Fractionation of sediment phosphate with chelating compounds: a simplification, and comparison with other methods

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Abstract

The NTA/EDTA fractionation of sediment-bound, inorganic phosphate was improved and made more effective. The iron-bound phosphate is now extracted with Ca-EDTA plus dithionite and followed by an extraction of calcium-bound phosphate by Na_2-EDTA at pH = 4.5. A comparison is made with two other sequential extraction procedures: the SEDEX and the Hieltjes & Lijklema extractions. The EDTA extractions have an advantage over the other two methods.

Abbreviations

o-P = ortho phosphate
ASOP = Acid Soluble Organic Phosphate
TRIS-buffer = tris (hydroximethyl)-amino methane
NTA = Nitrilo triacetic acid (or its salts)

EDTA = Ethylene dinitrilo tetraacetic acid(or its salts)
$Fe(OOH) \approx P$ = iron-bound phosphate
$CaCO_3 \approx P$ = calcium-bound phosphate
NaOH-P_{extr} = phosphate extracted with NaOH (etc.)

Throughout this article: M = mol dm^{-3}.

Introduction

In Europe a discussion has been started on the standardization of the fractionation of sediment-bound inorganic phosphates, 'inorg-P_{sed}', which consist of iron- and calcium-bound phosphate, $Fe(OOH) \approx P$ and $CaCO_3 \approx P$. As these compounds have different chemical and biological properties, it is essential for a better knowledge of the P-cycle in lakes to measure them separately. [1]

In principle two approaches to the fractionation are possible:

(A) Extractions with compounds such as NaOH and H_2SO_4, which produce operational results. The pH during the extraction is very different from the
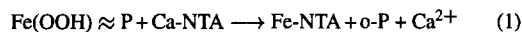
pH of the sediment, causing changes in the original P-composition.

(B) Extractions with chelating compounds supposed to react with specific compounds present in the sediments; these are carried out at a pH near to that of the sediment.
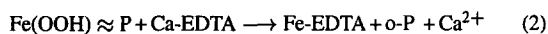
For several years we have used approach (B) using Ca-NTA (0.02 M) followed by Na-EDTA (0.05 M) to extract $Fe(OOH) \approx P$ and $CaCO_3 \approx P$ respectively. First Kouwe & Golterman (1976) used Ca-NTA and EDTA solutions, but, as the extraction takes a long time, it was performed with columns in which the sediment was extracted overnight. Ca-NTA was chosen for the first extraction as it gave more effective results than Ca-EDTA. Although the stability constant of Fe-EDTA is higher than that of Fe-NTA (See Annex 1), NTA produced better results, probably because the adsorption

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[1] These formulae are used to avoid a discussion on the true chemical nature of the compounds. See Golterman, 1995a & 1995b.

of NTA onto the sediment is stronger. Furthermore, the equilibrium constants for the reactions

$$Fe(OOH) \approx P + Ca\text{-}NTA \longrightarrow Fe\text{-}NTA + o\text{-}P + Ca^{2+} \quad (1)$$
and
$$Fe(OOH) \approx P + Ca\text{-}EDTA \longrightarrow Fe\text{-}EDTA + o\text{-}P + Ca^{2+} \quad (2)$$

differ less than for the same reactions with NTA and EDTA as reagents in (1) and (2) respectively; they depend on the pH and the reaction constant for (1) is maximal at pH = 6–8 (Golterman, 1982). Compared with Ca-EDTA, Ca-NTA is a stronger extractant than the conditional stability constants suggest.

Golterman (1982) added a reducing agent in order to accelerate the reaction. $NH_2OH.HCl$ or ascorbic acid were used, with the disadvantage that these compounds are only active when pH<5.5 and may then react with $CaCO_3 \approx P$. Later Golterman & Booman (1988) used dithionite as the reducing agent, which is active even at pH = 8, but is needed in excess and is an impractical compound being unstable even in closed pots. Ca-NTA has the disadvantage that it is insoluble at higher concentrations than 0.02 M. Therefore several extractions are often necessary (De Graaf & Golterman, 1989).

During discussions on standardization, it was pointed out that it is impractical to have to repeat the extractions several times. I have, therefore, tried to replace Ca-NTA by Ca-EDTA, the advantage of Ca- NTA over Ca-EDTA being less important than originally thought, since dithionite is added to the extractant and the extraction consequently takes no longer than 1 to 2 hours, depending on the type of sediment. In sediments with much $CaCO_3$ the extraction with EDTA at pH = 8 (the usual pH for $CaCO_3$ rich sediments) has also to be repeated several times, because the EDTA is rapidly saturated with respect to Ca. The pH needs to be relatively high in order to prevent the next extraction of acid soluble org-P ('ASOP', De Groot & Golterman, 1990). I checked whether a lower pH, but >4.5, which accelerates the $CaCO_3$ extraction, might be used without extracting the ASOP.

Concurrently, the experiments to test the efficiency of NTA and EDTA were used to compare this extraction with two other extraction techniques, i.e. the SEDEX method (Ruttenberg, 1992) and that of Hieltjes & Lijklema ('H & L', 1980). The SEDEX method uses Citric acid, Dithionite and Bicarbonate ('CDB') as extractants for $Fe(OOH) \approx P$ and Na-acetate followed by H^+ for the different forms of Ca-bound P. In the simplified version of the SEDEX extraction intermediate washings with $MgSO_4$ were omitted. 'H & L'

uses NH_4Cl, NaOH followed by H^+. Because in earlier work De Groot & Golterman (1990) noticed that 'H & L' extracts much lower quantities of $Fe(OOH) \approx P$ than Ca-NTA, the influence of the NaOH concentration was analysed first. For the results see Annex 2. Because only 0.5 M NaOH gets near to the results obtained by Ca-NTA, I used this concentration.

As the quantity of Fe(OOH) that can be extracted with Ca-NTA/Ca-EDTA will give useful information on the P equilibrium between $Fe(OOH) \approx P$ and $CaCO_3 \approx P$ (Golterman, 1995a) and on the degree of P saturation of sediments, I measured the extractable Fe as well.

The results presented here may stimulate the use of chelators for P fractionation, but also the development of chelators specific for Ca and Fe. Use of these compounds is at present limited by the influence of the pH on their specificity and by their price.

Methods

The following extractants are used:

(a) Ca-NTA, 0.02 M Dissolve 3.0 g of H_3NTA and 2.1 g of $CaCO_3$ together in 1 l of H_2O and remove the CO_2. Add 20 g of Tris-buffer and adjust the pH at 8 with HCl. Add 1% Na-dithionite ($\approx$0.05 M) just before the extraction.

(b) Ca-EDTA, 0.05 M Dissolve 18.6 g of Na_2-EDTA.$2H_2O$ (Titriplex III) together with 7.35 g of $CaCl_2.2H_2O$ in 1 l H_2O '$Ca_{(Cl_2)}$-EDTA'. Add Tris-buffer till the pH$\approx$9 (about 13 g). After the addition of 1% Na-dithionite, just before the extraction, the pH must be 7–8. In one experiment $CaCO_3$ was used: '$Ca(CO_3)$-EDTA'.

(3) Na_2-EDTA, 0.1 M Dissolve 37.2 g of $Na_2H_2EDTA.2H_2O$ in 1 l of H_2O. The pH is about 4.5.

(4) Na-acetate, 2 M

(5) Extractants according to Ruttenberg (1992) {i.e.: 5a) $MgSO_4$, 5b) Citrate-Dithionite-Bicarbonate-'CDB', 5c) Na acetate and 4) H_2SO_4}

(6) Extractants according to Hieltjes & Lijklema (1980).

o-P was measured using the blue-molybdate method (Method 5.6.2, Golterman et al., 1978). Fe was measured with o-phenanthroline using ascorbic acid as reductant (Method 4.5.1, Golterman et al., 1978) but including 1 ml 2 M Na-acetate buffer in the 50 ml final volume.

I worked only with fresh sediments, as freezing or drying changed the fractionation results (unpublished results). The disadvantage for a methodological study is that not all experiments are intercomparable: the sediments were taken from nearly the same place, but at different seasons. The different ambient temperatures in the laboratory (15–30 °C) too, may result in small differences in the $Fe(OOH) \approx P/CaCO_3 \approx P$ equilibria in the suspensions. About 0.5–1 g (d.w.) sediments were suspended in 5 ml of H_2O and mixed with the different extractants. Subsequent extractions were carried out with the pellets remaining in the centrifuge tube.

EDTA solutions have the disadvantage of interfering with the o-P and Fe determinations. For the o-P determination 2 ml 0.1 M of EDTA can be used without interference. For larger volumes, there are two options: (a) Heating (≈ 75 °C), which allows the normal procedure for up to 5 ml of 0.1 M (Ca-)EDTA. (b) Using 25 g of $(NH_4)_6Mo_7O_{24}.4\ H_2O$ per liter with 10 ml acetone added per 50 ml final volume and measuring the blue colour at 815 nm. With 7.5 ml EDTA 0.1 M, I found a calibration curve with a slope of 1% less than that of the normal calibration curve. Golterman (1995c) mentioned that with this stronger molybdate solution 10 ml EDTA could be present. This was true for the Philips spectrophotometer (PU 8600), but not for the (simpler) Spectronic 20. A different approach may be suggested i.e. extraction of the yellow phosphate-molybdate complex with butanol or hexanol, followed by reduction to the blue colour in the alcoholic phase (Golterman & Würtz, 1961) which is not suitable for routine analysis. With Camargue sediments this was not needed as sufficient P was always present to use small quantities of the extractants.

The Fe-phenanthroline colour develops slowly with EDTA, but not if 1 ml 2 M Na-acetate solution is added. 2 ml 0.1 M EDTA can then be used, if necessary, without any difficulty after dilution. As $Fe(OOH)$ is always present in much larger amounts than P, this dilution never poses a problem.

Results

In experiment 1, I tested whether an extraction with EDTA at pH = 4.5 extracts ASOP. I used the same sediment suspension for comparison with a modified (simplified) SEDEX extraction (Ruttenberg, 1992). The results are in Table 1. One extraction with acid EDTA extracted the same amount of P as five EDTA extractions at pH = 8 and no ASOP. The SEDEX method extracted the same quantity of $Fe(OOH) \approx P$, but less $Fe(OOH)$. NTA extracted more $Fe(OOH)$ than the CDB of the SEDEX method.

The mean subtotals of $EDTA + H_2SO_4$ are 423 μg g^{-1} for the first series and 451 $\mu g\ g^{-1}$ for the second series. The (acid) EDTA did not extract the ASOP. In the SEDEX extraction, $NaAc + H_2SO_4$ extracted somewhat less, 333 $\mu g\ g^{-1}$, which difference equals a loss of $\approx 10\%$ of Tot-P. This suggests an non-extractable apatite, or an organic $Ca \approx P$ complex.

In experiment 2, the efficiency of the Ca-EDTA was again tested against Ca-NTA and compared with the SEDEX method. As the SEDEX extraction yielded results identical to those of Experiment 1A, these are not given here. The results are in Table 2.

The 'old' and 'new' Ca-NTA solutions were made with old (a few months, depending on room humidity) or new dithionite (taken from a freshly opened pot). The influence of storing on the quantities of both P and Fe extracted is clear. The Ca-EDTA extracted slightly more $Fe(OOH) \approx P$ than Ca-NTA; the difference was larger for the $Fe(OOH)$ extracted. The extraction time for the 1st Ca-EDTA extraction must be 2 h while for the 2nd 1 h was sufficient.

In experiment 3, I again tested the difference between Ca-NTA and Ca-EDTA. This time, I used different amounts of sediments. I also analysed whether Ca-EDTA must be made with $CaCO_3$ ('Ca(CO_3)-EDTA') or can be made with $CaCl_2$ ('Ca$_{(Cl_2)}$-EDTA'). The results are in Table 3.

When 1.3 or 0.65 g of sediment was used, Ca-EDTA was slightly more efficient than Ca-NTA. Ca$_{(CO_3)}$-EDTA was slightly less effective if 1.3 g was used than Ca$_{(Cl_2)}$-EDTA, but this difference disappeared with 0.65 g of d.w. With Ca-EDTA 2 extractions were always sufficient. Ca-EDTA extracted $Fe(OOH)$ better than Ca-NTA. Ca$_{(CO_3)}$-EDTA and Ca$_{(Cl_2)}$-EDTA did not differ significantly.

In experiment 4, I tested whether larger amounts of sediments can be extracted with stronger (0.05 M) Ca-EDTA solutions, while in this experiment a comparison was also made with the H & L extraction. Results are in Table 4.

With 0.025 M or 0.05 M Ca-EDTA less $Fe(OOH) \approx P$ (87%, for the ratio of the two mean values) was extracted from 1.3 g sediment than from 0.65 g; from 0.65 g sediment 0.025 and 0.5 M Ca-EDTA extracted the same quantities; little more $Fe(OOH)$ was extracted. There are small differences in the quantities of Fe extracted, especially when larger quantities of sediments are used. With 0.65 g

Table 1. P and Fe extractions of 0.6 g sediment (d.w.) with Ca-NTA, EDTA pH = 8, Na$_2$H$_2$EDTA pH = 4.5 and with SEDEX. Tot-P = 970 ± 10 μg g^{-1}. Tot-Fe = 40.8 mg g^{-1}. (All extractions in duplicate; n.d. = not detectable.)

Compound	P(μg/g)						Fe (mg/g)			
	Extractant: Ca-NTA, THIO		MgSO$_4$ 'CDB' 30 30				Ca-NTA, THIO		'CDB'	
Fe(OOH) $\approx$ P	1st	1288 294	290	276			7.77	7.71	6.11	5.73
	2nd	35 37	44	46			2.04	2.34	1.79	1.69
	Sub-Total	323 331	334	322			9.81	10.1	7.9	7.42
	Extractant: EDTA 0.05 M		Na acetate		EDTA 0.1 M pH = 4.5					
CaCO$_3$ $\approx$ P	1st	81 83	52	53	295	310				
	2nd	108 102			n.d.	n.d.				
	3rd	46 45								
	4th	23 30								
	5th	34 34								
	Sub-total	292 294			295	310				
	Extractant: H$_2$SO$_4$									
'ASOP'	1st	120 140	278	284	150	147			0.37	0.40
	Sub-total	412 434			445	457				
	TOTAL	735 765	664	659						

Table 2. Comparison of P and Fe extractions of 1.3 g sediment with Ca-NTA and Ca-EDTA followed by extraction with Na$_2$-EDTA.

		P(μg/g)	Fe(mg/g)	P(μg/g)	Fe(mg/g)
	Extractant:	'Old' Ca-NTA, THIO		Ca-EDTA, THIO	
1st, 1h		256	4.45	300	5.35
1st, 1$\frac{3}{4}$h		299	5.07	322	5.40
	Extractant:	'New' Ca-NTA, THIO			
1st, 2 h		284	5.25	326	6.09
2nd, 1 h		134	0.55	125	0.73
Σ Fe(OOH) $\approx$ P		418	5.80	451	6.82
	Extractant:	Na$_2$-EDTA, 0.1 M, pH = 4.5			
1st, 2 h		235		194	
2nd, 15 h		170		153	
Σ Fe(OOH) $\approx$ P+CaCO$_3$ $\approx$ P		405		347	
		823		798	

per extraction the differences are not significant. The amount of extractable Fe(OOH) is about 10 mg g^{-1} (= 25% of Tot-Fe).

After 17 hr, with NaOH less Fe(OOH)$\approx$P was extracted, while the concentration of NaOH appeared to be important. The loss was recovered with the H$^+$ extraction. A subsequent extraction with 0.5 and 1.0 M NaOH yielded another 14 and 20% of the Fe(OOH)$\approx$P

respectively. Furthermore, the extraction time with NaOH was prolonged. The results are in the second part of Table 4. It seems likely that the NaOH-P$_{extr}$ was reprecipitated as Ca-bound P.

It can be seen that not only the NaOH concentration is important, but that the duration of the extraction also influences the quantity extracted. This quantity is much

Table 3A. P and Fe extractions of 0.65 or 1.3 g Camargue-sediment (containing about 30% $CaCO_3$) first with Ca-NTA, Ca(co₃)-EDTA or Ca(cl₂)-EDTA to extract Fe(OOH)≈P, followed by extraction of $CaCO_3$ ≈ P with Na₂-EDTA.

Extractant:		Ca-NTA		Ca(co₃)-EDTA		Ca(cl₂)-EDTA	
Sample:		1	1	1	2	1	2
P-extracted (μg/g) from 1.3 g sediments							
Compound	Extr. no.						
Fe(OOH)≈P	1st	218	215	242	180	265	206
	2nd	35	32	46	25	43	26
	3rd	30	30	n.d.	n.d.	n.d.	n.d.
∑Fe(OOH)≈P		283	277	288	205	308	232
$CaCO_3$≈P	1st	183	188	182		185	
	2nd			121		125	
	3rd			38		36	
∑ CaCO≈P				341		346	
∑Fe(OOH)≈P + $CaCO_3$≈P				629		654	
Fe-extracted (mg/g) from 1.3 g sediments							
Fe(OOH)	1st	4.41	4.34	5.59	9.80	6.13	9.86
	2nd	0.46	0.45	0.65	0.50	0.66	0.53
	3rd	n.d	n.d	n.d.	n.d.	n.d.	n.d.
∑Fe(OOH)		4.87	4.79	6.24[1]	10.3	6.79[1]	10.4

[1] Before improved Fe-determination

Table 3B.

Extractant:		Ca-NTA	Ca(co₃)-EDTA	Ca(cl₂)-EDTA		
Sample:		1	1	1	2	3
P-extracted (μg/g) from 0.65 g sediments						
Compound	Extr. no.					
Fe(OOH)≈P	1st	210	287	274	266	266
	2nd	40	n.d.	n.d.	32	38
	3rd	30	n.d.	n.d.	n.d.	n.d.
∑Fe(OOH)≈P		280	287	274	298	304
$CaCO_3$≈P	1st	228	235	160	170	
	2nd	100	101	150	152	
	3rd	n.d.	n.d.	39	30	
∑$CaCO_3$≈P		328	336	349	352	
∑Fe(OOH)≈P + ∑$CaCO_3$≈P		615	610	647	656	
Acid-soluble org.P		130	134	149	147	
∑Extractable		745	744	796	803	
Fe-extracted (mg/g) from 0.65 sediments						
Fe(OOH)	1st		7.2	7.4	6.69	6.62
	2nd		n.d.	n.d.	0.71	0.75
∑Fe(OOH)			7.2	7.4	7.4	7.37

lower than with Ca-NTA or Ca-EDTA, but the loss is recovered in the quantity of acid-P_{extr}.

Discussion

When discussing standardization of extraction methods it must be realized that the principle (i.e. the choice

Table 4. Experiment IVa. P and Fe extracted from 0.65 or 1.3 g of sediment; Extractant: 0.025 or 0.05 M (Ca(cl$_2$-EDTA; followed by Na$_2$-EDTA 0.1 M; 17 h stirring. The same with 1.0 M and 0.5 M NaOH (17 h) followed by 0.5 M H$^+$

Compound	Extr. no.	P (µg/g) 0.65 g Ca-EDTA 0.025 M	1.3 g 0.05 M	0.65 g 0.05 M	1.3 g 0.05 M	1.3 g NAOH 1 M	1.3 g 0.5 M
Fe(OOH)≈P	1st	287	241	281	239	149	101
	2nd	40	36	38	45	24	27
	Sum	327	277	319	284	173	128
CaCO$_3$≈P	1st			227	205	490	535
	2nd			100	110		
	Sum			327	315		
Fe(OOH)≈P + CaCO$_3$≈P				646	599	663	663
Fe(OOH)≈P	1st	271	226			239	205 (17 h)
	2nd	28	27			279	215 (24 h)
	Sum	299	253				
				Fe (mg/g)			
Fe(OOH)	1st	8.62	6.94	7.80	8.14		
	2nd	0.48	0.46	1.00	0.98		
	Sum Fe	**9.10**	**7.4**	**8.80**	**9.12**		
New suspension							
Fe(OOH)	1st			7.50	7.52		
	2nd			0.855	0.855		
	Sum Fe			**8.36**	**8.38**		

of the extractants) can be standardized, but not the extraction conditions such as duration of the extraction and the concentrations of the extractants, as these depend strongly on the nature of the sediments and on the concentrations of the different P fractions.

Comparison between old and new NTA/EDTA methods

The present experiments show that Ca-NTA and Ca-EDTA extract the same quantity of P from calcareous sediments, but that Ca-EDTA is more effective than Ca-NTA. The fact that the quantities are indeed the same is an argument for the use of complexing agents, the nature of which do not influence the quantities extracted. Ca-EDTA extracts more Fe(OOH) from these sediments than Ca-NTA, which suggests complexation of Fe(OOH) with organic matter. A further simplification is that Ca-EDTA can be made with CaCl$_2$, and not necessarily with CaCO$_3$. Na$_2$-EDTA at pH 4.5 is more effective than at pH = 8 and does not attack the ASOP. Galindo (pers. comm.) also found that Ca-

EDTA was more effective than Ca-NTA in sediments from the reservoir El Gergal (SW Spain) with a high percentage (40%) of Fe$_{extr}$. The Fe$_{extr}$ was higher with Ca-EDTA than with Ca-NTA. A disadvantage of the NTA/EDTA extractions is that they must be repeated, as one single extraction can never give complete recovery: in principle the extraction is an equilibrium reaction, the difference between the conditional stability constants of Fe^{2+}-NTA and Ca^{2+}-NTA being only $10^{-2.4}$. Although this difference is better for Fe^{2+}-EDTA and Ca^{2+}-EDTA, it is still only $10^{-3.5}$ (Golterman, 1982). Unfortunately, the quantity of Ca^{2+} liberated (reaction 1 and 2) shifts the equilibrium in the wrong direction. Furthermore, the second extraction is needed because some interstitial water remains in the pellet after the first extraction. It is always good laboratory practice to perform extractions twice to ensure complete extraction.

Comparison of different methods

The extraction most often used is that of Hieltjes & Lijklema (1980) with NaOH and HCl or H_2SO_4. The concentration of the NaOH and the influence of the duration have never been studied, while the back-precipitation onto $CaCO_3$, well documented in the literature (De Groot & Golterman, 1990), is not taken into account. De Groot (pers. comm.) reported the same phenomenon when extracting sediments labeled with ^{32}P. This procedure does not extract specific compounds and gives, therefore, no information on the quantity of P available for algae in several sediments (Golterman et al., 1969; Fabre et al., 1996), although in certain sediments it may do so, e.g. if no $CaCO_3$ and/or org-P is present. These sediments are rare.

With the new procedure I found that for both $Fe(OOH)\approx P$ and $CaCO_3\approx P$ two extractions are usually sufficient, but one should take care not to take too much sediment. It is considered an advantage of the 'H & L' method, that only one extraction is needed. Repeating the treatment with NaOH, however, I found that a second extraction may extract again $\approx 30\%$, while increasing the NaOH concentration again extracted more o-P. One may actually wonder what is extracted with the NaOH. Certainly not $Fe(OOH)\approx P$ only.

The SEDEX extraction gave results not different from those with Ca-EDTA or Ca-NTA, but extracted less $CaCO_3\approx P$. This difference cannot be explained with certainty, but it seems likely that the CDB extracted some $CaCO_3\approx P$. The fact that $Fe(OOH)$ is not completely extracted makes a re-adsorption onto $Fe(OOH)$ likely. Barbanti et al. (1994) in a comparison of different methods, came to the conclusion, that some forms of apatite behave differently. The SEDEX method must be tested to see whether repetition of an extraction (especially that with Na-acetate) will yield some extra P.

Williams et al. (1976, 1980) used an extraction method with CDB followed by NaOH. A subsequent extraction with HCl is supposed to extract $CaCO_3\approx P$. The Williams extraction was later changed by deleting the CDB step by the Geneva group (Favarger, pers. comm.) and the C.C.I.W. group (Manning, pers. comm.), but out of reverence the name of the procedure was not changed. The reason for this change was the belief that NaOH-P_{extr} reflects the P available to algae. However, in Williams et al. (1980) the amount of P used by the algae was not measured; it was only shown that a linear correlation was found between algal growth and NaOH-P_{extr} and the yield of the algal culture used, *Scenedesmus*, per mg of P is rather low: $\approx 20\%$ of what could be expected. These authors stated that they did not obtain a good extraction with Ca-NTA, but they used a Ca-NTA solution during a very short time only, and without a reducing agent. Golterman et al. (1969) and Fabre et al. (1996) have shown that NaOH-P is not equal to the P available for algae. Dorich et al. (1984, 1985) demonstrated a strong correlation between algal available P and NaOH-P_{extr}, but also that the NaOH extraction largely overestimated the available P. It seems very probably that this overestimation is caused by the hydrolysis of non-available org-P. If, as in the Williams method, NaOH is used as P-extractant, the method is not different[2] from 'H & L', which is essentially the old Chang & Jackson method with a preliminary washing with NH_4Cl at pH = 7 the function of which is rather dubious. The 'new' Williams method, therefore, need not be taken into account separately. The presence, in all sediments, of different forms of org-P which are partly hydrolysed by NaOH and HCl, is often not taken into account by geochemists, although these compounds behave biologically different from the iron- and calcium-bound phosphates.

It is suggested that the present method is called the 'EDTA method'. The present protocol is given in the annex.
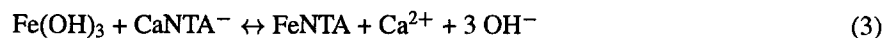
Acknowledgments

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[2] In their comparison with the Williams method, Hieltjes & Lijklema changed the sequence of NaOH and CDB.
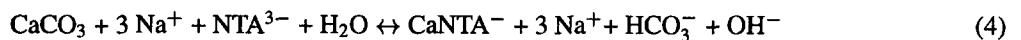
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Annex 1

Logarithms of conditional* ('Mixed') constants of calcium- and iron-EDTA and -NTA complexes, and of the two relevant equilibrium reactions (Golterman, 1982, quoted from Ringbom 1963). In this paper two equilibrium reaction constants are considered:

$$Fe(OH)_3 + CaNTA^- \leftrightarrow FeNTA + Ca^{2+} + 3\ OH^- \tag{3}$$

and

$$CaCO_3 + 3\ Na^+ + NTA^{3-} + H_2O \leftrightarrow CaNTA^- + 3\ Na^+ + HCO_3^- + OH^- \tag{4}$$

* That is, the influence of the pH is included.

The log. constants of these complexes are:

	pH				
	5	**6**	**7**	**8**	**9**
Ca-EDTA	3.2	4.7	6.1	7.1	8.1
Ca-NTA	0.5	1.3	2.3	3.3	4.2
Fe²⁺EDTA	6.6	8.3	9.7	10.7	11.7
Fe²⁺NTA	2.7	3.7	4.7	5.7	6.6
Fe³⁺EDTA	17.2	18.9	20.4	22.0	24.0
Fe³⁺NTA	12.3	14.3	16.3	18.3	20.1

Therefore the reaction constants of (3) and (4) are:

	pH		
	5	7	9
EDTA: Fe^{3+} minus Ca^{2+}	14.0	14.3	16.0
NTA: idem	11.8	14.0	16.0
EDTA: Fe^{2+} minus Ca^{2+}	3.4	3.6	3.6
NTA: idem	2.2	2.4	2.4

Protocol
At present the protocol for the EDTA method is as follows:

Step I: 500–1000 mg of fresh sediment (as d.w.) are extracted twice, with 30–50 ml of 0.05 M Ca-EDTA (reagent 2) each during 2 h.

Step II: Sufficient extractions with 30–50 ml of 0.1 M Na_2-EDTA (reagent 3) during 17 or 24 h, till the quantity extracted is less than 10% of the sum of the previous extractions with the same reagent.

Step III and IV: For the organic P-fractions this sequence may be followed by an extraction with 10–20 ml of 0.5 M H_2SO_4, at 20°C followed by 10-20 ml of 2 M NaOH at 90°C.

All extractions are carried out under occasional or constant shaking

Annex 2
The influence of the NaOH concentration and extraction duration in the 'H&L' extraction. Data from sediments from Doñana and El Gergal (Garcia & Galindo, respectively; pers. comm). Results expressed in $\mu g\ g^{-1}$ for the Camargue (17 h) and in percent of the 17 h extraction, with 0.1 M NaOH:

		Camargue			Doñana			El Gergal		
	$\mu g\ g^{-1}$	17 h	24 h	45 h	17 h	24 h	45 h	17 h	24 h	45 h
0.1 M	65	100	84	88	100	95	78	100	99	79
0.5 M	141	218	163	111	184	130	93	245	229	175
1.0 M	198	306	207	172	284	220	134	373	369	202