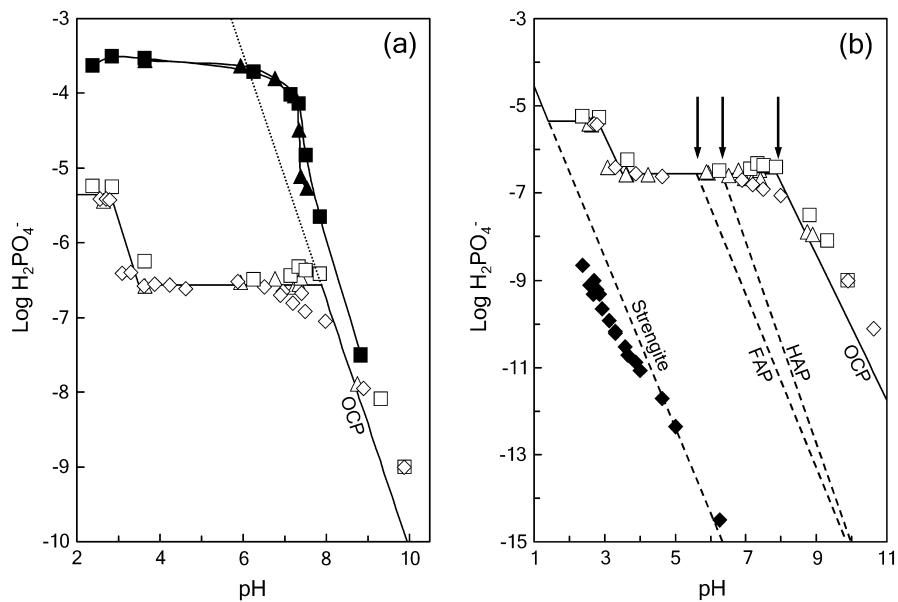


Figure 8 Enhanced dissolution of octacalcium phosphate in sediments by adsorption effects illustrated by the comparison of potential soluble P of the MF1 (\blacktriangle) and MF2 (\blacksquare) sediments and the theoretical solubility of octacalcium phosphate (a) and the pH values for the theoretical appearance/disappearance of fluorapatite, hydroxyapatite and octacalcium phosphate (arrows) in the sediments (b). The sediment isotherm is marked by the soluble phosphate concentrations of MF1 (Δ), MF2 (\square), HL1 and HL2 (\diamond) after 7 days of incubation. Strengite: strengite at 50 μM of Fe^{3+} . \blacklozenge : theoretical solubility of strengite calculated from measured soluble Fe concentrations. The isotherms of octacalcium phosphate, hydroxyapatite and fluorapatite are marked by OCP, HAP and FAP, respectively.

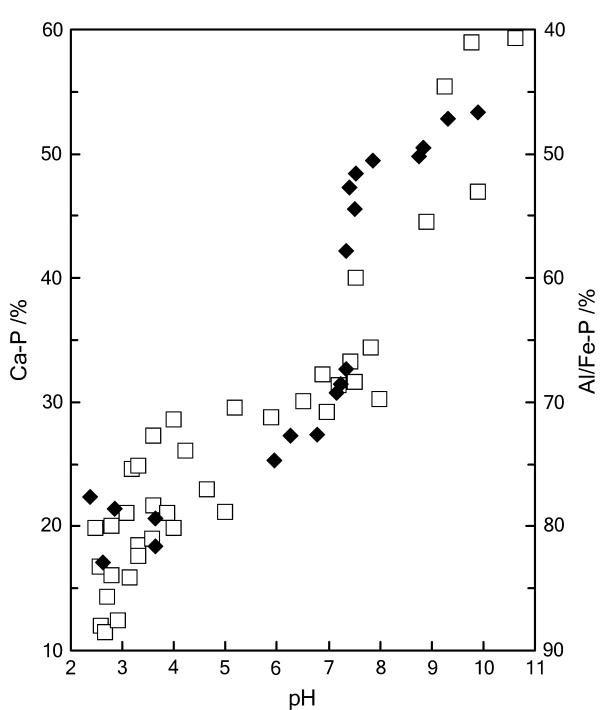


Figure 9 Effect of varying pH on the P composition in % of total inorganic P for all 7-day incubations of the sediment samples without P additions. MF sediments, which contained octacalcium phosphate naturally, are marked separately (\blacklozenge).

of FAP. In the pH range 2.4–2.8, Ca-P of MF2 did not further decrease with decreasing pH. Calcium phosphate, which remained at the lowest pH values used was operationally defined as residual P for all sediments and may include FAP. A possible precipitation of OCP during the fractionation after incubation is unlikely, because Ca^{2+} was eliminated prior to the fractionation. The fact that Ca was not detectable by AAS in the extracts of Al/Fe-bound P (data not shown) indicated that Ca had been removed completely.

All incubations, in which the original sediment pH had been increased to the range of possible OCP precipitation ($\text{pH} > 7$), showed formation of OCP after 7 days, as shown by increased Ca-P contents (e.g. Figure 5) and the corresponding solubility characteristics (Figure 3). The fast precipitation rate of OCP could be explained by a smaller phosphate adsorption in this pH range, as supported by the finding that soluble P concentration of MF was elevated in the pH range of enhanced OCP dissolution (Figure 8a). In view of the slow crystallization rate of HAP (Lindsay *et al.*, 1989), the CONVEX data support the hypothesis that authigenic apatite is formed via an OCP precursor in marine environments rather than by direct nucleation (Gunnars *et al.*, 2004). Direct nucleation of HAP may take place in the slightly acidic pH range below the pH of rapid OCP precipitation ($\text{pH} < 7$).

The largest changes in Ca-P content in relation to pH changes occurred by the precipitation or dissolution of OCP (Figure 9), and the amounts of Ca-P were highest for the MF sediments

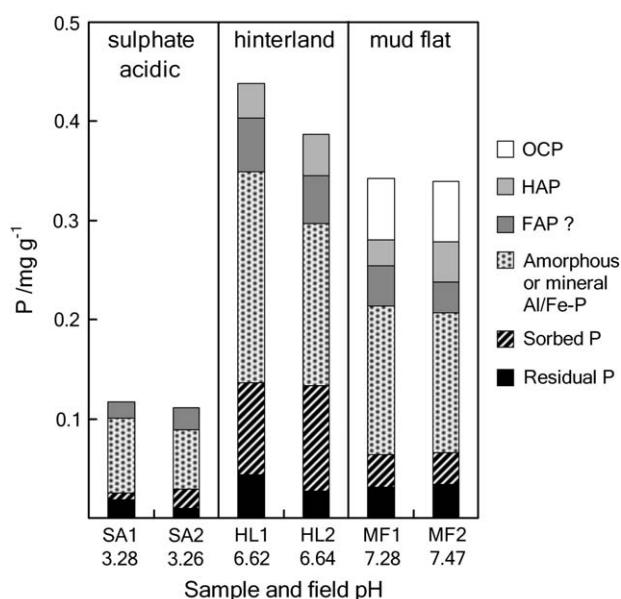


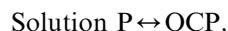
Figure 10 Phosphorus species composition of the selected sediments in relation to their sediment type and original, field-measured pH value as determined by the CONVEX method. Abbreviations: FAP, fluorapatite; HAP, hydroxyapatite; OCP, octacalcium phosphate.

mainly through the additional amounts of OCP (Figure 10). The formation of OCP is probably highly relevant for P cycling in alkaline environments. High values of aboveground biomass and bioavailable P are common at the fringe sites of mangroves (e.g. Boto & Wellington, 1984). Although this could be ascribed to sediment exchange or increased P availability by inundation-driven reduction of Fe-oxyhydroxides, the present results strongly suggest that P tidal inputs and subsequent OCP formation could represent a significant potential P source. Considering the narrow pH range of 7–8 at which OCP dissolution and precipitation can respectively occur, the alternation of its dissolution and formation by tidally induced changes in pore-water pH may increase dissolved P concentrations. In this context, previous field studies (J. F. Oxmann, unpublished data) also suggested that Ca-P could be a more effective source of P available for mangrove tree growth than Al/Fe-P. Bioavailable P (Morgan-P) correlated with Ca-P and leaf P in southern Vietnamese mangrove forests, whereas no correlation was found for bioavailable P versus Al/Fe-P. It is plausible that the rate of conversion reactions between solid phase inorganic P forms positively affects the rate of exchange with organic P mediated by plants and microbial activity.

The CONVEX procedure could be the method of choice to study dissolution and precipitation kinetics of mineral P compounds in soils and sediments and to explain results of previous studies on phosphate equilibria in soils. For example, Murrmann & Peech (1969b) pointed out that the rate of P mineral dissolution is too slow to account for the observed changes in phosphate solubility with pH. They found no change in solu-

ble phosphate after addition of HAP to acid soils and equilibration for 2 months and it seemed probable that no increase of soluble phosphate was observed because of the assumed extremely slow dissolution rate of HAP in soils. However, our fractionation data show that phosphate liberation during the conversion procedure through dissolution of Ca-P minerals, including HAP, is orders of magnitude greater than the measured changes in soluble phosphate. Murrmann & Peech (1969a,b) did not observe the masked liberation of phosphate through sorption after dissolution. The P adsorption capacity of the sediments was large enough to impede the increase of soluble phosphate concentrations in the pH range 3.5–7.8, even if Ca-P minerals had been completely dissolved during the conversion procedure.

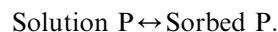
If it is assumed that the amount of phosphate liberated through dissolution and bound by subsequent adsorption is in solution, a hypothetical concentration of soluble phosphate can be calculated. The hypothetical soluble P shows the effect of adsorption on the dissolution behaviour of OCP and is further designated as potential soluble P. The concentration of the potential soluble P calculated for MF1 and MF2 without P additions was plotted on a solubility diagram together with the isotherm of OCP and the sediment isotherms of MF1, MF2, HL1 and HL2 (Figure 8a). This diagram compares soluble P with solid phase changes of P. It is evident that enhanced dissolution of Ca-P of MF1 and MF2, given by potential soluble P, occurred with decreasing pH at the point where the sediment isotherms and the OCP isotherm intersect. Below this pH, OCP dissolved to a much greater extent than expected from its theoretical isotherm. While the following equilibrium generally exists above pH 7.8:



an equilibrium with the mineral phase cannot be established below the pH value of the equilibration point of the three phases according to the equilibrium:



because the corresponding mineral completely dissolves. As the under-saturation is maintained throughout dissolution by adsorption effects, the mineral dissolves until the following equilibrium establishes:



The major reason for the under-saturation of the soil extracts with respect to the relevant minerals below the pH of the equilibration points can be referred to the adsorption of phosphate. For the selected sediments, the theoretical pH values at the equilibration points of the three phases for OCP, HAP and FAP are 7.8, 6.3 and 5.5, respectively (Figure 8b). Octacalcium phosphate was completely dissolved at pH 7 and half of the amount of added HAP was dissolved at pH 6.3 after 7 days of incubation. Thus, enhanced dissolution of these minerals through

adsorption effects indicates that they are less stable in soil or sedimentary environments compared with pure mineral incubations. The dissolution within narrower pH ranges may improve the discrimination of Ca-P minerals by the CONVEX method. Separate dissolution of the minerals would probably appear slightly below the pH values of the equilibration points, if the incubation time for dissolution is sufficiently long. As HAP was completely dissolved in the pH range 3–3.5 and the apparent FAP began to dissolve below pH 3.5 for all experiments, there was insufficient time for complete dissolution at greater pH values for these compounds.

The strongly acidic sediments contained relatively low amounts of Ca-P, presumably FAP, while HAP appeared additionally in the HL sediments and all Ca-P compounds were present in the MF sediments (Figure 10). For the quantification of mineral species by the CONVEX method it is not necessary to reproduce environmental dissolution conditions, as far as mineral amounts can be calculated by the difference in the amounts of Ca-P without dissolution and after complete dissolution of the corresponding mineral. Our results have shown a general conformity between the natural Ca-P species compositions of the six sediments determined by the CONVEX method and the Ca-P species compositions at the corresponding pH values after experimental pH adjustment. Incubations in pH adjusted 0.01 M CaCl₂ reflected natural conditions in terms of Ca-P mineral dissolution. Further CONVEX analysis of sediments with varying original pH will allow the extension of this experimental approach to a wider range of environmental settings. The dissolution of Ca-P compounds in the tidally affected environment of the sediments was probably influenced by a shift in pH, but generally the pH-related natural Ca-P species compositions in the sediments reflected the disappearance of Ca-P minerals observed by the mineral addition experiments.

The amount of adsorbed P in environmental settings may depend on the pH value, soil or sediment characteristics, phosphate inputs through material exchange and previously liberated phosphate through mineral dissolution. By excluding material exchange, it can be suggested that the greatest amounts of adsorbed P appear in the slightly acidic pH range. Lindsay (1979) suggested the coexistence of several P minerals in the pH range 6–6.5, in which soils generally reach the maximum phosphate solubility (Lindsay *et al.*, 1989). Thus, it is likely that the amount of adsorbed phosphate is highest in this pH range, if it is assumed that free phosphate will be adsorbed and the dissolution is further enhanced by adsorption. The previous considerations are supported by the determination of the greatest amounts of adsorbed P in the slightly acidic sediments. Moreover, the slightly acidic sediments contained the highest amounts of total P. In contrast, total P content was relatively low in the strongly acidic sediments and consisted mainly of amorphous or crystalline Al/Fe-P (Figure 10).

The fast precipitation of OCP and rapid adsorption of dissolved phosphate could be critical for the determination of P pools by some extraction schemes. For example, extraction at

elevated soluble phosphate concentrations in the pH range of possible OCP precipitation takes 26 hours by the SEDEX scheme. In contrast, changes in P composition can be observed by the CONVEX method and used for the determination of P species. The extraction time for the determination of the controls without preceding incubation takes 5 hours by the modified Kurmies' (1972) extraction, OCP precipitation is prevented during extraction by previous elimination of Ca and adsorbed or subsequently adsorbed P is quantitatively extracted by NaOH and H₂SO₄ plus additional Na₂SO₄ extractions. Benzing & Richardson (2005) argued that phosphate solubilized by the NaOH extraction of some methods could be precipitated as Ca minerals with an accompanied overestimation of calcium-bound P. In fact, this seems to cause errors in schemes with preliminary performed KCl extractions for 'loosely sorbed P' determination, which do not eliminate Ca prior to the NaOH extraction. We observed no overestimation using the Kurmies method, because the added strengite and variscite as well as the added soluble P were recovered almost completely within the Al/Fe-P fraction. Kurmies (1972) indicated that it is essential to eliminate Ca prior to such extraction methods.

It would be advisable to choose the CONVEX procedure for P species quantification of samples with characteristic features, especially if different Ca-P species are expected. Because of the time constraints involved in the determination of individual species, it should be used in combination with sequential extractions of higher sample throughput, e.g. in addition to the sequential extraction developed by Hedley *et al.* (1982) or the modified Kurmies' (1972) fractionation. Additionally, it is recommended that the CONVEX method be used for the preparation of reference soil or sediment samples. Standards of known species composition are necessary to evaluate different techniques used in P analysis (e.g. XANES).

As the described conversion-extraction method determines the actual P composition and changes in P composition with pH, the method should be effective in predicting changes in P composition and possible losses of P in regions where pH changes may occur. With respect to the high dissolution rates found in the present study, it can be concluded that high losses of P will appear by pH changes in natural systems, if the P buffering capacity through adsorption is insufficient. Our data additionally suggest that the efficiency of phosphate retention by adsorption has an influence on the pH values at which P minerals no longer occur. Therefore, as well as possible direct interactions and a probable reduction in calcium ion activity by humic material (e.g. Alvarez *et al.*, 2004), which may play a role in P mineralization, the P adsorption effect may have a large indirect influence on the P mineral composition of soils and sediments, simply by reducing the amount of soluble phosphate.

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