0038-075C/96/16111-770-785$03.00/0
Soil Science

November 1996
Vol. 161, No. 11
Printed in U.S.A.

A COMPARISON OF SOIL EXTRACTION PROCEDURES FOR ^{31}P NMR SPECTROSCOPY

B. J. Cade-Menun[1] and C. M. Preston[2]

The effect of extractants on phosphorus determination by ^{31}P NMR spectroscopy was examined using five forest floor samples. The extractants used were: 0.25 *M* NaOH, 1:6 soil to Chelex in water, 1:6 soil to Chelex in 0.25 *M* NaOH, and a 1:1 mix of 0.5 *M* NaOH and O.1 *M* EDTA. The broadest peaks were produced by the NaOH + EDTA extraction. However, NaOH + EDTA extracts contained the highest percentage of total phosphorus and the greatest diversity of P forms. These extracts were the only ones to show peaks for polyphosphates. Metals analysis indicated that NaOH + EDTA maintained Mn in solution, which seemed to be responsible for the line broadening. The sharpest peaks, with the best separation, were produced with Chelex + NaOH, and these were improved further by increasing the pH with NaOH prior to NMR analysis. Chelex + NaOH extracted 23 to 35% of the total soil P, Chelex in water extracted 10 to 13%, NaOH alone extracted 22 to 34%, and NaOH + EDTA extracted 71 to 90%.

This work suggests that, because the extractant used will affect the P forms, care must be taken when interpreting studies of P cycling in soils using ^{31}P NMR spectroscopy and when comparing studies using different extractants.

PHOSPHORUS-31 nuclear magnetic resonance (NMR) spectroscopy can be used to obtain both qualitative and quantitative estimates of the various forms of P in soils, including inorganic orthophosphate, polyphosphate, phosphonate, pyrophosphate, orthophosphate monoesters such as inositol phosphate, and orthophosphate diesters such as phospholipids. Additionally, it is analytically less complex than the detailed partition chromotography techniques otherwise required to identify specific organic P compounds. However, the natural P levels in soils are usually low. As ^{31}P NMR is relatively insensitive, requiring more than 100 μg P mL^{-1} for quantitative analysis (Adams and Byrne 1989), solution NMR is used for P, and extraction and concentration of this extract are required to produce clear spectra. An ideal extractant should remove virtually all of the P from a soil sample without altering in any way the forms of P found in the soil.

Most ^{31}P NMR studies have employed a rapid extraction technique involving ultrasonic dispersion in 0.5 *M* NaOH, which usually extracts less than 50% of the total phosphorus (Newman and Tate 1980; Tate and Newman 1982; Ogner 1983; Hawkes et al. 1984; Zech et al. 1985; Zech et al. 1987; Gil-Sotres et al. 1990; Forster and Zech 1993; Bedrock et al. 1994; Makarov et al. 1995). Others have used 0.5 *M* NaOH without sonication (Preston et al. 1986) or with a citrate-dithionite-bicarbonate pretreatment (Ingall et al. 1990). These treatments also removed less than 50% of the total P. Hinedi et al. (1989) used water, ice-cold $HClO_4$ and a combination of $HCl/HF/TiCl_4$, which showed only orthophosphate peaks on the NMR spectra. Condron et al. (1985) used a sequential extraction procedure of 0.1 *M* NaOH, 0.2 *M* aqueous acetylacetone, and 0.5 *M* NaOH, washing between with 0.5 *M* HCl. Up to 80% of the total organic P, as determined by ignition (Saunders and Williams 1955), was removed, mainly by the initial 0.1 *M* NaOH step of the extraction. Condron et al. (1990) also used a sequential extraction

[1] Dept. of Soil Science, The University of British Columbia, Vancouver, BC Canada V6T 1Z4. E-mail: menun@ce.berkeley.edu

[2] Pacific Forestry Centre, Natural Resources Canada, 506 West Burnside Rd., Victoria, BC Canada V8Z 1 M5. Dr. Preston is corresponding author. E-mail: cpreston@pfc.forestry.ca.

Received Feb. 23, 1996; accepted June 12, 1996.

procedure, with 0.5 *M* NaOH, 1 *N* HCl and 0.5 *M* NaOH, washing with water. This too removed about 80% of the total organic P. Emsley and Niazi (1983) tried tetra-n-butyl ammonium hydroxide (Bu_4NOH), hoping to utilize the salting-in effect of the large organic cation. However, this salting-in effect did not occur, and they concluded that Bu_4NOH was as effective, but no more so, as NaOH or KOH. Hinedi et al. (1989) tried a sequential treatment of trichloroacetic acid (TCA) and KOH and found that it extracted 86 to 99% of the total P from sewage sludge.

One drawback to these methods is that, in addition to P, they extract other paramagnetic ions, such as Mn and Fe, which cause line broadening and distortion of ^{31}P NMR spectra (Hawkes et al. 1984; Hutson et al. 1992). Adams and Byrne (1989), Adams (1990) and Condron et al. (1996) utilized Chelex™ (Bio-Rad Laboratories), a cation exchange resin, as an extractant in an attempt to remove these interfering ions. Chelex is a chelating cation exchange resin that shows a high preference for Fe and other polyvalent metal ions over cations such as Na or K. (The order of preference is: Fe > Al >> Ca >>> Na; Adams and Byrne (1989)). In the Na form, Chelex is alkaline (pH 11–12) and so can also solubilize organic P from the soil sample. This method extracted approximately the same amount of total P as the NaOH method (Adams and Byrne 1989).

Recently, Bowman and Moir (1993) proposed the use of a mixture of NaOH and EDTA as a one-step extractant to determine total soil organic P. The NaOH can solubilize the organic P, while EDTA chelates metal cations to increase the efficiency of P extraction. This method extracted as much as twice the amount of organic P as NaOH alone (Bowman and Moir 1993), but it has not yet been tested as an extractant for ^{31}P NMR spectroscopy.

With any soil extraction procedure, soil P compounds can be chemically altered during or after extraction. Hydrolysis, especially of orthophosphate diester to monoester, is thought to be a problem with NaOH (Tate and Newman 1982; Hawkes et al., 1984) and with Chelex (Adams and Byrne 1989), although there has been no systematic study of the recovery of added P compounds.

A detailed comparison of extraction methods has never been conducted on forest floor samples, which contain low levels of P in mainly organic form and relatively high levels of other paramagnetic ions. Therefore, one objective of this research was to compare several soil extraction procedures to determine the one most suitable for ^{31}P NMR analysis of forest floor samples from northern Vancouver Island. The second objective was to examine the method of Bowman and Moir (1993) to determine its effectiveness as an extractant for ^{31}P NMR spectroscopy.

MATERIALS AND METHODS

Five forest floor samples, collected in 1992 as part of a more general survey of soil P (Cade-Menun 1995), were used for this extraction study. These soils have been the subject of extensive investigation because of problems with growth stagnation and nutrient limitation in plantations established after clear-cutting and burning (Prescott and Weetman 1994). One sample (HA-OG) was from under an old-growth stand of western hemlock (*Tsuga heterophylla* (Raf.) Sarg.) - amabilis fir (*Abies amabilis* Dougl.), and one (CH-OG) was from under an old-growth stand of western red cedar (*Thuja plicata* Don.) - western hemlock. The other three were from cedar-hemlock sites 0 (CH-0), 5 (CH-5) and 10 (CH-10) years after logging and slash burning. These samples were all relatively high in total P, and had a range of other soil chemical properties (Table 1). These forest floor samples were air-dried and ground to pass through a 2-mm sieve.

Four different extractants were used. These were:

1) 0.5 *M* NaOH + 0.1 *M* EDTA (1:1) (Bowman and Moir 1993)
2) 0.25 *M* NaOH
3) 1:6 soil:Chelex (weight basis) in deionized water (Adams and Byrne 1989)
4) 1:6 soil:Chelex (wt. basis) in 0.25 *M* NaOH

For each extractant, 5 g of air-dried soil and 100 mL of liquid were used. The NaOH-EDTA and NaOH samples were extracted in 125-mL Erlenmeyer flasks at room temperature overnight with occasional stirring. For both of the Chelex procedures, samples were extracted in 250-mL plastic bottles overnight at room temperature on a reciprocal shaker. All samples were then filtered with Buchner funnels and Whatman 41 filter paper. A subsample of each was digested with persulphate (Bowman 1989) and was read using the Watanabe and Olsen (1965) method to determine the total amount of phosphorus that had been extracted. The remainder of each sample was freeze-dried.

To prepare the samples for NMR, approxi-

TABLE 1
Chemistry of the soils used for the extraction trials

Sample	HA-OG	CH-OG	CH-0	CH-5	CH-10
pH[a]	3.1	3.5	5.0	3.4	4.2
C (%)[b]	49.9	37.2	23.2	46.7	49.6
LOI	3500	695	213	1742	1006
Total N[c] (%)	1.159	0.851	0.879	0.807	0.995
C/N	43.1	43.7	26.4	57.9	49.8
Avail Ca (mg/kg)[d]	1906	3300	7395	3639	7188
Avail Mg (mg/kg)[d]	393.4	255.6	640.0	498.0	673.5
Avail Fe (mg/kg)[d]	139.6	182.6	220.0	118.3	112.2
Avail Al (mg/kg)[d]	380.7	649.1	720.0	364.9	193.9
Avail P[e] (mg/kg)	40.04	51.5	68.4	23.47	21.16
Total P[f] (mg/kg)	613.4	595.8	721.9	575.5	710.0
Inorg P[g] (mg/kg)	129.0	162.0	396.0	140.0	124.0
Org P[g] (mg/kg)	545.0	420.0	400.0	513.0	589.0

[a] pH measured in $CaCl_2$.
[b] C measured with Leco.
[c] Total N measured via modified Kjeldahl digest.
[d] Available Ca, Mg, Fe, and Al measured with Mehlich 3 (1984) extraction.
[e] Available P measured with Bray P1.
[f] Total P measured with Parkinson and Allen (1975) digest.
[g] Inorganic P and organic P measured with Saunders and Williams (1955) ashing.

mately 1 g of the freeze-dried extract was weighed into a 50-mL plastic centrifuge tube, with 2.5 mL of D_2O. Samples were vortexed for 2 min. A few of the Chelex + NaOH samples were prepared in duplicate, and to one of each pair, 1 pellet (approx. 0.5 g) of NaOH was added before vortexing. All of the samples were left to stand for 2 h and were then centrifuged. The supernatants were transferred into 10-mL NMR tubes and were refrigerated until used for NMR spectroscopy.

Phosphorus-31 NMR spectra were obtained at 101.27 MHz on a Bruker WM 250 high resolution NMR spectrometer using a 45° pulse with a 1.5-s delay and an acquisition time of 0.508 s. The ^{31}P spectra were proton decoupled using an inverse-gated pulse sequence to overcome the nuclear Overhauser enhancement in order to achieve quantitative results (Newman and Tate 1980; Preston et al. 1986). Accumulation times ranged from 24 to 48 h and were dependent on the length of time necessary to achieve a strong signal-to-noise ratio. The assignment of peaks was based on Newman and Tate (1980) and Adams and Byrne (1989). Peak areas were determined by integration.

To assess the effect of the chelators (EDTA and Chelex) on interfering paramagnetic ions, the concentrations of Fe and Mn in the solutions following extraction were measured by atomic absorption spectroscopy before freeze-drying. In addition, an adsorption trial was conducted using the CH-OG sample. Solutions containing Fe at concentrations of 0, 20, 30, 40, 60, and 100 mg/L or Mn at 0, 15, 20, 30, 40 and 80 mg/L were prepared in either 30 mL of 0.05 *M* EDTA or water, and a 30-mL aliquot of each solution was placed in a stoppered 100-mL centrifuge tube, with or without 1 g of air-dry CH-OG soil. To each water solution was added 5 g of Chelex. A blank containing 30 mL of water was used as a control. NaOH was not used in this trial because it caused the metals to precipitate. After shaking on a reciprocal shaker for 1 h, the samples were filtered through Whatman 42 filter paper, and metal concentrations of the supernatants were then deter-

mined using atomic absorption spectroscopy.

To determine the effect of the extractants on various P compounds, 5-g samples (air-dry basis) of CH-OG had 0.05 g of the following compounds added before extraction by 0.25 *M* NaOH + 0.05 *M* EDTA or by 1:6 soil:Chelex (wt. basis) in 0.25 *M* NaOH: ATP (adenosine 5′-triphosphate, disodium salt, Grade II, Sigma A-3377); glycerophosphate (disodium pentahydrate, Sigma G6504); or K-polyphosphate (formed by fusion of KH_2PO_4 as per Kulaev (1979)). These were then prepared for NMR analysis as described previously.

RESULTS

NMR Spectra

Figure 1, A-E, displays the NMR spectra generated from these extractant trials, and Table 2 shows a guide for the interpretation of the peaks. It should be noted that the lower pH in the Chelex + water extraction causes a peak shift, reversing the orthophosphate and the monoester peak positions relative to the other extraction procedures. This was also observed by O'Neill et al. (1980) and Adams and Byrne (1989). In some of the spectra for the extractant NaOH + EDTA, the peaks for orthophosphate and monoester P overlap. When there was clear peak separation with this extractant, there was a valley between the peaks at 6 ppm, and this, therefore, was used as the dividing line where overlapping occurred.

The sharpest peaks, with the best separation, were produced by the Chelex + NaOH extraction. These were further improved when extra NaOH was added to adjust the pH before NMR analysis. The broadest peaks were produced by the NaOH + EDTA extraction. These spectra also had the poorest separation of the orthophosphate and monoester peaks. The trends from sample to sample were quite consistent for each extraction method, but the results from each extractant were very different within each forest floor sample.

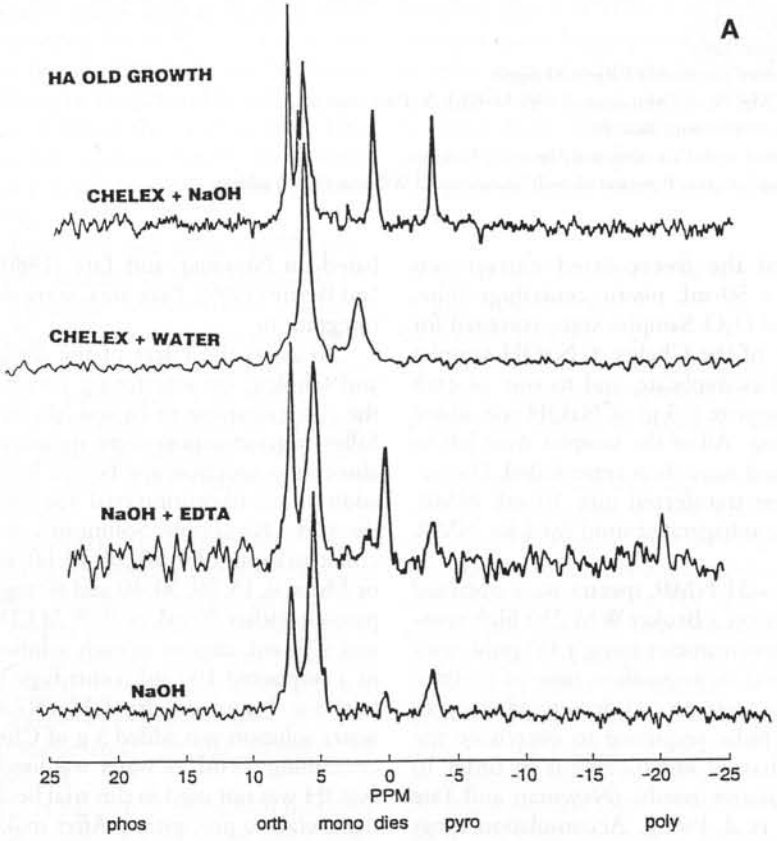

Fig. 1A Phosphorus-31 NMR spectra for HA old growth forest floor extracted with: Chelex + NaOH; Chelex + Water; NaOH + EDTA; and NaOH.

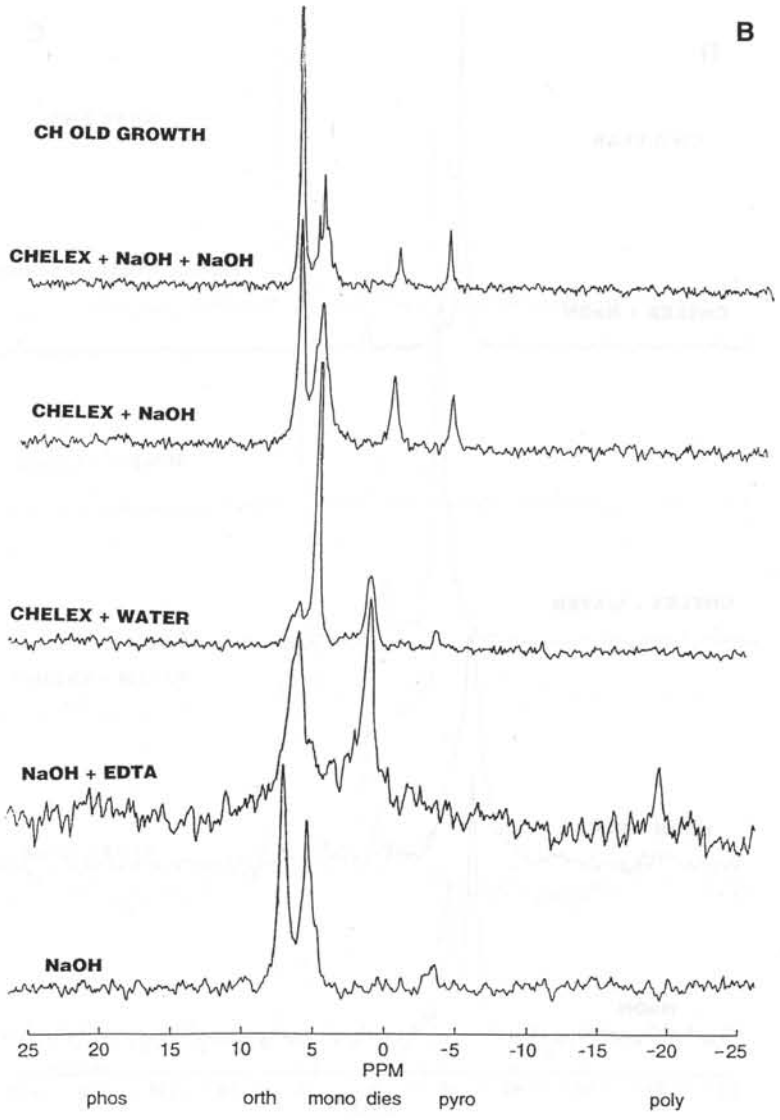

Fig. 1B Phosphorus-31 NMR spectra for CH old growth forest floor extracted with: Chelex + NaOH; Chelex + Water; NaOH + EDTA; and NaOH.

NaOH + EDTA was the only method to show polyphosphate peaks for three of the samples, whereas diester peaks in the NaOH extracts were seen only with the HA-OG samples (Fig. 1A). Phosphonate was only detected unambiguously in sample CH-5 (Fig. 1D) using the three extractions involving Chelex. Table 3 shows the percentage of P found within each class of compounds, calculated from the spectra by integration. The NaOH + EDTA extraction seems to have produced the greatest range of compounds. It also extracted the most P of all the extractants: 71.3–90.6% of P_T, compared with 22.1–34.5% extracted by NaOH, 9.6–12.6% by Chelex + water, and 23.1–35.3% by Chelex + NaOH (Table 4). The NaOH + EDTA also extracted more diester P than monoester P (Table 3), producing higher diester/monoester ratios than any other method except the Chelex + water extraction. The NaOH extraction method extracted the fewest types of P compounds, showing peaks for orthophosphate and monoesters in all samples but peaks for diesters and pyrophosphate in only a few samples. There were no peaks for phosphonates or polyphosphates with this extraction procedure.

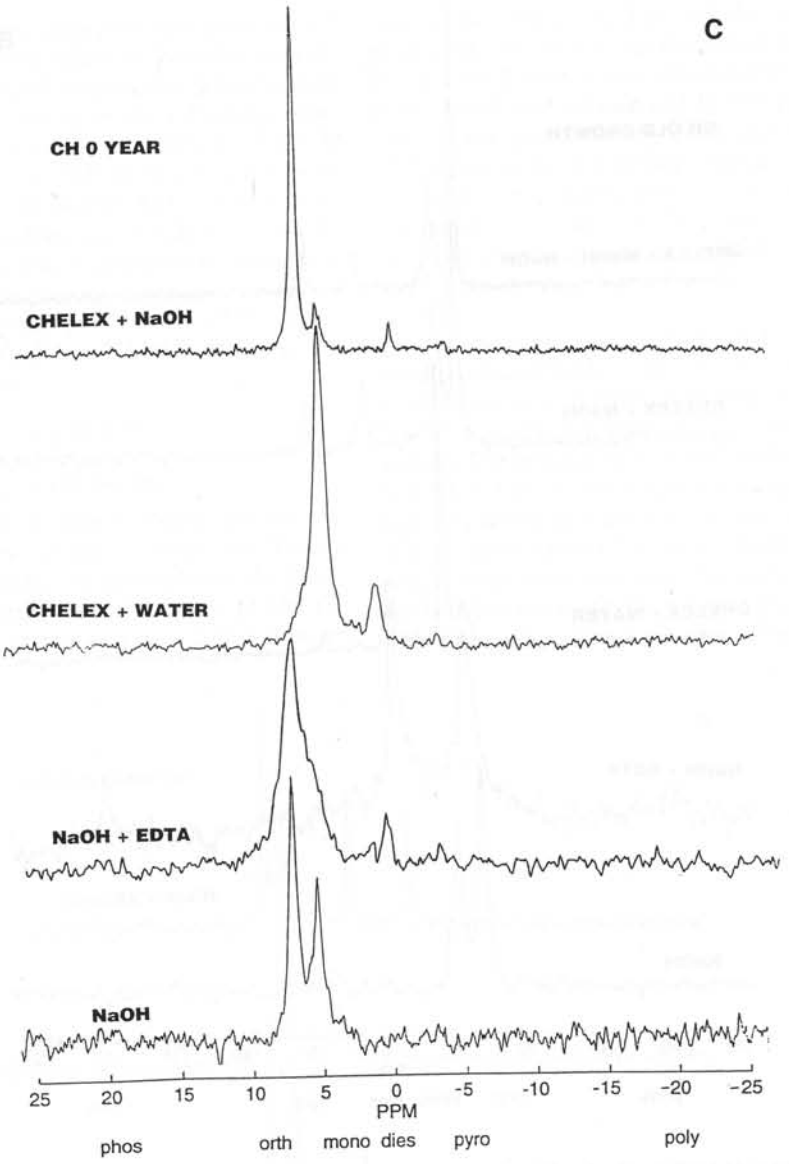

Fig. 1C Phosphorus-31 NMR spectra for CH 0-year forest floor extracted with: Chelex + NaOH; Chelex + Water; NaOH + EDTA; and NaOH.

Metals Analysis

Table 5 displays the analysis of Fe and Mn within each solution after extraction. The Fe concentrations extracted by NaOH alone and by H_2O + Chelex were comparable (8–36 vs. 4–29 mg/L). The Fe levels in the NaOH + EDTA solutions were intermediate, except for CH-0, the recent burn. NaOH + Chelex solutions contained the lowest levels of Fe. NaOH + EDTA extracted the greatest concentration of Mn, especially in the three postburn samples. The lowest Mn levels were found in the two Chelex extracts.

Figure 2 displays the results from the adsorption trial, using soil. For the samples without soil, the EDTA and water extracts contained the same levels of Fe or Mn that had been added to the samples, whereas the Chelex removed all of the Fe and Mn, leaving 0 mg/L in solution at all levels of addition. In Figure 2(A), the EDTA maintained all of the added Fe in solution, and also extracted additional Fe from the soil. The Chelex removed almost all of the added Fe from solution. With water alone, much of the Fe was adsorbed

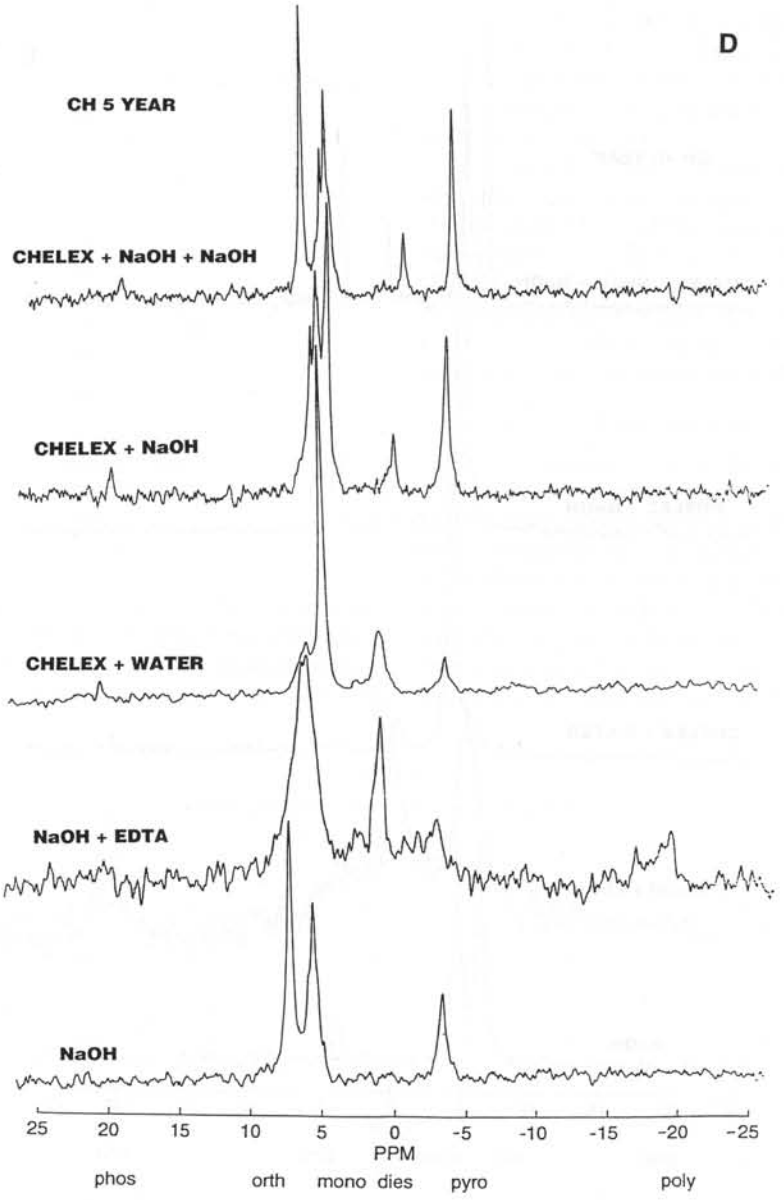

Fig. 1D Phosphorus-31 NMR spectra for CH 5-year forest floor extracted with: Chelex + NaOH; Chelex + Water; NaOH + EDTA; and NaOH.

onto the soil surfaces as the Fe in solution at all levels was lower than that that had been added to the sample. For Mn (Fig. 2(B)), the Chelex removed all of the added Mn from solution. The EDTA kept what had been added to the sample in solution, but it did not appear to extract any additional Mn from the soil. At the highest level of Mn addition (80 mg/L), there appeared to be some adsorption onto the soil. In water alone, all of the added Mn appeared to stay in solution.

P Addition

The results from the addition of P compounds to the NMR extracts are shown in Fig. 3, A and B. The P concentrations in each extract and the proportion of P in each compound class are found in Table 6.

The Chelex + NaOH extraction produced much sharper peaks than those produced by NaOH + EDTA, but peaks are seen at the same

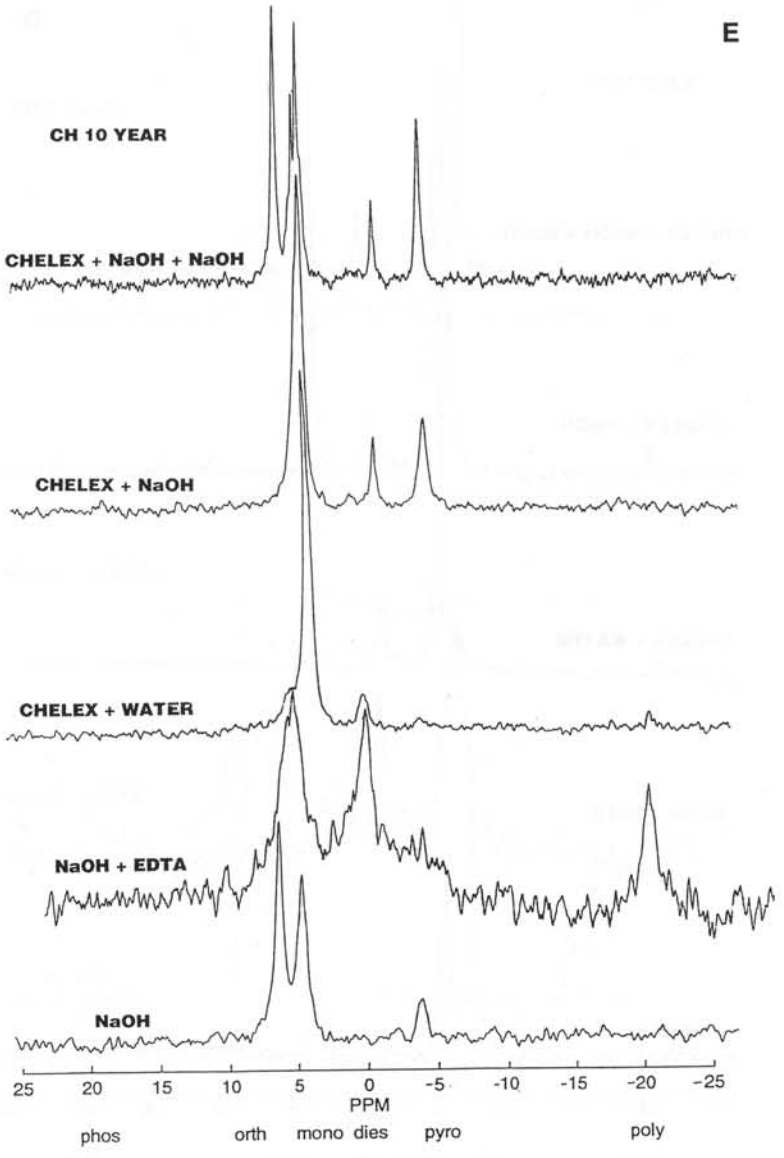

Fig. 1. E Phosphorus-31 NMR spectra for CH 10-year forest floor extracted with: Chelex + NaOH; Chelex + Water; NaOH + EDTA; and NaOH.

positions in the spectra regardless of extractant (Fig. 3, A and B). The added P compounds dominate each spectrum, changing the spectra from those of the original extract. When added polyphosphate was extracted with NaOH + EDTA (Fig. 3A), there was a large, broad peak at -20 ppm, the polyphosphate position. Pyrophosphate, diester, monoester, and orthophosphate peaks also appeared, but they were small and broad relative to the polyphosphate peak. In the Chelex + NaOH extract (Fig. 3B), there was a sharp polyphosphate peak, as well as sharp monoester and pyrophosphate peaks. The main difference between these two methods when polyphosphate was added was the amount of P extracted: 6500 mg/kg for NaOH + EDTA versus 3800 mg/kg by Chelex + NaOH (Table 6). NaOH + EDTA extracted more of the total P as orthophosphate and less as monoester.

When ATP was added to the sample, orthophosphate, monoester, and diester peaks appeared (Fig. 3, A and B). There were also three

TABLE 2
Interpretation of ^{31}P NMR spectra

PPM	Compounds	Abbrevation
15–20	phosphonates	phos
6–8	orthophosphate	orth
3–6	phosphate monoesters –inositol phosphates –sugar phosphates –mononucleotides	mono
1–(–1)	phosphate diesters –phospholipids –RNA, DNA	dies
(–3)–(–6)	pyrophosphate	pyro
(–20)	polyphosphate (ATP)	poly

approximately equal peaks at -5, -10 and -20 ppm. These represent the alpha, beta, and gamma phosphates in the ATP molecule. Both extraction methods yielded the same concentration of P in solution (Table 6) and approximately the same percentages of total P in the various compound classes, although NaOH + EDTA had slightly more orthophosphate and less monoester P than did Chelex + NaOH.

Glycerophosphate, a monoester formed in soil after hydrolysis of glycerophosphatides (Hance and Anderson 1963), appeared at the monoester position with both extraction methods (Fig. 3, A and B). The NaOH + EDTA solution (Fig. 3A) contained nearly twice as much P as the Chelex + NaOH solution (Table 6) and had more P as orthophosphate and less as monoester.

DISCUSSION

Of the reagents used in this study, NaOH + EDTA extracted the most P from each forest floor sample, with results comparable to those from sewage sludge extracted with TCA and KOH (Hinedi et al. 1989) or from sequential extraction of soil (Condron et al. 1990). This agrees with the findings of Bowman and Moir (1993) that it is a good extractant of organic P. The amount of P extracted by NaOH (22–34%) is

TABLE 3
Percentage of solution P found in various P forms

Sample	Extractant	Phos	Orth	Mono	Dies	Pyro	Poly	D:M[a]
HA-OG	Chelex + NaOH	0	26	44	15	15	0	0.34
	Chelex + H_2O	0	62	16	22	0	0	1.38
	NaOH + EDTA	0	21	49	15	7	7	0.31
	NaOH	0	36	51	4	9	0	0.08
CH–OG	Chelex + 2 NaOH	0	58	30	5	7	0	0.30
	Chelex + NaOH	0	36	40	14	10	0	0.35
	Chelex + H_2O	0	54	18	23	5	0	1.27
	NaOH + EDTA	0	17	33	39	0	11	1.18
	NaOH	0	51	43	0	6	0	0
CH–0	Chelex + NaOH	0	74	18	6	2	0	0.33
	Chelex + H_2O	0	73	13	13	1	0	1.00
	NaOH + EDTA	0	51	33	11	6	0	0.33
	NaOH	0	55	45	0	0	0	0
CH–5	Chelex + 2 NaOH	3	34	38	8	17	0	0.21
	Chelex + NaOH	2	31	39	9	19	0	0.17
	Chelex + H_2O	3	49	22	18	8	0	0.82
	NaOH + EDTA	0	23	40	18	7	12	0.45
	NaOH	0	49	33	0	18	0	0
CH–10	Chelex + 2 NaOH	0	34	42	8	16	0	0.19
	Chelex + NaOH	1	15	57	9	19	0	0.16
	Chelex + H_2O	0	68	14	10	4	4	0.71
	NaOH + EDTA	0	17	27	32	10	14	1.18
	NaOH	0	49	41	0	10	0	0

[a] The diester:monoester ratio.

TABLE 4

Phosphorus extracted by various methods

Extractant		HA–OG	CH–OG	CH–0	CH–5	CH–10
NaOH + Chelex	(mg/kg)	210.1	210.5	166.8	182.6	183.7
	%Total P[a]	34.3	35.3	23.1	31.7	25.9
H_2O + Chelex	(mg/kg)	77.4	66.2	82.8	55.0	71.6
	%Total P	12.6	11.1	11.5	9.6	10.1
NaOH + EDTA	(mg/kg)	526.4	424.8	643.5	451.6	643.1
	%Total P	85.8	71.3	89.1	78.4	90.6
NaOH	(mg/kg)	205.0	198.8	159.6	198.8	207.6
	%Total P	33.4	33.4	22.1	34.5	29.2

[a] Calculated from Table 1.

comparable to, or slightly lower than, that reported in the literature (Newman and Tate 1980; Tate and Newman 1980; Hawkes et al. 1984; Ingall et al. 1990; Gil-Sotres et al. 1990; Forster and Zech 1993; Bedrock et al. 1994). Sonicating during extraction might have increased these concentrations. The levels of P extracted by Chelex with both water and NaOH are lower than those obtained by Adams and Byrne (1989), Adams (1990), and Condron et al. (1996), which were comparable with NaOH levels, as was the amount extracted by Bu_4NOH (Emsley and Niazi 1983). There were also lower P levels in the Chelex + NaOH samples after P compounds such as polyphosphate and glycerophosphate were added before extraction.

The quality of the spectra produced by NaOH + EDTA was poor, however, relative to the other extractants used in this trial, with poor separation of the orthophosphate and monoester P peaks. This seems to be caused by the complexing by EDTA of paramagnetic ions other than P, particularly Fe and Mn. The one sample in which the peaks were clearly separated (HA-OG) contained the lowest concentrations of Fe and Mn. High concentrations of Mn seem to cause most of the peak overlap: spectra containing less than 200 μg/g of Mn have good separation between the orthophosphate and monoester P peaks. Other researchers have reported peak broadening in ^{31}P NMR spectra, by Mn (Hutson et al. 1992) and by Fe (Bedrock et al. 1994). This is one drawback to the NaOH-EDTA method — it removed cations from P compounds to allow more P to be extracted, but it did not remove the metals from solution as Chelex did. EDTA alone did not extract much Fe from the soil; however, more Fe was released when EDTA was combined with NaOH, probably because of the solubilization of organic matter (Stevenson 1994). The best spectra, in terms of peak separation and signal-to-noise ratio, were produced by the Chelex + NaOH extractions. These were improved further by adjusting the pH before analysis. However, overlapping of the orthophosphate and monoester P peaks was seen in all of the Chelex + H_2O spectra and in the spectra for the CH-10 sample extracted with Chelex + NaOH.

TABLE 5

Metal concentration (mg/L) in solution after extraction

Sample	Metal	NAOH	NaOH + EDTA	H_2O + Chelex	NaOH + Chelex
HA–OG	Fe	13	7	10	3
	Mn	2	8	0[a]	0
CH–OG	Fe	36	29	29	15
	Mn	5	24	2	0
CH–0	Fe	30	63	26	15
	Mn	5	32	3	2
CH–5	Fe	8	4	4	0
	Mn	4	30	2	0
CH–10	Fe	10	6	10	4
	Mn	7	51	2	0

[a] Readings below the instrumental detection limit are shown as 0.

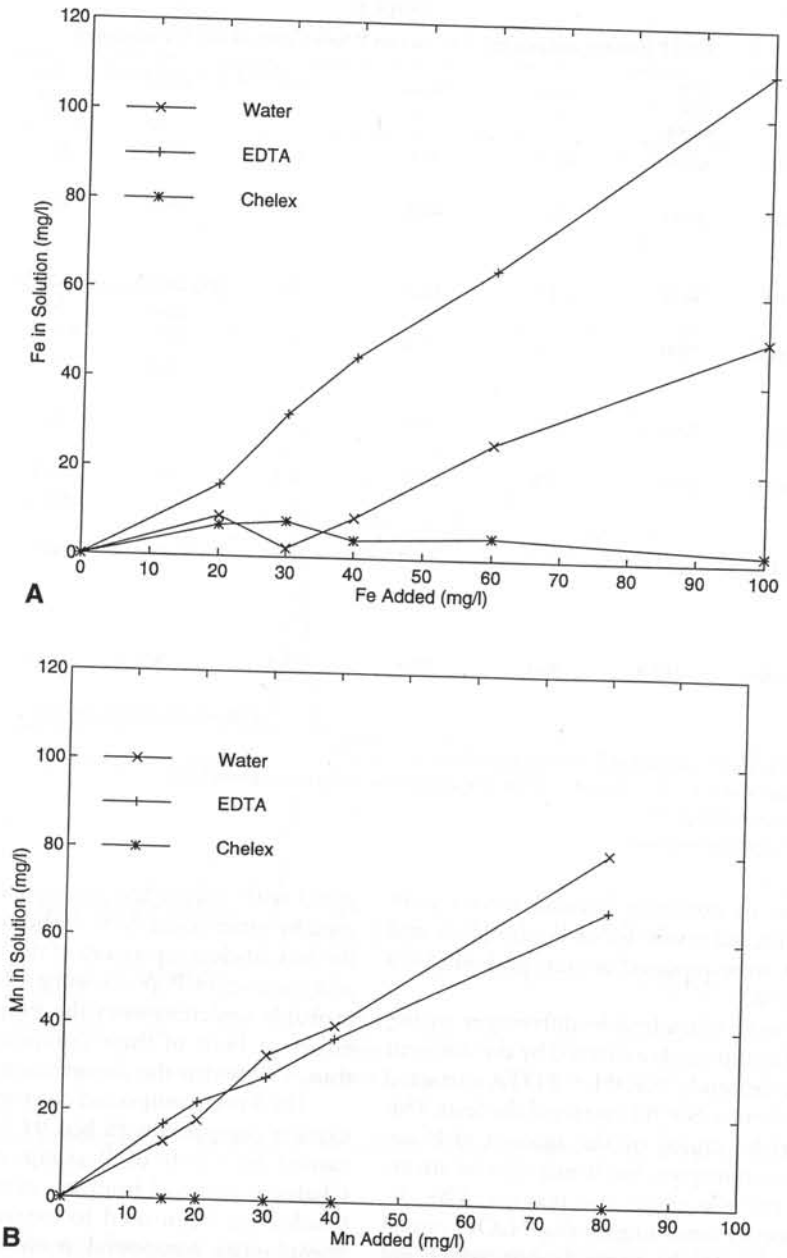

Fig. 2. Fe (A) and Mn (B) in solution following the adsorption trial.

The quality of spectra reported in the literature are quite variable. NaOH often produces poorly resolved resonances in the orthophosphate monoester region, with monoesters appearing as shoulders on the orthophosphate peak (Newman and Tate 1980; Zech et al. 1987; Hinedi et al. 1989; Condron et al. 1990; Gil-Sotres et al. 1990; Bedrock et al. 1994; Makarov et al. 1995). This has also been reported in Chelex extracts (Adams and Byrne 1989; Condron et al. 1996). The TCA and KOH extractions (Hinedi et al. 1989) produced clear, sharp spectra, as did the sequential extraction procedure of Condron et al. (1985). However, Hinedi et al. (1989) examined sewage sludge, which may not be comparable to forest floor. It is difficult to judge the quality of

TABLE 6

Total P content and percent P in various P forms after adding P compounds

Sample	P in Soil[a] mg/kg	Orth %	Mono %	Dies %	Pyro %	−10 peak %[b]	poly %
NaOH + EDTA + polyphos[c]	6500	11.7	6.3	4.3	12.8	n/a	64.9
NaOH + Chelex + polyphos	3800	0	40.3	4.9	16.1	n/a	38.7
NaOH + EDTA + ATP	3600	14.5	10.2	7.2	24.7 alph	21.7 beta	21.7 gam.
NaOH + Chelex + ATP	3500	2.8	17.8	4.7	28.0 alph	27.1 beta	19.6 gam.
NaOH + EDTA + glycero[d]	5900	26.4	63.9	9.7	0	n/a	0
NaOH + Chelex + glycero	3000	3.8	89.7	6.5	0	n/a	0
NaOH + EDTA orig. extract	424.8	33.3	16.7	38.9	0	n/a	11.1
NaOH + Chelex orig. extract	210.5	36.1	40.3	13.9	9.7	n/a	0

[a] Calculated from the amount of P in each extract.

[b] For ATP, the peaks at −5, −10 and −20 are the alpha, beta, and gamma phosphates.

[c] Polyphos is polyphosphate.

[d] Glycero is glycerophosphate.

Bu_4NOH as an extractant because spectra were not published, and results for orthophosphate and monoesters were reported as one peak (Emsley and Niazi 1983).

There were considerable differences in the diversity of compounds extracted by the different reagents in this study. NaOH + EDTA extracted the most, whereas NaOH extracted the least. This may in part be caused by the amount of P extracted by each reagent, but it may also be attributable to the nature of the reagent. The diester/monoester ratios suggest that NaOH caused hydrolysis of orthophosphate diesters, which did not occur with NaOH + EDTA. Hydrolysis has been reported by other NMR researchers (Ogner 1983; Adams and Byrne 1989; Ingall et al. 1990), and Hance and Anderson (1963) reported that phospholipids were readily hydrolyzed in alkaline solution. The diester/monoester ratios for NaOH are also comparable to reported values of 0.18–0.46 (Zech et al. 1985; Gil-Sotres et al. 1990; Forster and Zech 1993; Makarov et al. 1995). The lack of diesters in NaOH samples in this particular study is probably the result of the length of extraction, overnight in this case, compared with only a few minutes with sonication used by other researchers. It should be noted that the lack of clear separation of the orthophosphate and monoester P peaks using NaOH + EDTA probably underestimates the proportion of P in either or both of these compound classes and, thus, may widen the diester/monoester ratio.

The lower compound diversity in the Chelex extracts compared with NaOH + EDTA may be caused by a loss of P compounds when the Chelex is removed from the extracting solution. Chelex has been used to extract glyphosate, a phosphonate compound, from water, fruits, and vegetables (Cowell et al. 1985). When P compounds such as polyphosphate and glycerophosphate were added to the soil before extraction, the NaOH + EDTA extracted more P than Chelex + NaOH. This would suggest that P is removed along with the Chelex during filtration, possibly via cation linkages. The K^+ from the added K-polyphosphate may be exchanged with divalent cations such as Mn^{++} or Fe^{++}, which could then link the polyphosphate to the Chelex (Lévesque and Schnitzer 1967).

Polyphosphates are rarely seen in reported

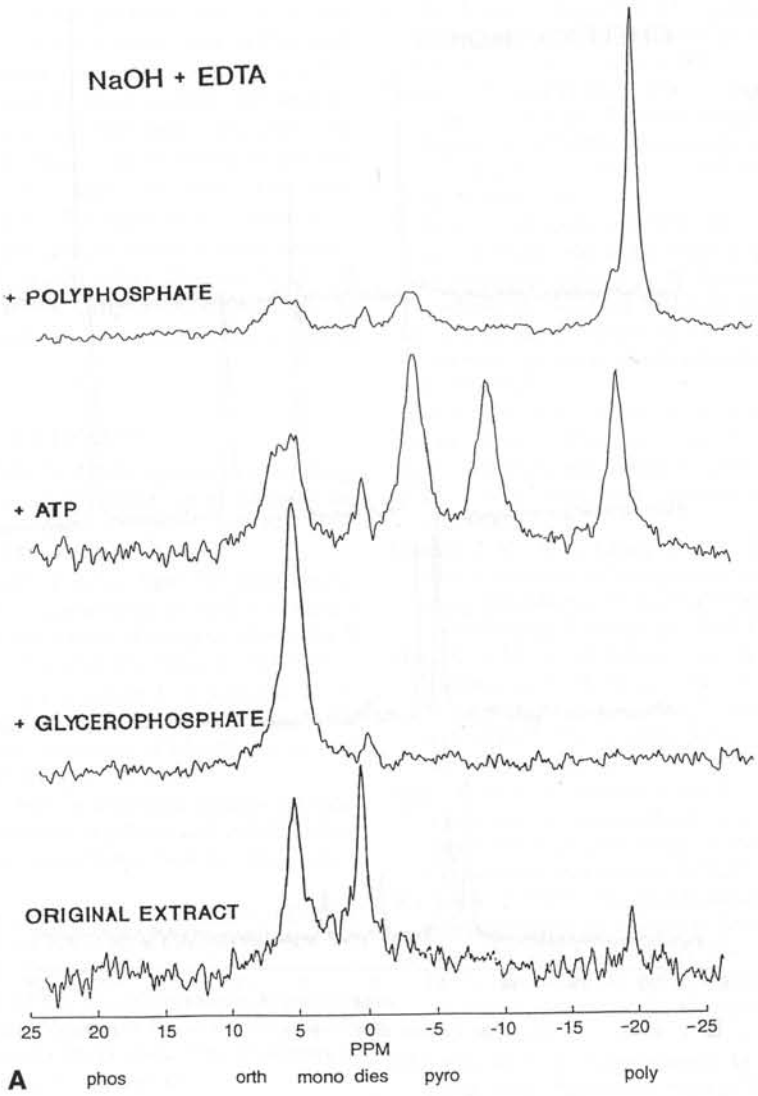

Fig. 3A Phosphorus-31 NMR spectra in soil and after the addition of polyphosphate, ATP, and glycerophosphate, extracted with NaOH + EDTA.

NMR spectra (Tate and Newman 1980; Emsley and Niazi 1983; Zech et al. 1987; Adams and Byrne 1989; Bedrock et al. 1994) and are usually at very low levels. This is surprising as they are widely distributed in nature, especially in forest soils (Kulaev 1979; Martin et al. 1985). Bedrock et al. (1994) report polyphosphate peaks in spectra from humic acid extracts but not from alkali extracts of peat and soil samples. They suggest that greater resolution of P compounds in the humic acids may be the result of their increased organic content and decreased Fe content relative to the peat and soil alkali extracts. The high amounts of polyphosphates extracted by NaOH + EDTA in this study may be attributable to the greater amount of total P extracted by this method as they are not seen in the spectra of the other extractants. They may be hydrolyzed by other reagents (Subbarao et al. 1977), they may be part of the more than 50% of the total P that was not extracted, or they may be lost during the extraction procedure.

This research suggests that we must be cautious when using ^{31}P NMR spectroscopy to examine P cycling in soils. The extractant used will influence both the concentration of P extracted

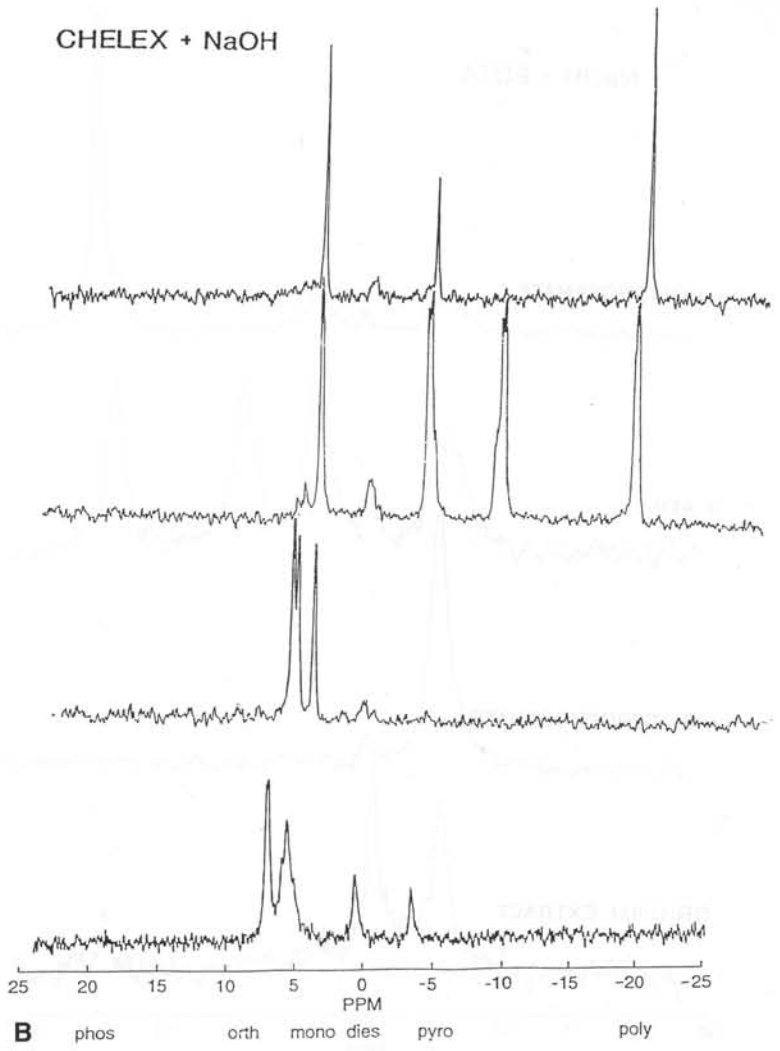

Fig. 3B Phosphorus-31 NMR spectra in soil and after the addition of polyphosphate, ATP, and glycerophosphate, extracted with Chelex + NaOH.

and the forms of P seen in NMR spectra, and, thus, may obscure important changes in some P forms. Care must also be taken when comparing studies using different extractants.

CONCLUSIONS

Phosphorus-31 NMR spectroscopy is a valuable tool for the direct identification of P compounds in soil extracts. Its major advantage is that it is less complex analytically than the detailed partition chromatography techniques otherwise required for identifying specific organic P compounds.

Extraction of P compounds from soil for ^{31}P NMR analysis is possible with a variety of reagents. The forms of P that dissolve depend on the reagent; the complex nature of soil organic P may make it impossible for a single extractant to dissolve all P compounds. It also may not be possible to produce high quality spectra without some alteration of P compounds by hydrolysis.

It appears from this study that the NaOH-EDTA extraction procedure for organic P (Bowman and Moir 1993) could be used as an extractant for ^{31}P NMR analysis. It extracted a higher concentration of P and a greater diversity of P compounds that any other extractant tested, with less apparent hydrolysis of compounds. However,

it also maintained other paramagnetic ions in solution, causing line broadening and overlapping peaks, thus reducing the quality of spectra. Consequently, it would be most suitable for samples with high P levels and low levels of interfering ions unless some way could be found to remove the EDTA-metal complexes after extraction without altering the P compounds in solution.

This study also demonstrated that the extractant used will greatly affect the results of ^{31}P NMR analysis of soil samples, making it difficult to compare results from studies using different extractants.

REFERENCES

Adams, M. A. 1990. ^{31}P-NMR identification of phosphorus compounds in neutral extracts of mountain ash (*Eucalyptus regnans* F. Muell.) soils. Soil Biol. Biochem. 22:419–421.

Adams, M. A., and L. T. Byrne. 1989. ^{31}P-NMR analysis of phosphorus compounds in extracts of surface soils from selected karri (*Eucalyptus diversicolor* F. Muell.) forests. Soil Biol. Biochem. 21:523–528.

Bedrock, C. N., M. V. Cheshire, J. A. Chudek, B. A. Goodman, and C. A. Shand. 1994. Use of ^{31}P NMR to study the forms of phosphorus in peat soils. Sci. Total Environ. 152:1–8.

Bowman, R. A. 1989. A sequential extraction procedure with concentrated sulfuric acid and dilute base for soil organic phosphorus. Soil Sci. Soc. Am. J. 53:362–366.

Bowman, R. A., and J. O. Moir. 1993. Basic EDTA as an extractant for soil organic phosphorus. Soil Sci. Soc. Am. J. 57:1516–1518.

Cade-Menun, B. J. 1995. Phosphorus forms of podzolic soils of northern Vancouver Island and their use by western red cedar. Ph.D. thesis, The University of British Columbia, Vancouver, BC.

Condron, L. M., M. R. Davis, R. H. Newman, and I. S. Cornforth. 1996. Influence of conifers on the forms of phosphorus in selected New Zealand grassland soils. Biol. Fert. Soils 21:37–42.

Condron, L. M., E. Frossard, H. Tiessen, R. H. Newman, and J. W. B. Stewart. 1990. Chemical nature of organic phosphorus in cultivated and uncultivated soils under different environmental conditions. J. Soil Sci. 41:41–50.

Condron, L. M., K. M. Goh, and R. H. Newman. 1985. Nature and distribution of soil phosphorus as revealed by a sequential extraction method followed by ^{31}P nuclear magnetic resonance analysis. J. Soil Sci. 36:199–207.

Cowell, J. E., J. L. Kunstman, P. J. Nord, J. R. Steinmetz, and G. R. Wilson. 1985. Validation of an analytical residue method for analysis of glyphosate and metabolite: An interlaboratory study. J. Agric. Food Chem. 34:955-960.

Emsley, J, and S. Niazi. 1983. The analysis of soil phosphorus by ICP and ^{31}P NMR spectroscopy. Phosphorus and Sulfur 16:303–312.

Forster, J. C., and W. Zech. 1993. Phosphorus status of a soil catena under Liberian evergreen rain forest: Results of ^{31}P NMR spectroscopy and phosphorus adsorption experiments. Z. Pflanzenernahr. Bodenk. 156:61–66.

Gil-Sotres, F., W. Zech, and H. G. Alt. 1990. Characterization of phosphorus fractions in surface horizons of soils from Galicia (N.W. Spain) by ^{31}P NMR spectroscopy. Soil Biol. Biochem. 22:75–79.

Hance, R. J., and G. Anderson. 1963. Identification of hydrolysis products of soil phospholipids. Soil Sci 96:157–161.

Hawkes, G. E., D. S. Powlson, E. W. Randall, and K. R. Tate. 1984. A ^{31}P nuclear magnetic resonance study of the phosphorus species in alkali extracts of soils from long-term field experiments. J. Soil Sci. 35:35–45.

Hinedi, Z. R., A. C. Chang, and R. W. K. Lee. 1989. Characterization of phosphorus in sludge extracts using phosphorus-31 nuclear magnetic resonance spectroscopy. J. Environ. Qual. 18:323–329.

Hutson, S. M., G. D. Williams, D. A. Berkich, K. F. LaNoue, and R. W. Briggs. 1992. A ^{31}P NMR study of mitochondrial inorganic phosphate visibility: Effects of Ca^{2+}, Mn^{2+} and the pH gradient. Biochemistry 31:1322–1330.

Ingall, E. D., P. A. Schroeder, and R. A. Berner. 1990. The nature of organic phosphorus in marine sediments: New insights from ^{31}P NMR. Geochim. Cosmochim. Acta 54:2617–2620.

Kulaev, I. S. 1979. The biochemistry of inorganic polyphosphates. John Wiley & Sons, Toronto.

Lévesque, M., and M. Schnitzer. 1967. Organo-metallic interactions in soil: 6. Preparation and properties of fulvic acid metal phosphates. Soil Sci. 103:183–185.

Makarov, M. I., G. Guggenberger, H. G. Alt, and W. Zech. 1995. Phosphorus status of Eutric Cambisols polluted by P-containing immissions: Results of ^{31}P NMR spectroscopy and chemical analysis. Z. Pflanzanernahr. Bodenk. 158:293–298.

Martin, F., J.-P. Marchal, A. Timinska, and D. Canet. 1985. The metabolism and physical state of polyphosphates in ectomycorrhizal fungi. A ^{31}P nuclear magnetic resonance study. New Phytol. 101:275–290.

Mehlich, A. 1984. Mehlich 3 soil test extractant: A modification of Mehlich 2 extractant. Commun. Soil Sci. Plant Anal. 15:1409–1416.

Newman, R. H., and K. R. Tate. 1980. Soil phosphorus characterization by ^{31}P nuclear magnetic resonance. Commun. Soil Sci. Plant Anal. 11:835–842.

Ogner, G. 1983. ^{31}P-NMR spectra of humic acids. A comparison of four different raw humus types in Norway. Geoderma 29:215–219.

O'Neill, I. K., M. Sargent, and M. L. Trimble. 1980. Determination of phytate in foods by phospho-

rus-31 Fourier transform nuclear magnetic resonance spectroscopy. Anal. Chem. 52:1288–1291.

Parkinson, J. A., and S. E. Allen. 1975. A wet oxidation procedure suitable for the determination of nitrogen and mineral nutrients in biological material. Commun. Soil Sci. Plant Anal. 6:1–11.

Prescott, C. E., and G. F. Weetman. 1994. Salal Cedar Hemlock Integrated Research Program: A Synthesis. Faculty of Foresty, University of British Columbia, Vancouver, BC.

Preston, C. M., J. A. Ripmeester, S. P. Mathur, and M. Lévesque. 1986. Application of solution and solid-state multinuclear NMR to a peat-based composting system for fish and crab scrap. Can. J. Spectrosc. 31:63–69.

Saunders, W. M. H., and E. G. Williams. 1955. Observations on the determination of total organic phosphorus in soils. J. Soil Sci. 6:254–267.

Stevenson, F. J. 1994. Humus chemistry: Genesis, composition, reactions, 2nd Ed. John Wiley & Sons, Toronto.

Subbarao, Y. V., R. Ellis, Jr., G. M. Paulson, and J. V. Paukstelis. 1977. Kinetics of pyro- and tripolyphosphate hydrolyses in the presence of corn and soybean roots as determined by NMR spectroscopy. Soil Sci. Soc. Am. J. 41:316–318.

Tate, K. R., and R. H. Newman. 1982. Phosphorus fractions of a climosequence of soils in New Zealand tussock grassland. Soil Biol. Biochem. 14:191–196.

Watanabe, F. S., and S. R. Olsen. 1965. Test of an ascorbic acid method for determining P in water and $NaHCO_3$ extracts from soil. Soil Sci. Soc. Am. Proc. 29:677–678.

Zech, W., H. G. Alt, L. Haumaier, and R. Blasek. 1987. Characterization of phosphorus fractions in mountain soils of the Bavarian Alps by ^{31}P NMR spectroscopy. Z. Pflanzenernahr. Bodenk. 150:119–123.

Zech, W., H. G. Alt, A. Zucker, and I. Kögel. 1985. ^{31}P-NMR spectroscopic investigations of NaOH extracts from soils with different land use in Yucatan (Mexico). Z. Pflanzenernahr. Bodenk. 148:626–632.