

BASIC EDTA AS AN EXTRACTANT FOR SOIL ORGANIC PHOSPHORUS

R. A. BOWMAN* AND J. O. MOIR

Abstract

Soil organic P is an important source of plant-available P in the Great Plains. It is, therefore, important in conservation tillage agriculture, that we know the size and the potential availability for crop use of this large P pool, which could exceed one-half of the total P. We propose a one-step procedure with hot basic EDTA (ethylene diaminetetraacetic acid) for total soil organic P that requires minimum manipulation and analytical prowess. Soil samples (0.5 g) were incubated for 2 h at 85 °C with 25 mL of 0.25 M NaOH + 0.05 M Na₂EDTA, after which time the organic P in the extracts was determined by persulfate oxidation. Results from the evaluation of nine soils from various parts of the USA and one Canadian soil showed significant correlations with an existing wet sequential extraction and a dry high-temperature ignition method for total organic P determinations. With the high-organic-matter Canadian soil, the EDTA method extracted 32% more organic P than the wet extraction method, and essentially the same as the dry ignition method. The new method appears to be especially useful for soils where organic acids and chelation mechanisms with P may be more dominant than ester-type formations.

PHOSPHORUS is the second most limiting soil nutrient for crops, and plays an important role in dry matter production, as evidenced by its primary role in energy-mediated reactions. Simplistically, two forms of P exist: organic P associated with the soil organic matter (SOM), and inorganic P associated with mineral and adsorbed solid phases (Olsen and Khasawneh, 1980). Both forms of P can also be present in the solution phase (Frossard et al., 1989).

Even though plants take up inorganic P, the importance of organic P as a reservoir for plant-available P in the Great Plains has been well documented (Dor Maar, 1972; Stewart and Tiessen, 1987). Various studies have shown that a large part of the total P loss during cultivation comes from the organic P fraction (Tiessen et al., 1982; Bowman et al., 1990). It is, therefore, important in conservation tillage agriculture that we know the size and potential availability of this large organic P pool, which could exceed 50% of the total P (Bowman and Cole, 1978).

Present methods to evaluate this organic P pool size are generally tedious and at times not very reproducible. The combination of Na₂EDTA and NaOH appears to possess chemical properties for a one-step determination for total organic P. The base can solubilize organic P associated with the SOM, and the

EDTA can chelate metal cations to increase the efficiency of SOM extraction. Harrap (1963) used EDTA for organic P determination; however, his procedure required multiple wet extraction steps. Other wet digestion methods (Mehta et al., 1954; Bowman, 1989) and dry combustion methods (Saunders and Williams, 1955; Legg and Black, 1955) also required multiple steps.

The primary objective of this research was to develop an easy and reproducible methodology with basic EDTA for total soil organic P, and to evaluate and compare basic EDTA as an extractant for organic P with established organic P procedures such as acid and base extractions and dry combustion followed by acid extractions.

Materials and Methods

The surface horizon (0–15 cm) of 10 (nine U.S. and one Canadian) uncultivated soils of 2-mm mesh size was selected to evaluate and compare total organic P content from a proposed procedure to two established procedures (Bowman, 1989; Saunders and Williams, 1955). In the Ascalon soil (Table 1), two cultivated phases (20 and 60 yr in cultivation) were also part of the evaluation. In all extracts, inorganic P concentrations were determined colorimetrically (Watanabe and Olsen, 1965), and organic P concentration by difference (total P by persulfate oxidation of the organic P [Bowman, 1989] minus inorganic P). All analyses were replicated three times. The rudimentary steps and relevant modifications for the above procedures are given below. For full details, the reader is advised to consult the original references. Other pertinent soil data collected on the 10 soil samples included soil organic C content (dry combustion), total N (Kjeldahl), pH (2:1 in water), and texture (hydrometer, Gee and Bauder, 1986).

Two grams of finely ground soil (2-mm mesh) was weighed and placed into a 250-mL centrifuge tube. Three milliliters of concentrated (18 M) H₂SO₄ were added to the tube, which was swirled to completely wet the soil sample. Four 1-mL portions of distilled water were then added to the tube, with swirling of the tube after each 1-mL delivery. Ten milliliters of water were added to the tube, which was then shaken vigorously, and set aside for 2 to 5 min before the addition of 33 mL of water for a total of 50 mL. The suspension was then centrifuged at 12 000 × g for 10 min, and the filtered extract was analyzed for inorganic P and total P concentrations. Base extraction was essentially the same as that described by Bowman (1989).

Two 1.00-g soil samples were placed into 250-mL Erlenmeyer flasks. One sample was ignited at 550 °C for 2 h for a measure of total P while the other sample remained at room temperature. Both samples were then extracted by shaking with 50 mL of 1 M H₂SO₄ for 16 h. Inorganic P concentrations of both extracts were determined, with the difference in concentrations being attributable to organic P.

Samples from three different soils (Ascalon, Haverson, and Promise) were used to measure optimum extraction of organic P by evaluating equilibration times (0.5, 2, and 16 h), temperatures (room temperature and 85 °C), and different concentrations of EDTA and NaOH (0.1 M EDTA with no NaOH, 0.075 M EDTA with 0.125 M NaOH, 0.05 M EDTA with 0.25 M NaOH, 0.025 M EDTA with 0.375 M NaOH, and no EDTA and 0.5 M NaOH). Total extractant volume was 25 mL; sample size parameter had been established previously (Bowman et al., 1991). Efficiency of SOM extraction was

R.A. Bowman, USDA-ARS, P.O. Box 400, Akron, CO 80720; and J.O. Moir, Dep. of Soil Science, Univ. of Saskatchewan, Saskatoon, Canada. Received 4 May 1992. *Corresponding author.

Table 1. Selected physical and chemical properties of nine test soils used in the organic P extraction study.

Soil	Series subgroup	pH	Sand	Silt	Clay	Total	
						Organic C	Kjeldahl N
						g kg ⁻¹	
Ascalon sandy loam	(0)† Typic Argiustoll	6.6	630	230	140	9.0	0.95
	(20)	6.9	710	160	130	4.9	0.59
	(60)	6.7	790	90	120	3.3	0.42
Cecil sandy loam	Typic Kanhapludult	4.8	770	150	90	5.0	0.53
Duroc loam	Pachic Paplustoll	7.3	410	460	130	22.0	1.89
Haverson loam	Ustic Torrifluent	7.9	350	390	260	12.0	1.20
Nunn clay loam	Aridic Argiustoll	7.4	330	420	250	14.0	1.00
Palouse silty clay loam	Pachic Ultic						
	Haploxeroll	5.8	120	610	270	30.0	3.20
Promise clay	Vertic Haplustoll	6.4	100	400	500	32.0	3.20
Redfeather sandy loam	Lithic Cryoboralf	5.2	640	270	90	6.0	0.73
Vona sandy loam	Ustollic Haplargid	6.5	790	120	90	6.0	0.70
Meeting Lake clay loam	Typic Cryoboralf‡	6.4	—	—	—	281	13.7

† Ascalon soil in cultivation for 0, 20, and 60 yr.

‡ Canadian Luvisol; not analyzed for texture; predominantly organic; ash content = 447 g kg⁻¹.

estimated from absorbance at 550 nm. Total and organic P concentrations were also determined from the extracts (Watanabe and Olsen, 1965). The 16-h equilibrium did not show significant improvement in extractability of SOM and P over the 2-h equilibration. Table 2 presents the data obtained using 0.50 g of soil and 2 h of equilibration at 85 °C.

One-half gram of finely ground soil sample (<1-mm mesh) was weighed into a heat-resistant 50-mL polypropylene screw-top centrifuge tube. Sample weight can decrease as the content of organic matter or lime increases. Twenty-five milliliters of 0.25 M NaOH + 0.05 M Na₂EDTA was added to the tube, which was then mixed for 15 s (a mechanical mixer is ideal). The loosely capped tube was placed for 10 min in an incubator or oven set at 85 °C. This initial loose capping is needed to minimize gas buildup. After 10 min, the tube was tightly capped and heating was continued until a total of 2 h had elapsed. The tube was removed from the oven, cooled, mixed, and the extract was centrifuged at 10 000 rpm for 10 min. The supernatant was filtered through a no. 40 filter paper, and inorganic P and total P were determined colorimetrically on the filtrate. Aliquot size should not exceed 2 mL, since excessive EDTA can retard color development and could require more persulfate for complete oxidation. Appropriate blanks were determined.

For comparison of methods, and method × soil interactions, an analysis of variance was conducted by Tukey's honestly significance difference test at $P = 0.05$. Coefficients of variation for replicates among soil types were also determined to assess precision (Gomez and Gomez, 1984).

Results and Discussion

The 10 soils (Table 1) represented a broad range of soil physical and chemical properties. Soils were obtained from different parts of the U.S., with one soil (Ascalon) varying in years of cultivation (native grassland to >50 yr of cultivation). Soil organic C varied from very low (Cecil and Vona) to high for mineral soils (Palouse and Promise), to very high for the Canadian organic soil. Soil reaction (pH) ranged from 4.8 in the Cecil soil to 7.9 in the calcareous (80 g CaCO₃ equivalent kg⁻¹ soil) Haverson loam. Clay content was lowest in the Cecil and Vona soils (90 g kg⁻¹) and highest in the Promise soil (500 g kg⁻¹). Reduction in fines (silt plus clay) in the cultivated Ascalon soils, compared with the original native soil, illustrate the significant effect of erosion in the Great Plains as reported by Bowman et al. (1990). Erosion also accounts for some of the losses of organic matter (Bowman et al., 1990; Schimel et al., 1985; van Veen and Paul, 1979).

The extractants (EDTA and NaOH) have been used separately to measure P concentration. Even though the Na salt of EDTA is a good extractant of organic matter (it complexes metal cations), interest in Na₂EDTA is low because it cannot accurately measure organic C or organic N because C and N are part of the EDTA molecule. Organic P, however, is extracted without confounding. Harrap (1963) measured organic P by initially removing basic cations with EDTA, and extracting the soil residue with base at room temperature followed by hot base extractions.

The EDTA alone (pH ≈ 4.6) extracted very little SOM, as measured by the intensity of the color (Table 2). Although more SOM was extracted with base alone (pH ≈ 13) than with EDTA, the combination of the two simultaneously eliminated cationic bridges with SOM and solubilized the organic matter, resulting in a more colored extract in much the same way acid pretreatment does. The concentrations of EDTA and NaOH most effective in extracting SOM and organic P were 0.05 M EDTA + 0.25 M NaOH and 0.025 M EDTA + 0.375 M NaOH.

Both organic and inorganic P pools were measured in

Table 2. Color and P extractability with varying proportions of 0.1 M Na₂EDTA and 0.5 M NaOH. Values represent average of three samples for 2-h extraction at 85 °C.

Soil	Extractant (% EDTA)	Relative optical density	mg kg ⁻¹	
			Inorganic P	Organic P
Ascalon	0	0.19	35	33
	25	0.43	53 (56)†	56 (58)†
	50	0.46	57 (60)	61 (63)
	75	0.25	55	17
	100	0.03	86	17
Haverson	0	0.29	16	37
	25	0.53	56 (58)	73 (68)
	50	0.59	89 (84)	78 (73)
	75	0.32	140	24
	100	0.06	198	36
Promise	0	2.71	56	230
	25	4.91	97 (110)	276 (270)
	50	5.11	100 (114)	287 (285)
	75	2.47	76	50
	100	0.13	107	53

† Values in parentheses represent 16-h extraction at 85 °C.

Table 3. Comparison of total organic P measured by the sequential acid-base extraction, dry ignition, and the proposed NaOH-EDTA procedures. Values represent average of three replications.

Soil	Acid-base	Ignition	NaOH-EDTA
	mg total organic P kg ⁻¹ †		
Ascalon (0)†	70	84	61
(20)†	40	60	35
(60)†	28	45	25
Cecil	34	42	31
Duroc	160	193	172
Haverson	104	106	96
Nunn	144	170	125
Palouse	232	238	230
Promise	308	350	285
Redfeathers	75	112	82
Vona	48	60	42
Meeting Lake	628	856	835
Average (U.S.)	113	132	109
(all soils)	156	193	168
Average CV, %	7.8	6.9	4.6

† Years in cultivation.

‡ Tukey's HSD ($P = 0.05$) to compare three methods within soils = 21; within methods = 8.

the five different extracts with varying concentrations of EDTA and NaOH for the three soils (Table 2). Inorganic P extractability generally increased as the concentration of EDTA increased. Results also indicated that, singly, base is more effective than EDTA in releasing organic P. However, some EDTA was necessary for more efficient release of organic P. The above combination of EDTA and NaOH, which was effective in extracting the dark organic matter color, also extracted the most organic P.

Analysis of variance of the data (Table 3) for the U.S. soils and for all soils showed significant differences among soils and methods. There was also a significant interaction between soils and methods. Among the U.S. soils, there was no significant difference between the wet sequential extraction procedure and the basic EDTA procedure for the U.S. soils. Only the Duroc and the Redfeathers soils exhibited higher values for the EDTA procedure, with the Palouse essentially the same and the Promise slightly lower. The higher results for the dry combustion were not unexpected since the dry combustion method usually gives higher values than wet extraction methods because of increases in inorganic P solubilities on high-temperature ignition of mineral soils (Soltanpour et al., 1987).

When the Canadian soil was included in the comparisons, significant differences were observed among all three methods because of much lower organic P extractability by the wet extraction procedure than by the other two procedures. However, a simple comparison of replicates for all three methods showed no difference between dry combustion and the EDTA procedure, both of which were significantly higher than the wet extraction. The closeness of results for the former two procedures could be attributed to low interference by increases in inorganic P solubilities on dry ignition of the high-organic Canadian soil. Even though the averages for the EDTA and wet extraction methods were still close, the EDTA method extracted some 8% more organic P because of the large amount of organic P in the one Canadian soil.

The fact that the proposed basic EDTA procedure required only one extraction step makes it easier than previously established total organic P methods. It must be borne in mind, however, that even though there is a single extraction the evaluation of total organic P always requires an oxidation of all organic P in the extract since, colorimetrically, only inorganic orthophosphate is measured.

The EDTA method might be especially more effective with soils high in organic matter, where chelation is a major factor in organic P tie-up. This was borne out on a Canadian Luvisol (Boralf) soil, where the EDTA procedure extracted 35% more organic P than the sequential extraction procedure, and essentially extracted the same as the dry ignition procedure. The Redfeathers soil developed under forested conditions is similar to the Canadian soil in the A horizon, and it also showed a 9% increase with the EDTA procedure over the sequential wet extraction procedure.

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