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On the presence of organic phosphate in some Camargue sediments: evidence for the importance of phytate

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Abstract

The organic phosphate pool of some Camargue sediments (South of France) was studied, after removal of inorganic phosphate, with Ca-NTA/dithionite (Fe bound phosphate) and Na-EDTA (Ca bound phosphate). The organic phosphate was divided into an acid soluble organic phosphate fraction (ASOP) and a residual organic phosphate fraction (ROP). The extraction of organic matter with 2.0 M NaOH (90 °C) from ROP yielded considerable quantities of Org-P. In this extract the presence of phytate (inositol hexa phosphate) could be demonstrated using phytase to hydrolyse the phytate. Phytate was shown to account for a considerable part of organic phosphate in sediments of freshwater marsh sediments as well as in the sediment of the brackish/salt water lake 'Etang de Vaccares'. In laboratory experiments phytate was found to precipitate with all poly-valent cations tested. Furthermore, phytate was found to be strongly adsorbed onto Fe(OOH), which may explain its accumulation and its stability in sediments.

Considerable quantities of ASOP were found; the chemical stucture of this pool remains unknown.

Abbreviations: In this paper the standard abbreviations are used, as suggested by the editor (see page vi):

$\text{chem-X}_{\text{phys}}$ ($\text{inorg-P}_{\text{diss}}$, $\text{Tot-N}_{\text{sed}}$)

Additional abbreviations used:

ASOP	= acid soluble organic phosphate
$CaCO_3 \approx P$	= calcium bound phosphate
Fe(OOH)	= iron hydroxide
$Fe(OOH) \approx P$	= iron bound phosphate
$NaOH_{extr}$-P	= phosphate extracted with NaOH (90 °C) after ASOP extraction.
Phyt-P	= phytate bound phosphate
ROP	= residual organic phosphate

Introduction

Organic-P (Org-P) is the P-fraction which has often been neglected and underestimated in sediment chemistry. Holtan *et al.* (1988) did not even mention the question of the forms and quantities of Org-P in sediments in their review. Boström *et al.* (1982) presented an 'idealized' distribution of P_{sed} fractions in which Org-P was estimated at approximately 16%. Recent work has shown the presence of large quantities of Org-P, accounting for more than 50% of Tot-P in most sediments (Boström *et al.*, 1985; De Groot & Golterman, 1990; De Groot, 1990; De Groot & Fabre, 1993; Moutin *et al.*, 1993), values which correspond to the values of Org-P found in soils (Stevenson, 1982; Tate, 1985). De Groot & Golterman (1990) and De Groot (1990) showed that a general underestimation of Org-P occurred, because of hydrolysis of Org-P by insufficiently specific extractants used in the extraction of inorganic P-fractions from the sediment. The extraction schemes used, all utilize NaOH and HCl or H_2SO_4 for the extraction of inorganic P (Hieltjes & Lijklema, 1980; Psenner *et al.*, 1985; Psenner *et al.*, 1988). Golterman & Booman (1988) and De Groot & Golterman (1990) have proposed an extraction scheme for the fractionation of inorg-P_{sed} utilizing less aggressive extractants: Ca-NTA/dithionite for the extraction of $Fe(OOH) \approx P$ and Na-EDTA for the extraction of $CaCO_3 \approx P$. De Groot (1990) showed the existence of an acid hydrolisable Org-P fraction (ASOP) in sediments. This fraction was obtained after removal of inorg-P with Ca-NTA/dithionite and NaEDTA. In schemes using HCl or H_2SO_4 for the extraction of $CaCO_3 \approx P$ ASOP is extracted together with the apatite-P.

Phytate is an organic phosphate which is widespread in nature. In plant seeds phytin (Ca, Mg-phytate) is common and plants can stock phosphate as phytate (Hess, 1975). Phytate has been found in soils all over the world and has been accepted to be the largest Org-P fraction, identified in soils (Stevenson, 1982; Tate, 1985).

In this study the Org-P present in Camargue sediments (Rhone delta, France), rich in $CaCO_3$ (20–40%), was studied. After the removal of $Fe(OOH) \approx P$ and $CaCO_3 \approx P$, according to the method of Golterman & Booman (1988) and De Groot & Golterman (1990), followed by the extraction of ASOP (De Groot, 1990), the residual Org-P was studied, using methods comparable to those used in soils. The organic matter was extracted using hot NaOH and Tot-P was measured. After precipitation of humic acids with H_2SO_4 from the extract enzyme essays were carried out on the supernatant in order to try and detect phytate in the extracts.

Materials and methods

All determinations were carried out according to the IBP Manual Nr 8 (Golterman *et al.*, 1978). Iron was determined colorimetrically as Fe^{2+} by means of o-phenantroline (IBP, method 4.5.1), using ascorbic acid as reducing agent. Orthophosphate (o-P) was determined by means of a molybdate antimony reagent (IBP method 5.6.1.). Ca^{2+} was determined by means of atomic emission spectrophotometer (Jenway PFP7 Flame Photometer).

Sediment samples were taken from several marshes, a drainage ditch and a shallow brackish lake and transported directly to the laboratory. The samples were divided into two parts: part A was used to analyse o-P in the interstitial water, from part B a sediment suspension was prepared containing 100–200 g sediment per liter. Suspensions were sieved over 200 μm meshsize.

Interstitial water was obtained by centrifugation (7000 rpm) of the sediment under exclusion of air. The supernatant was collected with a syringe and passed over a 0.45 μm GM/F millipore filter into a vacutainer.

For the determination of Tot-C and Tot-N 0.25–0.5 g d.w. was digested with $K_2Cr_2O_7$/H_2SO_4. The volume of the digestion mixture was adjusted to 50 ml. Assuming $C_5H_7NO_2$ as general formula of organic matter (Golterman, 1975), Org-C was calculated from COD which was determined by measuring Cr^{3+} (method 7.3.2). On a aliquot from the same digestion mixture Tot-N

was determined as NH_3: an equal volume of 10 M NaOH was added, after which the NH_3 was distilled into a reaction vessel and determined acidimetrically. For the determination of Tot-P and Tot-Fe 0.25–0.5 g d.w. was digested with H_2O_2/H_2SO_4. The volume of the digestion mixture was adjusted to 50 ml and o-P and Fe were determined after centrifugation and neutralization.

P-fractionation

From an aliquot containing approximately 0.25 g d.w. of sediment (pellet 0; Fig. 1) Fe(OOH)≈P was extracted with a mixed solution of Ca-NTA (0.02 M) and dithionite (0.045 M), buffered with 24 g l^{-1} of TRIS (pH = 8) and $CaCO_3 \approx P$ with Na-EDTA (0.05 M; pH = 8) (Golterman & Booman, 1988; De Groot & Golterman 1990). ASOP was extracted (from pellet I; Fig. 1) with 0.25 M H_2SO_4 (De Groot, 1990). There after an extraction with 2.0 M NaOH (90 °C for 30 minutes) was carried out (on pellet II; Fig. 1). After centrifugation a digestion with $K_2S_2O_8$ and H_2SO_4 was performed on the supernatant to determine the $NaOH_{extr}$-P, which was measured as o-P after neutralization. After adjusting the pH, the NaOH-extract was incubated with a phytase

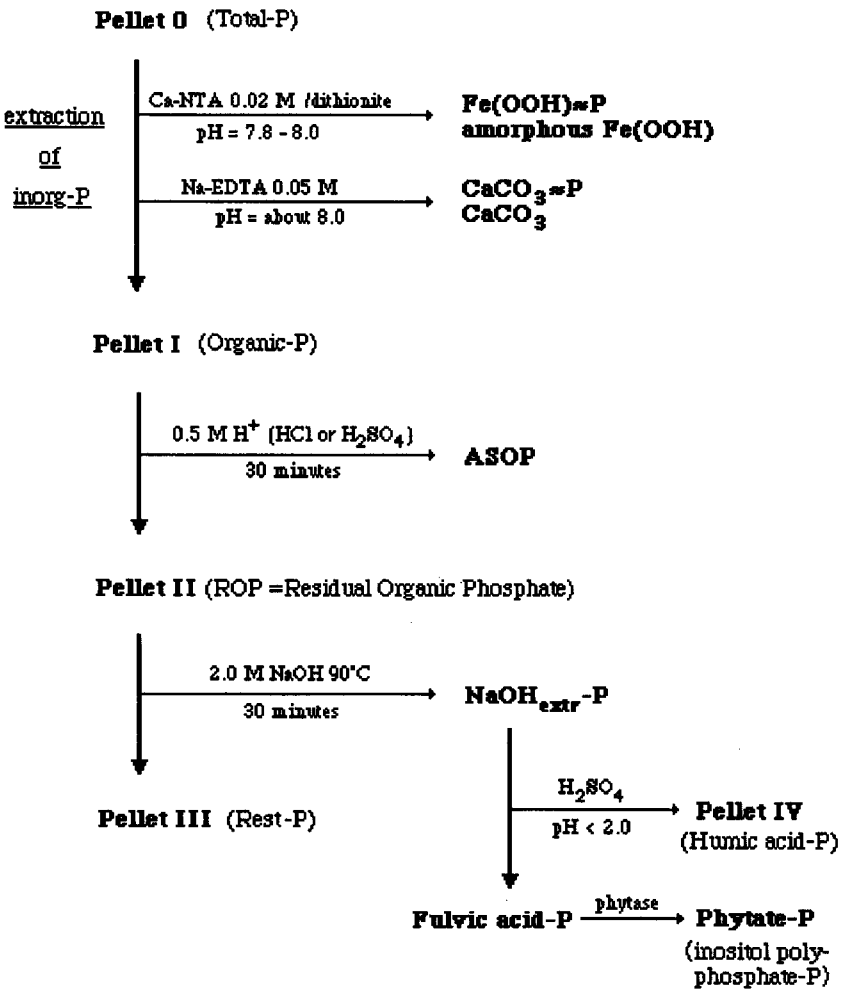

Fig. 1. Fractionation scheme.

and with an alkaline phosphatase. Since interferences of the enzymes by the organic matter present in the extract occurred, the humic acid was precipitated (pellet IV; Fig. 1) in acid medium, before the enzyme essay. The Org-P present in the pellet IV was determined after digestion with H_2O_2. The enzyme essay was carried out with the supernatant, after adjusting the pH.

Enzyme essays

Both a phytase from aspergillus with an optimum of pH 2.8 (Sigma P-9792) and an alkaline phosphatase with an optimum of pH 10.8 (Sigma P-4252) were used. The samples were buffered with 0.2 M acetic acid for phytase and with 0.5 M bicarbonate for alkaline phosphatase; the pH was adjusted if necessary. After 17 h of incubation o-P was determined. The enzyme essays, carried out on the NaOH extract were often partly, or completely inhibited by the organic matter present in the extract. Therefore, part of the humic substances were precipitated in acid medium and removed by centrifugation and the enzyme essay was carried out on the supernatant containing 'Fulvic acid-P' only. After precipitation of humic acid, often a partial inhibition of the enzyme still occurred, whereas in sediments rich in organic matter complete inhibition occasionally occurred; this inhibition was measured by adding a known quantity of phytate to the samples in the enzyme essays.

Phytate salt formation and solubility experiments

Phytate salts were prepared by mixing 1 ml 0.01 M phytate with respectively 6 and 9 ml of a 0.06 M solution of either Mn^{2+}, Mg^{2+}, Fe^{2+} or Ca^{2+}, or with 4 and 6 ml of a 0.01 M solution of either Fe^{3+} or Al^{3+}. After 48 h the suspensions were centrifuged and the supernatant was filtered (0.45 μm GM/F millipore). The concentration of dissolved phytate and pH were measured. The phytate salts were resuspended in 10 ml of 0.5 M HCl or in 10 ml of 0.05 M Na-EDTA. In case of incomplete dissolution of the precipitate the concentration of dissolved phytate was measured.

Phytic acid adsorption

A suspension of Fe(OOH) containing 6.62 mmol of Fe per liter was prepared by dissolving $FeCl_3$ in H_2O and adjusting the pH to about 8 by adding 10 M NaOH. After sedimentation the overlaying solution was decanted and replaced by H_2O in order to remove Na^+ and Cl^-. This was repeated until dissolved Cl^- was lower than to 1 μM. Three series of 4 phytate additions (0, 0.01, 0.033 and 0.1 mmol) were prepared of approximately 100 ml; A) without Ca^{2+} and o-P addition, B) 4 mg Ca^{2+} no o-P addition and C) 4 mg Ca^{2+} and 1.98 mg o-P (as KH_2PO_4). To all flasks 1.0 mmol of H_3BO3 was added. pH was adjusted to 8.9 with NaOH. NaCl and KCl were added in order to obtain equal quantities of K^+ and Na^+ in all flasks: 0.064 mmol K^+ and 0.114 mmol Na^+. The final volume was 112 ml.

After two weeks pH, o-P, Ca^{2+} and phytate were determined (in duplicate) in solution, as well as Fe(OOH)$\approx$P and Fe(OOH)$\approx$phytate. Furthermore the suspensions were sequentially fractionated with: (a) 0.02 M Ca-NTA/0.045 M dithionite, (b) 0.25 M H_2SO_4 and c) 2.0 M NaOH 90 °C. Enzyme essays with phytase were carried out both for the solution, the NaOH-extract and for the solid Fe(OOH)$\approx$phytate.

Results

Laboratory experiments with phytate

Since Ca, Al, Mg, Fe and Mn are present in considerable quantities in most sediments, the interactions of theirs ions with phytate were tested in the laboratory: phytate was found to form precipitates with ions of all of these metals (Table 1). Results of the solubility experiments carried out on these phytate salts are also presented in Table 1. The ferric iron and the aluminium salts were little soluble in 0.5 M HCl, whereas the Ca, Mg

Table 1. Some properties of phytate salts. Phytate concentration and pH as measured in equilibrium, metal concentration estimated by assuming a stoichiometric formation of the salt. For the presentation of the results of the solubility experiment the following codes are used: + = complete dissolution of salt within 2 h, +/− = complete dissolution of salt within 40 h.

Metal	Salt	pH	[phytate] $\mu M\,l^{-1}$	[metal] $\mu M\,l^{-1}$	EDTA	HCl
Ca^{2+}	Ca_6phytate	7.12	2.57	16	+	+
		6.98	1.28	$9\ 10^3$		
Fe^{2+}	Fe_6phytate	5.94	0.08	0.5	+/−	+/−
		4.66	0.17	$9\ 10^3$		
Fe^{3+}	Fe_4phytate	3.58	0.74	3	+/−	14.2 μM of phytate
		2.79	42.62	$5.9\ 10^3$		
Mg^{2+}	Mg_6phytate	6.29	9.89	59	+	+
		6.94	5.55	$9\ 10^3$		
Mn^{2+}	Mn_6phytate	6.77	1.78	11	+	+
		6.32	0.94	$9\ 10^3$		
Al^{3+}	Al_4phytate	5.21	24.14	97	+/−	0.63 μM of phytate
		3.48	4.02	$5.7\ 10^3$		

and Mn salts dissolved readily in 0.5 M HCl (Table 1).

In the adsorption experiment phytate added was adsorbed onto Fe(OOH) (Table 2). Furthermore, Ca^{2+} enhanced the adsorption of phytate on Fe(OOH) (Fig. 2). The presence of o-P did not affect the adsorption (Table 2); the differences found in phytate adsorbed between the flasks with only Ca^{2+} and those with Ca^{2+} and o-P were probably due to difference in pH. With the phytate adsorption some Ca^{2+} was found to be adsorbed; the precipitation of Ca-phytate could be excluded to occur, as no phytate dissolved in the acid extraction.

The adsorption of phytate on the Fe(OOH) had an inhibitory effect on the o-P adsorption: per mmol phytate adsorbed 1.7 mmol o-P was found to be released or inhibited to be adsorbed (Table 2).

In the subsequent fractionation (Ca-NTA, HCl, Residual-P), at least 99% of the phytate adsorbed on the Fe(OOH) was recovered as Residual-P. During the extraction with Ca-NTA-dithionite in the samples with phytate a fraction

Table 2. Results of the phytate adsorption experiment (phyt-P = remaining dissolved phytate).

Treatment	pH	o-P ($mg\,l^{-1}$)	phyt-P ($mg\,l^{-1}$)	Ca^{2+} ($mg\,l^{-1}$)	Fe(OOH)≈P ($mg\ flask^{-1}$)	Fe≈phyt-P ($mg\ flask^{-1}$)	P/Fe ($g\,g^{-1}$)
Phyt	8.91	0.00	0.00	0.00	0.00	0.00	0.000
Phyt	8.92	0.00	0.14	0.00	0.00	1.75	0.052
Phyt	8.70	0.00	25	0.00	0.00	3.12	0.092
Phyt	8.85	0.00	136	0.00	0.00	2.82	0.084
Phyt, Ca	8.90	0.00	0.00	12.7	0.00	0.00	0.000
Phyt, Ca	8.98	0.00	0.00	7.98	0.00	1.77	0.052
Phyt, Ca	8.68	0.00	6.22	2.77	0.00	5.15	0.15
Phyt, Ca	8.77	0.00	122	5.60	0.00	4.37	0.13
Phyt, Ca, o-P	8.73	0.17	0.00	7.23	1.96	0.00	0.000
Phyt, Ca, o-P	8.71	3.45	0.00	5.08	1.59	1.77	0.052
Phyt, Ca, o-P	8.51	13.8	3.11	2.72	0.41	5.49	0.16
Phyt, Ca, o-P	8.54	15.3	108	5.84	0.24	5.92	0.18

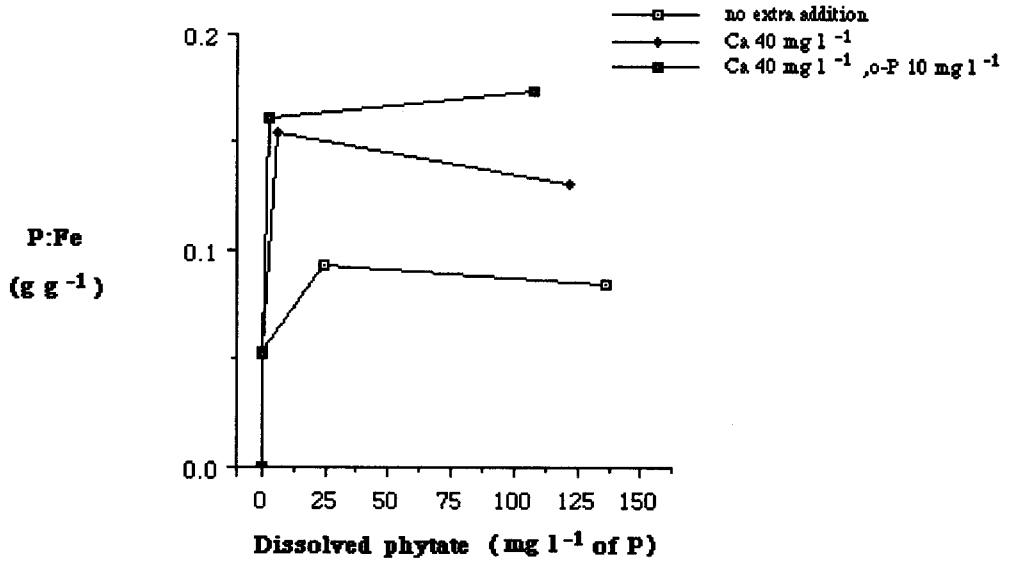

Fig. 2. Adsorption isotherms of phytate on Fe(OOH).

of the orange precipitate containing approximately all phytate did not dissolve, whereas most of the Fe(OOH) and all Fe(OOH)≈P dissolved. In the samples without phytate all iron completely dissolved in the Ca-NTA-dithionite. It seems that, to some extent, phytate protects a part of the Fe(OOH) against reducing agents. During the extraction with 0.5 M HCl the rest of the Fe(OOH) disappeared, but a white precipitate remained. This precipitate was found to contain 4.3 molecules of Fe per 6 molecules of P, which suggests that Fe_4phytate has been formed during the extraction.

Phytase was found to be incapable of hydrolyzing phytate from the Fe(OOH)≈phytate complex. 2.0 M NaOH (90 °C) was found to dissolve phytate from the Fe(OOH)≈phytate complex.

Org-P in sediments

Some properties of the sediments studied are presented in Tables 3a and 3b. After the removal of Fe(OOH)≈P and $CaCO_3$≈P with Ca-NTA/dithionite and Na-EDTA, the Org-P could be divided into an acid soluble fraction (ASOP), accounting for 59–229 $\mu g\ g^{-1}$ of P, a hot-NaOH soluble fraction, accounting for 102–291 $\mu g\ g^{-1}$ of P, and a Rest fraction, accounting for 6–79 $\mu g\ g^{-1}$ of P.

Table 3a. Tot-P, Tot-Fe, Org-C, Tot-N concentrations and C/N ratio of the sediments studied.

	Tot-P ($\mu g\ g^{-1}$ d.w.)	Tot-Fe (mg g^{-1} d.w.)	Org-C (mg g^{-1} d.w.)	Tot-N (mg g^{-1} d.w.)	C/N (g g^{-1})
Garcines Nord I	660	49.9	24.1	2.73	8.8
Garcines Nord II	632	43.2	97.2	7.88	12.3
Garcines Sud	488	26.1	13.9	1.15	12.1
Relongues	986	13.4	76.5	7.48	10.2
Buisson Verte	419	23.2	15.1	1.14	13.2
Baisse Salee	689	40.7	21.2	2.07	10.2
Ditch ORF	3230	47.5	46.2	4.78	9.7
Etang de Vaccares	639	39.2	20.6	2.68	7.7

Table 3b. pH, dissolves interstitial o-P and Fe^{2+} and inorg-P fractions of sediments studied.

	pH	o-P ($\mu g\,l^{-1}$)	fe^{2+} ($mg\,l^{-1}$)	$Fe(OOH) \approx P$ ($\mu g\,g^{-1}$)	$CaCO_3 \approx P$ ($\mu g\,g^{-1}$)
Garcines Nord I	8.4	1	4.0	53	210
Garcines Nord II	7.0	757	2.4	69	243
Garcines Sud	7.2	270	0.9	35	140
Relonges	7.2	557	0.6	49	277
Buisson Verte	7.3	419	9.1	34	131
Baisse Salee	7.1	622	15.8	34	185
Ditch ORF	7.6	189	0.5	2070	442
Etang de Vaccares	7.7	527	3.0	109	200

Often some Ca^{2+} was extracted with the ASOP. This Ca^{2+} seems to originate from traces Ca-EDTA which remained present in the interstitial water after centrifuging and removal of the EDTA-extract; the sediments used were rich in $CaCO_3$ (20–40%). After washing the pellet between the Na-EDTA and the ASOP extraction, there was a strong decrease in the Ca^{2+} and only traces of Ca^{2+} remain in the extract (unpublished data).

The extract obtained by means of the hot NaOH extraction had a dark brown colour. In a direct essay on the pellet of the Garcines sediment Org-P could be hydrolysed from ROP for 34% using phytase, whereas alkaline phosphatase hydrolysed no more than 4.6% of Org-P. Since from all sediments tested alkaline phosphatase hydrolysed only small quantities of Org-P, the essay was not carried out systematically. The quantities of Org-P, found in the different fractions are presented in Table 4. Phytate was found to be present in substantial quantities in all sediments studied (24–150 $\mu g\ g^{-1}$ of P).

Even though the humic acid was precipitated, in some cases a partial inhibition of the enzyme by fulvic acid in solution still occurred (Table 5).

In the pellet III, which remained after the NaOH extraction less than 10% of Tot-P (between 1.6 and 17% of Org-P) was recovered, for all sediments tested.

Discussion

In a shallow freshwater marsh in the Camargue (France) considerable quantities of ASOP were found in different sediment layers (De Groot & Fabre, 1992). In the deepest layer, ASOP was the largest P-fraction accounting for up to 60% of Tot-P. On desiccation of the sediment specific mineralization of ASOP was observed (De Groot & Van Wijck, 1993; De Groot & Fabre, 1993),

Table 4. Org-P fractions measured in the sediments studied.

Sediment	ASOP P ($\mu g\,g^{-1}$)	$NaOH_{extr}$-P P ($\mu g\,g^{-1}$)	Phytate-P P ($\mu g\,g^{-1}$)	Humic acid-P P ($\mu g\,g^{-1}$)	Rest-P P ($\mu g\,g^{-1}$)
Garcines Nord I	219	165	149	15.4	5.9
Garcines Nord II	88	123	45	8.8	22.3
Garcines Sud	137	143	65	6.0	22.3
Relonges	221	271	117	102	66.6
Buisson Verte	145	102	24	7.1	12.3
Baisse Salee	163	169	90	39.4	42.4
Ditch ORF	173	290	107	107	78.7
Etang de Vaccares	58	170	92	8.8	32.3

Table 5. The inhibition of phytase in the presence of NaOH extract from different sediments.

Sediment	Volume NaOH extract	μg of o-P after hydrolysis		% inhibition
		1.85 μmol phytate	9.25 μmol phytate	
Control	0	346		0
0			1730	0
Buisson Verte	5	204		41
Baisse Salée	5	177		49
Vaccares	5	171		51
Garcines Sud	5	192		45
Buisson Verte	5		895	48
Baisse Salée	5		882	49
Vaccares	5		875	49
Garcines Sud	5		934	46
Buisson Verte	10		676	61
Baisse Salée	10		471	73
Vaccares	10		534	69
Garcines Sud	10		559	68

whereas no significant mineralization occurred in the ROP fraction ($NAOH_{extr}$ P + Rest pellet P). In the top layer a mineralization of about 40% (about 70 μg g^{-1}) and in the deepest layer a mineralization of about 60% (about 200 μg) of ASOP was observed (De Groot & Fabre, 1993). Under these condition the ASOP was shown to be the only active Org-P fraction and must therefore be considered as an ecologically important fraction.

The nature of the ASOP fraction has not been elucidated yet. ASOP exists likely of easily acid-hydrolysable Org-P. We have no data to decide whether the organic residue went into solution during the extraction or remained in the pellet. The UV-spectra of the ASOP extracts were measured, but no information concerning the nature of ASOP was obtained. The results of experiments with suspensions of *Scenedesmus spec* (unpublished data), showed no significant release of o-P from the cells during a 30 minute extraction with 0.5 M HCl, which was performed after treatment with Ca-NTA/dithionite and EDTA. The hypothesis of a release of phosphate after lysis of living cells, as suggested by De Groot (1990), should therefore be rejected. Since a considerable decrease in Ca^{2+} extracted with ASOP occurred after washing of the pellet I, the hypothesis of a Ca≈Org-P complex, as suggested by De Groot (1990), should be rejected as well.

Phytate has been found in considerable quantities in soils all over the world (Cosgrove & Tate, 1963; Anderson, 1964; Martin & Wicken, 1966; Williams & Anderson, 1968; Omosoto & Wild, 1970; Anderson & Malcolm, 1974). Several isomers of inositol were detected in soils. Cosgrove (1966) detected myo-, DL-, scyllo- and neo-inositol in Scottish and Californian soils, by means of ion-exchange chromatography and paper chromatography. In sediments however phytate has never been detected before, although some studies were carried out (Lijklema, pers. comm.). Herbes *et al.* (1975) showed the presence of phytate in fresh water. The hot-NaOH extractable P may contain humic bound P and phytate-P, as well as traces of nucleic acid-P (Anderson, 1970). The quantities of phytate obtained from different Camargue sediments (24.0–150 μg g^{-1} of P), correspond to values found by Islam and Ahmed (1973) in Bangladesh soils (17.5–150 μg g^{-1}). The values found in our study should be considered to be minimum values as an underestimation may have occurred due to partial inhibition of the phytase (Table 5).

Anderson & Malcolm (1974) studied the nature of hot alkali-soluble Org-P in some Scottish soils. The different inositol-phosphates accounted for 53.3% of Org-P: inositol hexaphosphate accounted for 44.3% of Org-P, inositol penta-

phosphate for 6.6%, inositol tetraphosphate for 1.9% and inositol tri-phosphate for 0.6 %, plus some traces of inositol diphospate. Furthermore, they detected four isomers of inositol: myo-, scyllo-, chiro- and neo-inositol. In addition to the inositol phosphates, they detected traces of desoxinucleoside diphosphates (0.6 % of Org-P) and pyrophosphate (0.6% of Org-P), as well as several unidentified compounds (2.7% of Org-P). Since we used phytase it was neither possible to distinguish the different forms of inositol phosphate, nor the different isomers.

Islam & Ahmed (1973) found that prolonged submersion increased the mineralization of phytate from soils. The fact that their time curves tend towards an asymptote at substantial phytate concentrations may indicate that: (1) the increase of mineralization was due to an increase in mircrobial activity in general and not to a specific increase in phytate mineralization, or (2) that phytate is present in different forms, which are not equally mineralized. Remoisting of soils was found to cause a temporary increase of microbial activity (Stephenson, 1956; Barlett & James, 1980).

Since most naturally occurring Org-P compounds are easily metabolized by microorganisms their stability in the sediment will depend on the ability of the molecules to form complexes with sediment components, which will protect them from mineralization. Two forms of complexation seem to be of importance:

(1) complexation of phosphate groups with metal ions and hydroxides and
(2) complexation of functional groups with organic matter.

Phytate was shown to form insoluble complexes with all poly-valent cations tested.

Furthermore, phytate was found to be strongly adsorbed onto Fe(OOH). Small quantities of phytate were completely adsorbed (Table 2; Fig. 2). Golterman *et al.* (1991) found that the presence of metal ions and especially bi-valent ions such as Ca^{2+} and Mg^{2+}, in solution had a positive effect on the phosphate adsorption onto Fe(OOH). We observed a similar effect of Ca^{2+} on the adsorption of phytate on Fe(OOH). The addition of phytate to a suspension of Fe(OOH)$\approx$P caused the release of part of the adsorbed o-P (Table 2; Fig. 3), indicating that inositol hexa-phosphate has an affinity towards Fe(OOH), stronger than that of o-P.

Furthermore, on reduction of the Fe(OOH) no phytate was released, but Fe_4phytate was formed. Therefore phytate seems likely to accumulate and to be stable in sediments. Whether the integration into larger organic complexes is of importance remains to be seen. Williams & Anderson (1968) reported organo-mineral complexes containing nucleic acid and inositol phosphates.

Although plants produce considerable quantities of inositol phosphates and have been shown to be the main source of soil inositol phosphates (Ballou *et al.*, 1963), the role of microorganisms in the production of soil-phytate should not be neglected (Cosgrove, 1966; Stevenson, 1982). Scyllo-inositol is found in microorganisms but not in higher plants (Stevenson, 1982), therefore scyllo-inositol phosphates are certain to have been produced by microorganisms.

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