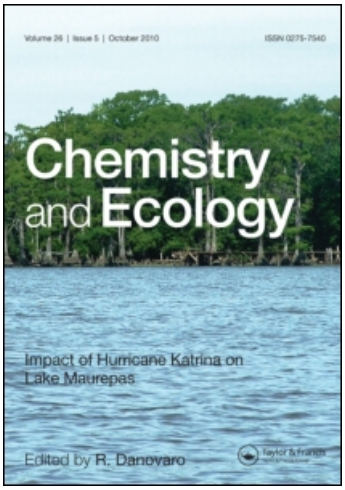

Chemistry and Ecology
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713455114

An improved technique for the determination of organic phosphorus in sediments and soils by ^{31}P nuclear magnetic resonance spectroscopy

R. W. McDowell[a]; I. Stewart[b]
[a] AgResearch Ltd, Invermay Agricultural Centre, Mosgiel, New Zealand [b] Department of Chemistry, University of Otago, Dunedin, New Zealand

To cite this Article McDowell, R. W. and Stewart, I.(2005) 'An improved technique for the determination of organic phosphorus in sediments and soils by ^{31}P nuclear magnetic resonance spectroscopy', Chemistry and Ecology, 21: 1, 11 — 22
To link to this Article: DOI: 10.1080/02757540512331334942
URL: http://dx.doi.org/10.1080/02757540512331334942

Chemistry and Ecology
Vol. 21, No. 1, February 2005, 11–22

Taylor & Francis
Taylor & Francis Group

An improved technique for the determination of organic phosphorus in sediments and soils by ^{31}P nuclear magnetic resonance spectroscopy

R. W. McDOWELL†* and I. STEWART‡

†AgResearch Ltd, Invermay Agricultural Centre, Private Bag 50034, Mosgiel, New Zealand
‡Department of Chemistry, University of Otago, PO Box 56, Dunedin, New Zealand

(Received 31 August 2004; in final form 18 November 2004)

Knowledge of organic phosphorus (P) species quantity and distribution in sediments and soils is needed to determine the potential for aquatic and terrestrial organism growth. This can be achieved with ^{31}P nuclear magnetic resonance spectroscopy (NMR), but resolving peaks within spectra can be problematic because of broadening via paramagnetics, which can cause peaks to overlap. We compared ^{31}P NMR spectra of NaOH-EDTA extracts of three sediments and three soils to those that had first been pre-treated with Ca-EDTA-dithionite to remove Fe and Mn, paramagnetics that cause broadening of peaks, but leave organic P alone. Broadening of peaks in Ca-EDTA-dithionite pre-treated samples decreased by 46% and revealed peaks that were hidden compared to untreated samples. The spectrum of one pre-treated soil was similar if not better than the same soil that had received pre-treatment with Chelex resin (also to remove paramagnetics). Therefore, pre-treatment with Ca-EDTA-dithionite is recommended as a simple and cost-effective method for improving organic P identification and determination in subsequent NaOH-EDTA extracts of sediments and soils rich in Fe and Mn.

Keywords: ^{31}P NMR; Monoesters; Diesters; Paramagnetics; Broadening

1. Introduction

The cycling of phosphorus (P) in sediments and soils is central to the growth of aquatic and terrestrial organisms. In addition to inorganic forms of P, many P transformations occur via organic P. Many techniques are available for the study of P forms. However, techniques such as high-performance liquid chromatography, which can be used to identify specific organic P compounds such as inositol phosphates [1], are analytically complex. In contrast, ^{31}P nuclear magnetic resonance spectroscopy (NMR) has been successfully used to identify many classes of compounds at once. These compounds include inorganic orthophosphate, pyrophosphate and polyphosphate and organic monoesters, diesters, phosphonates and phospholipids [2–4]. One major drawback to ^{31}P NMR is sensitivity, with P concentrations >100 mg/l required to generate clear spectra. Consequently, current techniques aim to extract as much P from

*Corresponding author. Email: richard.mcdowell@agresearch.co.nz

Chemistry and Ecology
ISSN 0275-7540 print/ISSN 1029-0370 online
http://www.tandf.co.uk/journals
DOI: 10.1080/02757540512331334942

the sample as possible. The most commonly used extractant is a combination of NaOH and EDTA, proposed by Bowman and Moir [5] as a single-step extractant to determine organic P. However, another drawback is that during extraction for P, paramagnetic ions such as Fe and Mn are also extracted. This is enhanced by using the chelator EDTA. These ions cause broadening of peaks, thus distorting spectra and causing otherwise sharp peaks to overlap, creating uncertainty in peak assignments and the total area occupied by the peak [6].

To minimize broadening of peaks, a number of researchers have used chelating resins such as Chelex™ (Bio-Rad Laboratories), Chelating Resin™ (Hampton Research) or Dowex M4195 (Dow Chemicals) to remove paramagnetics prior to NMR spectroscopic analysis [7, 8]. These chelating resins house ions such as iminodiacetate or Bis-picolylamine ions that are highly selective for divalent ions over monovalent ions, and exhibit a very high attraction for transition metals, even in concentrated solutions. For the analysis of P in forest soils, Cade-Menun and Preston [9] and Cade-Menun *et al.* [10], used Chelex in combination with either water or NaOH to remove Fe and Mn and showed that this produced much sharper peaks. However, while treatment with Chelex partially corrects broadening, six times as much Chelex was used as soil extracted, representing a labour-intensive approach and considerable expense for regular use. For example, treating 5 g of soil with Chelex would cost US $96 based on 25 g of Chelex shipped to New Zealand. Cade-Menun and Preston [9] also expressed concern that the Chelex may remove polyphosphates from solution and that the effect on other soil P compounds during extraction was unknown.

In addition to ^{31}P NMR, other methods such as sequential extraction have been used to classify P either by bioavailability to plants or aquatic biota or by chemical form (e.g. associated with Fe). One such method has been developed by Golterman and co-workers [11] and, unlike many sequential extraction regimes, has been tested and calibrated for specific chemical P forms (e.g. Fe- and Ca-associated P [11]). One of the fractions uses the chelating compound EDTA in Ca form to bind Fe released under reducing conditions by the addition of dithionite. This extraction liberates P bound with Fe under mild conditions (pH 7.5), without removing organic P [11]. Consequently, our objective was to test Ca-EDTA-dithionite as a pre-treatment to remove Fe and Mn and decrease broadening of peaks for the better identification and quantification of remaining (largely organic) P species. A secondary objective was to determine the effect of Ca-EDTA-dithionite pre-treatment on standard organic and inorganic P compounds and to compare the proposed pre-treatment to Chelex treatment of NaOH extracts, either with or without EDTA.

2. Materials and methods

2.1 *Sediment and soil sampling and analysis*

Three sediments from the oxic (determined by O_2 microprobe) top 2 cm depth of stream bed, and three soils under pasture (top 7.5 cm) were sampled for study. Two of the sediments were taken from the main reach of the Bog Burn in central Southland, New Zealand, one from the forested headwaters (25% of catchment land use) and the other downstream near the outlet (Winton highway), surrounded by dairy land (75% of catchment land use). A third sediment was sampled from a continuously flowing wet area used as a wallowing site for farmed red deer (*Cervus elaphus*) on the Invermay deer farm, near Mosgiel, Otago, New Zealand. The three New Zealand soils were a Podzol taken from Northland (Wharekohe), an Allophanic soil from Taranaki (Stratford) and a Pallic soil from Otago (Waitahuna). Both soils and sediments represent a wide range of landscapes, parent materials, and organic matter concentrations. All sediments and soils were air-dried, crushed and ground $<150\,\mu$m before analysis. In addition

Table 1. Selected chemical and physical properties of studied sediments and soils.

Sample	pH	Total Al (g/kg)	Total F (g/kg)	Total Ca (g/kg)	Total P (g/kg)	Organic C (g/kg)	Sand (g/kg)	Silt (g/kg)	Clay (g/kg)
Sediments									
Forest	5.8	34.2	28.9	8.2	0.37	10.4	740	220	40
Winton Hwy	6.0	58.7	35.4	12.5	0.41	0.8	480	460	60
Wallow	5.7	64.9	25.9	7.5	0.50	20.0	550	400	50
Soils									
Wharekohe	5.4	7.5	1.5	7.5	0.46	33.6	180	680	140
Stratford	5.4	90.2	50.1	25.4	2.91	67.8	520	270	210
Waitahuna	5.8	70.9	39.9	10.1	0.93	39.9	240	540	220

to analysis for P, Fe and Mn, other analyses included sediment and soil pH (in water), organic C by Shimadzu total organic C analyser, total Al and Ca, and particle size. A list of these results is given in table 1.

2.2 *Pre-treatment*

Prior to extraction with NaOH-EDTA for later analysis by ^{31}P NMR, a portion of each sample (5 g) was pre-treated by extracting with 100 ml Ca-EDTA-dithionite reagent. For 1L of this reagent, 18.6 g of EDTA was mixed with 7.35 g of $CaCl_2$ and the pH adjusted to about 8 with Tris-buffer (about 19 g). Just before extraction, 1% Na-dithionite was added and the pH adjusted to pH 7.5. The soil-pre-treatment solution was shaken for 2 h, centrifuged (4000 $\times$ *g*), and the supernatant decanted for determination of Fe and Mn by ICP-MAS and orthophosphate by colorimetry [12]. Total P was determined after a persulphate digestion and the difference between orthophosphate and total P defined as organic P. A maximum of 2 ml could be used for orthophosphate determination (i.e. without digestion) before EDTA interferes with the Mo–P colorimetric reaction. At present, 100 ml of pre-treatment solution costs US$0.15, compared to US$96 for Chelex treatment, and although Chelex resin is recoverable, this involves additional costs for labour and chemicals.

For Chelex-treated samples, 30 g of resin (Na form) was placed in a 50 μm nylon mesh bag and included in either a 0.25 M NaOH or 0.25 M NaOH + 0.05 M EDTA extraction (both used for later analysis by ^{31}P NMR). After extraction, resin was cleaned using 0.5 M HCl and regenerated with 1 M NaOH.

2.3 *^{31}P NMR*

Analysis of pre-treated and untreated sediments and soils by ^{31}P NMR was made on re-suspended NaOH + EDTA extracts. Briefly, samples (5 g) were shaken with 100 ml of 0.25 M NaOH + 0.05 M EDTA (Na form) for 16 h, centrifuged (4000 $\times$ *g*), and the supernatant filtered (Whatman # 42). The extract was analysed via ICP-MAS for Al, Ca, Fe, and Mn, and orthophosphate colorimetrically before and after digestion by $K_2S_2O_8$. Each extract was then frozen and freeze-dried.

Solution ^{31}P NMR spectra were obtained using a Varian 500 MHz Inova NMR spectrometer with a 51 mm standard Oxford superconducting magnet, FTS temperature controller, and a 28 shim set. A 5 mm Varian z-axis PFG direct detection probe was used for all the samples. The Sun Ultra 10 workstation uses Solaris 8 OS and Varian VNMR 6.1C NMR software.

Table 2. Peak assignments for known P compounds in NaOH-EDTA with and without pre-treatment with Ca-dithionite-EDTA.

Compound	Peak assignments: pre-treated sample (ppm)	Peak assignments (ppm)
Inorganic P		
Orthophosphate	6.5	6.5
Na-pyro-phosphate	−3.7	−3.7
Na-poly-phosphate	−3.6, −19.1, −20.5	−3.6, −19.1, −20.5
Monoesters		
α-D-Glucose-1-phosphate	3.7	3.7
D-Glucose-6-phosphate	5.7	5.7
Guanosine-5-monophosphate	5.1, 5.0	5.1, 5.0
Phytate	5.6, 5.1, 4.7, 4.6	5.6, 5.0, 4.7, 4.6
Diesters		
Lecithin	0.6	–[a]
L-α-Phosphatidyl-L-serine	1.6	1.6
Phosphonates and others		
N-(Phosphonomethyl)glycine	17.1	17.1
Phosphocreatine (N–P)	−0.4	−0.4

[a] Not detected.

Each sample was prepared to a pH > 13 by taking 0.2 g of the dried extract and adding 600 µl of D_2O and 100 µl of 10 M NaOH. Samples were ultrasonicated (Crest model 175T) for 3 min, equilibrated for 20 min, then centrifuged (Qualitron 6 place mini-centrifuge) for 5 min. The supernatant was transferred to a 5 mm NMR tube and ^{31}P NMR spectra obtained at 202.298 MHz at 20°C. A similar number of scans were generated for each sample (7064–10,000 scans, Waitahuna untreated soil = 12,056 scans) using a pulse angle of 45°, a pulse delay of 5 s, and an acquisition time of 1.99 s with 64 K data points. This yielded a total delay of 6.99 s and was sufficient to realize the spin-lattice relaxation time (T_1) and yield quantitative spectra. Chemical shifts were recorded relative to an external phosphoric acid standard ($\delta = 0$ ppm) in a capillary tube.

Spectra were deconvoluted using a Lorentzian line shape of 2 Hz and measured using Mestre-C software [13]. To elucidate the likely peak assignments and the effect of NaOH-EDTA extraction, 11 known P compounds were sourced (table 2) and 10 mg of each compound added to 100 ml of 0.25 M NaOH + 0.05 M EDTA, and shaken overnight. The solution was frozen, freeze-dried, and analysed as per the sediment and soil samples. The effect of pre-treatment was also examined by dissolving each P compound (10 mg) in 100 ml Ca-EDTA-dithionite, which was frozen, freeze-dried, and then extracted with 100 ml 0.25 M NaOH + 0.05 M EDTA, frozen and freeze-dried before analysis by ^{31}P NMR.

Organic P compounds within the spectra were determined semi-quantitatively using the peak assignments of standard compounds and those of Makarov *et al.* [4], Cade-Menun and Preston [9], and Turner *et al.* [14], the percentage spectral area occupied by each compound, and the total P concentration in the extract.

2.4 *Statistical analyses*

All statistical procedures (means and standard error of the mean) were conducted using Genstat v6.0 [15]. Due to the expense and time necessary for ^{31}P NMR, replication was not possible.

3. Results and discussion

3.1 *Effect of pre-treatment on known P compounds*

Before extracting samples, an experiment was conducted to determine if Ca-EDTA-dithionite pre-treatment affected P forms, either by altering the chemical shift (largely due to changes in salt concentration or pH) or degrading specific P species. Eleven compounds (three inorganic P compounds (orthophosphate, polyphosphate and pyrophosphate)), four orthophosphate monoesters (α-D-glucose-1-P, D-glucose-5-mono-P, guanosine-5-mono-P, and phytate), two orthophosphate diesters (lecithin and phospho-L-serine), one phosphonate (*N*-(phosphonomethyl)glycine) and phosphocreatine were combined and added to solutions containing either Ca-EDTA-dithionite or NaOH-EDTA and freeze-dried. The pre-treated sample (Ca-EDTA-dithionite) was then extracted with NaOH-EDTA and again freeze-dried. The spectrum of the re-solubilized pre-treated extract is given in figure 1 along with the absolute difference between the pre-treated spectrum and the spectrum for P compounds in NaOH-EDTA without pre-treatment. Peak assignments given in table 2 were based on a combination of literature values [14] and preliminary ^{31}P NMR spectra of each compound dissolved in 0.25 M NaOH. Subtle differences in chemical shift to literature values will occur due to a higher salt concentration and pH in our samples compared to those assigned by Turner *et al.* [14]. Re-solubilization of our extracts was in 10 M NaOH + D_2O and not 1 M NaOH + D_2O as in Turner *et al.* [14].

Pre-treated and untreated spectra are similar, but several small differences were noted after subtracting the untreated spectrum from the pre-treated spectrum (figure 1). The main difference is in the lecithin peak (−0.6 ppm), while minor differences occurred in the orthophosphate monoester region, and among polyphosphate and orthophosphate peaks (figure 1). Turner *et al.* [14] noted that lecithin completely degraded into constituent compounds within 24 h. Our samples were in contact with NaOH-EDTA for about 16 h and, coupled with the time taken for re-solubilization and NMR acquisition, could account for the loss of the lecithin peak

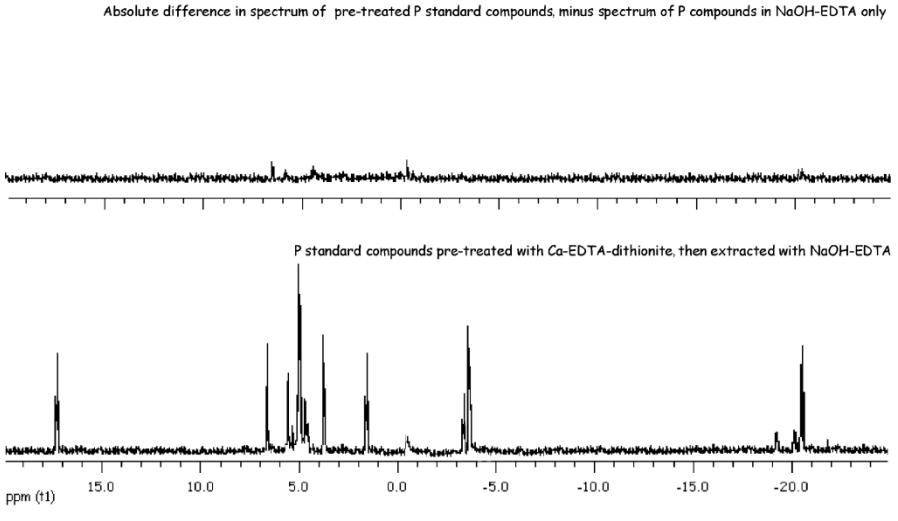

Figure 1. Spectra showing peak assignments for known P compounds pre-treated with Ca-EDTA-dithionite before extraction with NaOH-EDTA (bottom) and the absolute difference between the pre-treated spectrum and the spectrum for P compounds in NaOH-EDTA alone (i.e. untreated).

and redistribution of P within the monoester region of the spectrum. Unfortunately, due to a cluster of peaks in this region, constituents are hard to identify, although some differences arose at 5.6 and 4.8 ppm (figure 1). A change in orthophosphate concentration may have occurred via a small change in polyphosphate concentration.

Overall, the absolute difference spectrum in figure 1 is unremarkable, indicating that for P standard compounds and, by inference, P compounds in sediments and soils, pre-treatment with Ca-EDTA-dithionite was not detrimental to the distribution or form of P present. While we expect pH to be the main factor in determining the stability of P forms in extracts, we did not test known P compounds in soil NaOH-EDTA extracts either with or without pre-treatment. Hence, we cannot rule out the effect of other compounds such as Al and Ca, although saturation with Na and alkaline conditions will render hydrolysis and the formation of Al–P and Ca–P minerals unlikely.

3.2 *Effect of pre-treatment on total Fe and P in sediments and soils*

In NaOH-EDTA extracts of untreated samples, total Fe ranged from 1466 mg/kg in the strongly leached Wharekohe Podzol to 8183 mg/kg in the Forest sediment, whereas Mn was greatest in the volcanic-ash derived Stratford soil (492 mg/kg) and least in the Wharekohe Podzol (10 mg/kg) (table 3). In contrast, the Forest sediment exhibited the least total P concentration (272 mg/kg), while the greatest concentration was in the Stratford volcanic-derived soil (2736 mg/kg). By pre-treating samples with Ca-EDTA-dithionite, on average only 52% of the Fe and 48% of the Mn was present in the resulting NaOH-EDTA extracts, compared to untreated samples. For P, NaOH-EDTA extracts of pre-treated samples had 85% of the extracts of untreated samples.

Concentrations of Al decreased by an average of 13%, but Ca increased by an average of 56% due to Ca added during pre-treatment. Although precipitation of Ca–P minerals by pre-treatment cannot be discounted, the good efficiency of P extraction coupled with the non-uniformity of Ca concentrations in the NaOH-EDTA extract of pre-treated soils, means that the chelating efficiency of NaOH-EDTA (Na form) was not compromised by the added Ca (table 3).

Further examination of data shows that 182–2292 mg/kg of P was extracted by NaOH-EDTA in untreated samples, while for pre-treated samples, 147–1965 mg/kg of P was extracted (table 3). Obviously, pre-treatment affected P extractable by NaOH-EDTA. However, further fractionation of P in NaOH-EDTA extracts into inorganic and organic P via colorimetry indicated that, on average, the organic P concentration changed only 14% (10, 12, 10, 4, 22, and 22% change for Forest, Winton highway, Wallow, Wharekohe, Stratford, and Waitahuna samples, respectively).

The use of Ca-EDTA-dithionite is recommended for extraction of inorganic P, but not organic P [16]. This was based on the premise that extraction, buffered at near neutral pH with chelating agents and a reducing agent, removes only reducible inorganic P (e.g. associated with Fe and Mn) and does not cause acid or alkali hydrolysis of organic matter [17]. This has the advantage over regular alkaline extractions that remove organic P (largely as phytate) plus P bound to Fe and Mn. Since Fe and Mn are paramagnetic, they have the potential to cause broadening of peaks, and broad peaks may obscure adjacent peaks (i.e. organic P compounds). Following pre-treatment to remove Fe and Mn, the P:Fe+Mn ratio in NaOH-EDTA extracts increased by 24–105%, compared to untreated NaOH-EDTA extracts (table 3).

Chelating resins have been used for 25 years to remove paramagnetics before analysis by NMR [8,10,18]. Chelex is a chelating resin with a high affinity for polyvalent ions, such as Fe, over monovalent ions. Consequently, as a comparison, the Waitahuna soil was also extracted

Table 3. Total Fe, Mn, and P (mg/kg) in extracts of untreated and pre-treated (Ca-dithionite-EDTA) sediment and soils.

	Untreated					Pre-treated									
	NaOH-EDTA extracts for ^{31} P NMR					Ca-EDTA-dithionite					NaOH-EDTA extracts for ^{31}P NMR				
Sample	Fe	Mn	Al	Ca	P	P: Fe + Mn	Fe	Mn	P	Fe	Mn	Al	Ca	P	P: Fe + Mn
Sediments															
Forest	520	100	1200	1570	182 (22)[a]	0.29	3444	654	93	359	50	1026	3070	147 (27)	0.36
Winton Hwy	312	70	1210	2340	257 (14)	0.67	2475	1186	119	161	10	992	3940	206 (15)	1.20
Wallow	650	60	1750	4010	324 (23)	0.46	3098	110	127	306	20	1570	5560	237 (29)	0.73
Soils															
Wharekohe	390	10	640	3520	416 (46)	1.04	797	55	111	191	5	540	5510	305 (50)	1.56
Stratford	1194	492	13,010	5540	2292 (38)	1.36	2302	600	96	663	252	11,370	7900	1965 (43)	2.15
Waitahuna	1829	20	3580	3210	913 (54)	0.63	2596	120	193	649	18	3140	5610	861 (60)	1.29

[a]Data in parentheses are the percentage of total P as organic P.

with Chelex, either with or without EDTA. For soil extracts, Cade-Menun and Preston [9] showed that by combining NaOH with Chelex during extraction, Fe and Mn concentration decreased on average 69 and 92%, respectively, in five samples compared with NaOH + EDTA extracts. However, while decreasing broadening or peaks, the total P concentration was only 37% of NaOH-EDTA extracts, implying that the organic P fraction was not efficiently extracted. The total Fe and Mn concentration for the Waitahuna soil NaOH + Chelex extract was 1087 and 10 mg/kg, 75 and 50% of the NaOH-EDTA extract, while total P was only 43% (302 mg/kg) of the NaOH-EDTA extract. When EDTA was also incorporated, the chelating capacity of Chelex was negated, and 2411 mg/kg of Fe, 22 mg/kg of Mn, and 574 mg/kg of P were extracted. Compared to Ca-EDTA-dithionite pre-treatment, less Fe and P were extracted by Chelex resin treatment (table 3). More Mn was extracted, but Mn was on average only 17% of Fe concentration in NaOH-EDTA extracts of untreated samples. Consequently, given the added expense and labour associated with NaOH + Chelex resin extraction, there appears little advantage in using Chelex in place of Ca-EDTA-dithionite, to decrease line-broadening and improve organic P analysis by ^{31}P NMR, in this example.

3.3 *P forms in sediments and soils*

Examination of NaOH-EDTA extracts by ^{31}P NMR revealed a number of P compound classes (table 4). In untreated samples, orthophosphate accounted for 46% of total P in the strongly leached Wharekohe soil to 91% of total P in the forest sediment. The next most abundant compound class was orthophosphate monoesters (on average, 32%), followed by diesters (as DNA, 4%) and pyrophosphate (2%). Trace amounts of phosphonates were found in the wallow sediment. Phosphonates (direct C–P bond) are attributed to microbial activity, and

Table 4. Concentration (mg/kg), and percentage in parentheses, of P forms in sediments and soils ± Fe as detected by ^{31}P liquid state NMR.

Depth (cm)	Phosphonates (20)	Orthophosphate (6.26)[a]	Monoesters (3–6)[b]	Phospholipids (0.6–2)	DNA (1 to −1)	Pyrophosphate (−3 to −6)
Untreated						
Forest	–	142 (78)	37 (21)	–	–	2 (1)
Winton Hwy	–	233 (91)	17 (6)	–	–	7 (3)
Wallow	6 (2)	234 (71)	83 (27)	–	–	–
Wharekohe	–	193 (46)	204 (49)	–	14 (3)	6 (1)
Stratford	–	1587 (69)	613 (27)	–	62 (3)	28 (1)
Waitahuna	–	439 (48)	453 (50)	–	6 (1)	15 (2)[b]
Mean	–	471 (63)	237 (32)	–	27 (4)	12 (2)
SEM	–	92.6	41.8	–	10.1	2.1
Pre-treated						
Forest	–	104 (70)	34 (23)	–	–	8 (6)
Winton Hwy	–	176 (85)	22 (10)	2 (1)	–	5 (3)
Wallow	6 (2)	144 (61)	87 (34)	–	1 (1)	5 (2)
Wharekohe	5 (1)	145 (48)	141 (46)	3 (1)	8 (3)	3 (1)
Stratford	–	1042 (53)	859 (44)	–	33 (2)	31 (2)
Waitahuna	–	373 (43)	376 (44)	–	11 (1)	22 (3)
Mean	6	331 (53)	267 (43)	3 (1)	11 (2)	12 (2)
SEM	–	60.2	51.8	–	2.6	1.9
Chelex[c]	–	149 (49)	148 (49)	–	2 (1)	3 (1)
Chelex + EDTA	–	245 (43)	322 (55)	–	2 (1)	5 (1)

[a]Chemical shift (δ ppm).
[b]Total percentage >100 due to rounding.
[c]Both Chelex samples were extracts of the Waitahuna soil.

their presence in the Wallow sediment could be due to enhanced microbial input via deer faeces [19].

Compared to untreated samples, pre-treated samples exhibited a greater diversity of P compound classes and a greater concentration of organic P detectable in the spectra. As an example of the greater diversity of peaks in the pre-treated samples, orthophosphate diesters that could only be resolved as DNA in untreated samples could, with pre-treatment, be resolved into DNA and phospholipids. A compound just down-field of orthophosphate at about 6.9 ppm was found in the spectra of pre-treated samples but rarely in the spectra of untreated samples (figure 1). Amelung *et al.* [20] found a similar peak in NaOH-NH_4F extracts of zonal steppe soils in Russia and tentatively assigned it to aromatic P diesters. However, Turner and Richardson [21] have reclassified these compounds as undefined inositol phosphates. In addition to these compounds, pyrophosphate and phosphonates could be detected in more samples when pre-treated, compared to the corresponding untreated spectra (figures 2 and 3).

The increase in spectral definition is evident in the comparison of spectra for untreated and pre-treated samples (figures 2 and 3). Using orthophosphate as an internal standard (i.e. present in all spectra at a known chemical shift) and by comparing the line-width at half peak height, a measure of broadening is gained. Although this comparison should be done in samples with the same signal-to-noise ratio, a preliminary experiment indicated that the change in width with increasing number of scans from 512 (39.2 Hz) to 7240 (37.2 Hz) in an untreated Waitahuna soil was negligible compared to the difference between untreated (36.9 Hz) and pre-treated (10.5 Hz) samples. The data shows that pre-treatment decreased line-broadening by, on average, 37% (table 5). This is a considerable improvement over untreated samples and allows for good separation of peaks especially in the monoester region where compounds such as phytate exhibit many peaks [14].

Using Chelex to treat extracts, McDowell [22] also noted an increase in spectral definition for soil solution (0.01 M $CaCl_2$ soil extracts) from pasture samples of different management. Cade-Menun and Preston [9] and Cade-Menun *et al.* [10] noted a similar effect for Chelex-treated NaOH extracts of forest soils. However, they noted that the diversity of P compounds decreased and attributed this to the removal of P with Chelex resin via cation linkages. In our samples, polyphosphates were not detected (chemical shift about 19–20 ppm), despite

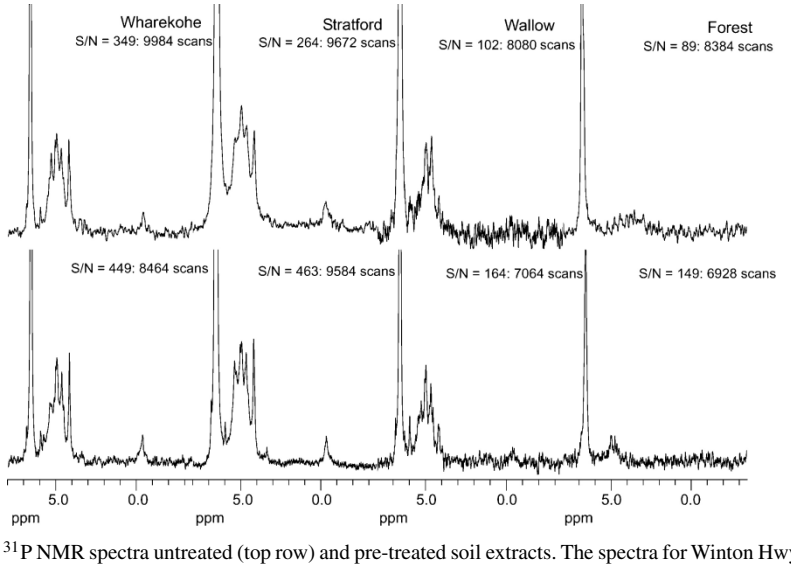

Figure 2. ^{31}P NMR spectra untreated (top row) and pre-treated soil extracts. The spectra for Winton Hwy untreated (signal-to-noise ratio = 79 : 8024 scans) and pre-treated (signal-to-noise ratio = 121 : 7948 scans) sediment is not given for clarity, but is similar to the Forest sediment.

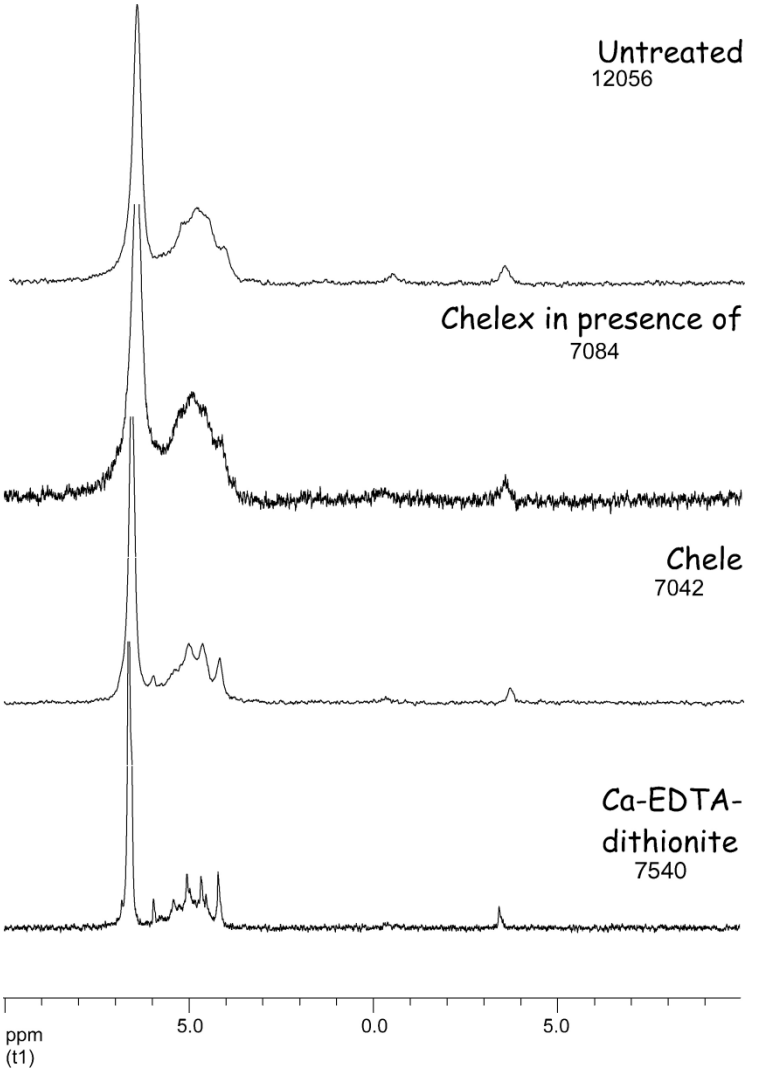

Figure 3. ^{31}P NMR spectra (from top down) of untreated and Chelex-treated Waitahuna soil extracts, either with or without EDTA present, or after pre-treatment with Ca-EDTA-dithionite showing the decrease in line-broadening caused by pre-treatment to remove Fe and Mn. The width of the orthophosphate peak at half peak height, a measure of broadening of peaks, for untreated and pre-treated (Ca-EDTA-dithionite) samples was 36.9 and 10.4 Hz, respectively.

being common in sediments and soils [7, 23] where biota have a P surplus resulting in luxury uptake and storage as polyphosphate. Polyphosphates sorb strongly, and some loss would be expected if associated with Fe. However, pre-extraction of Waitahuna soil (1 g) that had been left to incubate with 50 ppm of Na-polyphosphate overnight indicated that only 20% of polyphosphate added was removed, with 85% of the remaining polyphosphate removed in subsequent NaOH-EDTA extraction.

In addition to pre-treatment with Ca-EDTA-dithionite or Chelex resin, Robinson *et al.* [24] noted that the quality of spectra of wetland soils was enhanced by pre-extraction with 1 M KCl and 0.5 M $NaHCO_3$. While this was attributed to the removal of inorganic P and some labile organic P, it is well known that bicarbonate extracts Al and Fe forms of P [25], suggesting that Fe concentration was probably also decreased. Unfortunately, Fe concentrations were not measured.

Table 5. Width (Hz) of the orthophosphate peak at half height in spectra of untreated and pre-treated sediments and soils.

Sample	Untreated	Pre-treated
Sediment		
Forest	26.15	21.62
Winton Hwy	22.20	14.50
Wallow	25.53	19.38
Soils		
Wharekohe	24.47	12.86
Stratford	27.23	23.03
Waitahuna	36.91	10.48
Mean	27.08	17.00

In contrast to these data showing that removal of paramagnetics improves spectral resolution, Shand *et al.* [26] concluded that removal of up to 50% of Fe from organic rich, freeze-dried soil solutions produced little change in spectral resolution. However, by decreasing the concentration of paramagnetic compounds in solution, not only is the signal gained, but the relaxation rates (T_1) needed to generate quantitative spectra increase. An investigation of T_1 times for several P compound classes by Cade-Menun *et al.* [10] indicated that the minimum T_1 was 0.31 s, with most between 0.5 and 1.5 s. The pulse delay used by Shand *et al.* [26] was 0.2 s, meaning nuclei had insufficient time to relax, irrespective of Fe concentration, and that spectra were not quantitative. A previous investigation of T_1 for our samples indicated that a total delay (delay plus acquisition time) of 6.99 s was adequate (the maximum T_1 was 0.91 s for the orthophosphate peak in pre-treated Stratford soil) for quantitative spectra.

4. Conclusions

The dissolution of known P compounds in Ca-EDTA-dithionite indicated that the use of pre-treatment was unlikely to have a deleterious effect on P form. When employed before extraction with NaOH-EDTA, and compared to samples without pre-treatment, the number and distribution of peaks were better defined due to less overlapping of peaks. This effect was attributed to the removal of paramagnetics (largely Fe) by pre-treatment. Compared to an alternative method such as the use of chelating resins (e.g. Chelex), the proposed method was as efficient, but less costly and labour intensive. It is recommended that pre-treatment with Ca-EDTA-dithionite be used to better examine the nature and distribution of organic P compound classes in NaOH-EDTA extracts of sediments and soils.

Acknowledgements

Funding for this work was provided by the New Zealand Foundation for Research Science and Technology under contract AGRX002. We thank Drs Alan Hayman and Mervyn Thomas for helpful comments.

References

[1] M. Espinosa, B.L. Turner, P.M. Haygarth. Preconcentration and separation of trace phosphorus compounds in soil leachate. *J. Environ. Qual.*, **28**, 1497 (1999).

[2] R.H. Newman, K.R. Tate. Soil phosphorus characterisation by ^{31}P nuclear magnetic resonance. Communications in. *Soil Sci. Plant Anal.*, **11**, 835 (1980).

[3] L.M. Condron, K.M. Goh, R.H. Newman, J.W.B. Stewart. Nature and distribution of soil phosphorus as revealed by a sequential extraction method followed by ^{31}P nuclear magnetic resonance analysis. *J. Soil Sci.*, **41**, 41 (1985).
[4] M.I. Makarov, L. Haumaier, W. Zech. Nature of soil organic phosphorus: An assessment of peak assignments in the diester region of ^{31}P NMR spectra. *Soil Biol. Biochem.*, **34**, 1467 (2002).
[5] R.A. Bowman, J.O. Moir. Basic EDTA as an extractant for soil organic phosphorus. *Soil Sci. Soc. Am. J.*, **57**, 1516 (1993).
[6] R.J. Smernick, J.M. Oades. Effects of added paramagnetic ions on the ^{13}C CP/MAS NMR spectrum of a de-ashed soil. *Geoderma*, **89**, 219 (1999).
[7] M.A. Adams, L.T. Bryne. Phosphorus-31 NMR analysis of phosphorus compounds in extracts of surface soils from selected karri (*Eucalyptus diversicolor* F. Muell.) forest. *Soil Biol. Biochem.*, **21**, 523 (1989).
[8] W.J. Ray, J.W. Burgner, C.B. Post. Purification of NMR reagents. *Biochemistry*, **29**, 2770 (1990).
[9] B.J. Cade-Menun, C.M. Preston. A comparison of soil extraction procedures for ^{31}P NMR spectroscopy. *Soil Sci.*, **161**, 770 (1996).
[10] B.J. Cade-Menun, C.W. Liu, R. Nunlist, J.G. McColl. Soil and litter phosphorus-31 nuclear magnetic resonance spectroscopy: Extracts, metals, and phosphorus relaxation times. *J. Environ. Qual.*, **31**, 457 (2002).
[11] H. Golterman, J. Paing, L. Serrano, E. Gomez. Presence of and phosphate release from polyphosphate or phytate phosphate in lake sediments. *Hydrobiologia*, **364**, 99 (1998).
[12] F.S. Watanabe, S.R. Olsen. Test of an ascorbic acid method for determining phosphorus in water and $NaHCO_3$ extracts from soil. *Soil Sci. Soc. Am. Proc.*, **29**, 677 (1965).
[13] J.C.C. Gómez, F.J.S. López. Mestre-C. *Nuclear Magnetic Resonance Companion.* v. 3.5.1. Available at http://www.mestrec.com (accessed February 2004).
[14] B.L. Turner, N. Mahieu, L.M. Condron. Phosphorus-31 nuclear magnetic resonance spectral assignments of phosphorus compounds in soil NaOH-EDTA extracts. *Soil Sci. Soc. Am. J.*, **67**, 497 (2003).
[15] Genstat Committee 6. *Genstat for Windows*, 6th edn. Rothamsted Experimental Station, Lawes Agricultural Trust, Harpenden, UK (2002).
[16] J. Kassila, M. Hasnaoui, A. Yahyaoui. Sequential extractions of inorganic and org-Phosphate from fish pond sediments (Deroua station, Beni Mellal, Morocco) by different fractionation methods. *Hydrobiologia*, **431**, 51 (2000).
[17] C.J. De Groot, H.L. Golterman. On the presence of organic phosphate in some Camargue sediments: Evidence for the importance of phytate. *Hydrobiologia*, **252**, 117 (1993).
[18] M.F. Dunn, S.E. Pattison, M.C. Storm, E. Quiel. Removal of metals from enzyme solutions. *Biochemistry*, **19**, 718 (1980).
[19] L.M. Condron, E. Frossard, H. Tiessen, R.H. Newman, J.W.B. Stewart. Chemical nature of organic phosphorus in cultivated and uncultivated soils under different environmental conditions. *J. Soil Sci.*, **41**, 41 (1990).
[20] W. Amelung, A. Rodionov, I.S. Uresevskaja, L. Haumaier, W. Zech. Forms of organic phosphorus in zonal steppe soils of Russia assessed by ^{31}P NMR. *Geoderma*, **103**, 335 (2001).
[21] B.L. Turner, A.E. Richardson. Identification of scyllo-inositol phosphates in soil by solution phosphorus-31 nuclear magnetic resonance spectroscopy. *Soil Sci. Soc. Am. J.*, **68**, 802 (2004).
[22] R.W. McDowell. Identification of phosphorus species in extracts of soils with contrasting management histories. *Commun. Soil Sci. Plant Anal.*, **34**, 1083 (2003).
[23] M. Hupfer, R. Gächter. Polyphosphate in lake sediments: ^{31}P NMR spectroscopy as a tool for its identification. *Limnol. Oceanogr.*, **40**, 610 (1995).
[24] J.S. Robinson, C.T. Johnston, K.R. Reddy. Combined chemical and ^{31}P-NMR spectroscopic analysis of phosphorus in wetland organic soils. *Soil Sci.*, **163**, 705 (1998).
[25] H. Tiessen, J.O. Moir. Characterization of available P by sequential extraction. In *Soil Sampling and Methods of Analysis*, M.R. Carter (Ed.), pp. 75–86. Lewis, Boca Raton, FL (1993).
[26] C.A. Shand, M.V. Cheshire, C.N. Bedrock, P.J. Chapman, A.R. Fraser, J.A. Chudek. Solid-phase ^{31}P NMR spectra of peat and mineral soils, humic acids and soil solution components: Influence of iron and manganese. *Plant and Soil*, **214**, 153 (1999).