

Development of a New Soil Extractant for Simultaneous Phosphorus, Ammonium, and Nitrate Analysis

R. L. Haney

United States Department of Agriculture, Agricultural Research Service,
Temple, Texas, USA

E. B. Haney

Railroad Commission of Texas, Surface Mining and Reclamation
Division, Austin, Texas, USA

L. R. Hossner

Department of Soil & Crop Sciences, Texas A&M University, Texas
Agricultural Experiment Station, College Station, Texas, USA

J. G. Arnold

United States Department of Agriculture, Agricultural Research Service,
Temple, Texas, USA

Abstract: A new soil extractant (H^3A) with the ability to extract NH_4 , NO_3 , and P from soil was developed and tested against 32 soils, which varied greatly in clay content, organic carbon (C), and soil pH. The extractant (H^3A) eliminates the need for separate phosphorus (P) extractants for acid and calcareous soils and maintains the extract pH, on average, within one unit of the soil pH. The extractant is composed of organic root exudates, lithium citrate, and two synthetic chelators (DTPA, EDTA). The new soil extractant was tested against Mehlich 3, Olsen, and water for extractable P, and 1 M KCl and water-extractable NH_4 and NO_2/NO_3 . The pH of the extractant after adding soil, shaking, and filtration was measured for each soil sample (5 extractants \times 2 reps \times 32 soils = 320 samples) and was shown to be

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Address correspondence to R. L. Haney, United States Department of Agriculture, Agricultural Research Service, 808 E. Blackland Road, Temple, Texas 76502, USA.
E-mail: rhaney@spa.ars.usda.gov

highly influential on extractable P but has no effect on extractable NH_4 or NO_2/NO_3 . H^3A was highly correlated with soil-extractable inorganic N (NH_4 , NO_2/NO_3) from both water ($r = 0.98$) and 1 M KCl ($r = 0.97$), as well as being significantly correlated with water ($r = 0.71$), Mehlich 3 ($r = 0.83$), and Olsen ($r = 0.84$) for extractable P.

Keywords: Chelate, extractant pH, Mehlich 3, Olsen, soil extractant, soil pH

INTRODUCTION

Soil nutrient data from soil testing and research laboratories is a valuable tool available to producers and research scientists. Currently, soil test procedures require at least two soil extractants to analyze for ammonium (NH_4), nitrate (NO_2)/nitrite (NO_3), and phosphorus (P). Generally, 1–2 M KCl or water is used for $\text{NH}_4/\text{NO}_2/\text{NO}_3$, and Bray, Mehlich 3, and Olsen for P, although others can be used depending on soil type, soil pH, and climatic conditions. The use of a single extractant would increase laboratory productivity and decrease analysis cost. Few of the soil extractants currently available are capable of multinutrient extraction without sacrificing accuracy for one nutrient or another (Holford 1980). Soils are highly variable and complex; therefore, developing a multinutrient extractant that does an acceptable job of accurately identifying plant-available nutrients is difficult and time consuming. However, the need for such an extractant does exist.

Mehlich 3 is currently a popular multinutrient extractant because of its ability to extract a number of nutrients (with the exceptions of ammonium and nitrate/nitrite) and was primarily developed for neutral to acid soils (Mehlich 1984). The Olsen extractant was developed primarily for calcareous soils (Olsen et al. 1954).

An extractant that has the ability to extract nutrients near the soil pH is a desirable trait because soil pH and P solubility are highly interrelated (Golterman 1998, Sharply 1993). After a literature review, we decided that a good soil extractant would mimic the soil environment that has actively growing roots, because the target for fertilizer recommendations is plant yield. While it would be impossible to understand all the processes that occur in the rhizosphere, we chose to focus on plant root exudates to develop a soil extractant. Plants have the ability to deliver organic exudates to the soil solution to acquire necessary nutrients (Rengel 2002; Baudoin, Benizri, and Guckert 2003). Under certain conditions, plants can increase production of root exudates to overcome nutrient deficiencies such as phosphorus, iron, zinc, and manganese (Azaizeh et al. 1995; Rengal 1997; Subbarao, Ae, and Otani 1997). Ion toxicity and pathogen attack can also stimulate an exudate response from plants (Ryan et al. 1997, Zheng and Ma 1998; Mehta, Sharma, and Sindhan 1992). When plants encounter phosphorus deficiency, they have the ability to exude a wide range of both organic and inorganic compounds to increase the availability of phosphorus in the soil

solution (Rengel 2002). The objective of this study was to develop a soil extractant that meets the following initial criteria: 1) it should contain compounds that have been identified as common organic root exudates; 2) it should be able to extract soil, on average, within one unit of the soil pH; 3) it should be compatible with colorimetric and ICP methods for determining nutrient concentration; and 4) it should be significantly correlated with results of currently used soil test methods.

MATERIALS AND METHODS

Extractant Development

Different chemicals were experimented with, in many combinations and concentrations, including hydroquinone, citric acid, oxalic acid, acetic acid, lithium citrate, sucrose, instant tea (for tannins), sodium citrate, malic acid, ethylenedinitrilotetraacetic acid (EDTA), diethylenetriaminepentaacetic acid (DTPA), ascorbic acid, aluminum chloride, and lithium chloride. The soft drink 7-Up was even tried, and it actually compared quite well with Olsen and Mehlich 3 for extractable P.

It was reasoned that the organic compounds contained in root exudates were the most important, but we did not want to add potassium (K), sodium (Na), or calcium (Ca) in the extractant because metals, and other nutrients as well, might eventually be extracted. Lithium citrate was chosen because lithium is rarely tested in soil and lithium would act somewhat like K in KCl for replacing NH_4 from exchange sites. In the soils tested, lithium citrate alone was an excellent extractant in calcareous soils for P. The amount of lithium citrate in the extractant was proportional to the amount of extractable P in soil with pH greater than 7.

Next, three organic acids were added to the extractant that plants most commonly use to overcome deficiencies of various nutrients; oxalic acid, malic acid, and citric acid (Rengel 2002; Baudoin, Benizri, and Guckert 2003; Shenker, Hadar, and Chen 1999) and balanced the acidic solution with lithium citrate. The lithium citrate acted as a weak buffer when the acids were added, and it was a delicate balancing act to determine the proportion of the three acids to lithium citrate. It was known that lithium citrate was working well for extractable P in calcareous soils. However, some of the extracting power was lost after the acids were added for calcareous soils, but the addition of the organic acids made the extractant more flexible for use across a wider range of soil pH. Combinations of chemicals were chosen based on extractant pH. EDTA and DTPA were also added to help with the extraction of P and possibly other metals. Chelators help protect certain cations such as iron (Fe), zinc (Zn), copper (Cu), and manganese (Mn) from reacting with soil by forming chelate complexes, which can be taken up by plants. The aim was to create a soil extractant

based on organic acids (citric and oxalic acid are chelators), lithium citrate, and two synthetic chelators (EDTA and DTPA) that would extract nutrients near the soil pH. Therefore, the final combination of chemicals were based on extractant pH. The extractant H³A (Haney, Haney, Hossner, Arnold) was designated, which is produced by dissolving the following ingredients in one liter of water. Molarities are also stated. Lithium citrate: 5.0 g = 0.02 M; citric acid: 0.5 g = 0.0024 M; malic acid: 0.5 g = 0.004 M; oxalic acid: 0.5 g = 0.004 M; EDTA: 0.25 g = 0.002 M; and DTPA: 0.25 g = 0.001 M.

Soils

Soils were collected from ten states in the USA. They were from California (3), Texas (6), Illinois (6), Oklahoma (5), Pennsylvania (4), Colorado (4), Arizona (1), Alaska (1), Mississippi (1), and Wyoming (1) for a total of 32 soil samples. Soil characteristics are listed in Table 1. These soils were collected from pastureland (5 soils) and croplands, with the majority in conventional tillage (23 soils) and some in no-till (4 soils). The soils had a wide range in soil pH (4.7–8.4), organic C (0.1–2.6 g C kg⁻¹), and clay content (6–59%).

Extraction and Analytical Methods

Each soil was dried at 55°C for 24 h and ground to pass a 2-mm sieve. Each soil was weighed (4 g) in duplicate in 50-mL plastic centrifuge tubes and extracted with 40 mL using five extractants (1 M KCl, water, Olsen, Mehlich 3, and H³A) for a total of 320 samples. Samples were shaken for 30 min (5 min for Mehlich 3), centrifuged at 3000 rpm for 8 min, and filtered through Whatman 2V pleated filter paper. Each of the 320 soil extracts was tested for pH. The samples were then analyzed for NH₄-N, NO₂/NO₃-N, and PO₄-P on an OI Analytical, Flow IV, rapid-flow colorimetric analyzer.

RESULTS AND DISCUSSION

The amount of P extracted from soil is dependent upon many factors. Among those are soil pH, clay content, and concentrations of calcium, iron, and aluminum (Cox 2001). Extractable P is strongly influenced by soil pH but is also highly influenced by the pH of the soil extractant (Golterman 1988). In an effort to demonstrate this phenomenon, it was decided to manipulate the pH of an extractant on four acid soils (soil pH 5.5–6.8). Organic acids were used to drop the extractant pH to 2.6, and then we increased the pH incrementally to pH 9.0 with a combination of acids and lithium citrate and lithium citrate alone (details in Table 2). Extractable P ranged from 60–95 ppm at

Table 1. Soil characteristics

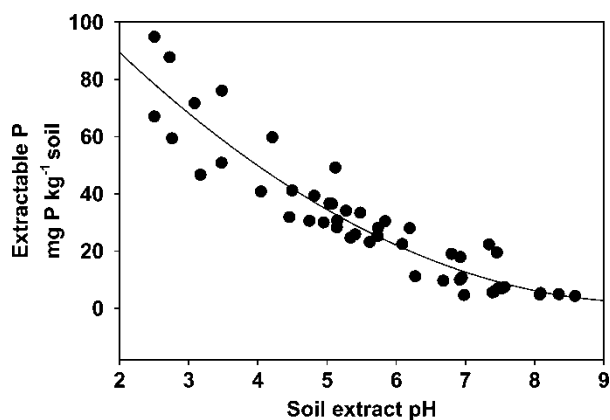
Soil number	Soil series	% Clay content	% Soil organic C	Soil pH
1	Perkins	15	0.59	4.7
2	Pella	32	1.37	5.4
3	Ellis	15	2.54	5.7
4	Chelsea	12	0.83	5.9
5	Bresser	15	3.19	5.9
6	Mardin	34	2.17	6.0
7	Kichatna	11	2.58	6.0
8	Hagerstown	34	1.78	6.1
9	Platner	23	0.95	6.1
10	Berks	31	2.41	6.2
11	Gilford	15	2.13	6.2
12	Anton	23	0.94	6.2
13	Lindon	23	1.00	6.3
14	Rosetta	22	1.11	6.3
15	San Ysidro	20	0.52	6.3
16	Leland	13	0.59	6.3
17	Morocco	10	0.57	6.4
18	Belmond	18	1.84	6.6
19	Chelsea	12	0.17	6.7
20	Griffy	17	1.12	6.9
21	Wheatwood	20	1.32	7.4
22	Beckman	42	0.96	7.6
23	Houston pasture	59	1.77	7.8
24	Ardep	6	0.78	8.0
25	Casa grande	13	1.08	8.0
26	Houston pasture fertilized	50	1.80	8.0
27	Quinlan	12	0.35	8.2
28	Houston con-till corn	55	1.38	8.2
29	Weswood con-till corn	28	0.72	8.3
30	Pratt	13	0.56	8.3
31	Weswood con-till sorghum	28	0.37	8.3
32	Houston no-till corn	52	1.64	8.4

an extractant pH of 2.6 for the four acid soils and decreased to less than 15 ppm at an extracting pH of 8.5 (Figure 1). The pH of the extracting solution had a considerable impact on the amount of extractable P. In addition, we included two soils, one with a soil pH of 8.2 (low organic C, low nutrients) and one with a soil pH of 6.5 (high organic C, high nutrients), and repeated the process of manipulating the extractant pH to demonstrate the impact of extractant pH on extractable phosphorus. The pH

Table 2. Manipulation of soil-extracting solution pH by varying organic acid and lithium citrate concentrations

Extractant	Ingredients: dissolved in 1 l	pH
1	5.0 g lithium citrate	9.0
2	2.5 g lithium citrate	8.0
3	5.0 g lithium citrate, 0.25 g malic acid	7.5
4	5.0 g lithium citrate, 0.5 g malic acid	7.0
5	2.5 g lithium citrate, 0.5 g citric acid	6.5
6	5.0 g lithium citrate, 0.5 g malic acid, 0.5 g oxalic acid	6.0
7	5.0 g lithium citrate, 0.5 g malic acid, 0.5 g oxalic acid, 0.5 g citric acid	5.5
8	2.5 g lithium citrate, 0.5 g citric acid, 0.5 g malic acid, 0.5 g oxalic acid	4.5
9	1.5 g lithium citrate, 0.5 g citric acid, 0.5 g malic acid, 0.5 g oxalic acid	4.2
10	1.5 g lithium citrate, 0.75 g citric acid, 0.75 g malic acid, 0.75 g Oxalic acid	3.5
11	0.5 g citric acid, 0.5 g malic acid, 0.5 g oxalic acid	2.6
12	0.75 g citric acid, 0.75 g malic acid, 0.75 g oxalic acid	2.35

6.5 soil increased from 20 ppm extractable P to 95 ppm as the extractant pH decreased, while the soil pH 8.2 increased from 2 ppm extractable P to 3.8 ppm as the extractant pH decreased (Figure 2). These results indicate that we can manipulate the extractable phosphorus by increasing or decreasing the pH of the soil extractant. Because Mehlich 3 and Olsen extract at such low and high pH, respectively, it seems that extracting the soil near the pH (more

**Figure 1.** Impact of soil extract pH (2.4–9.0) on extractable P from four acid soils (pH 5.5–6.5).

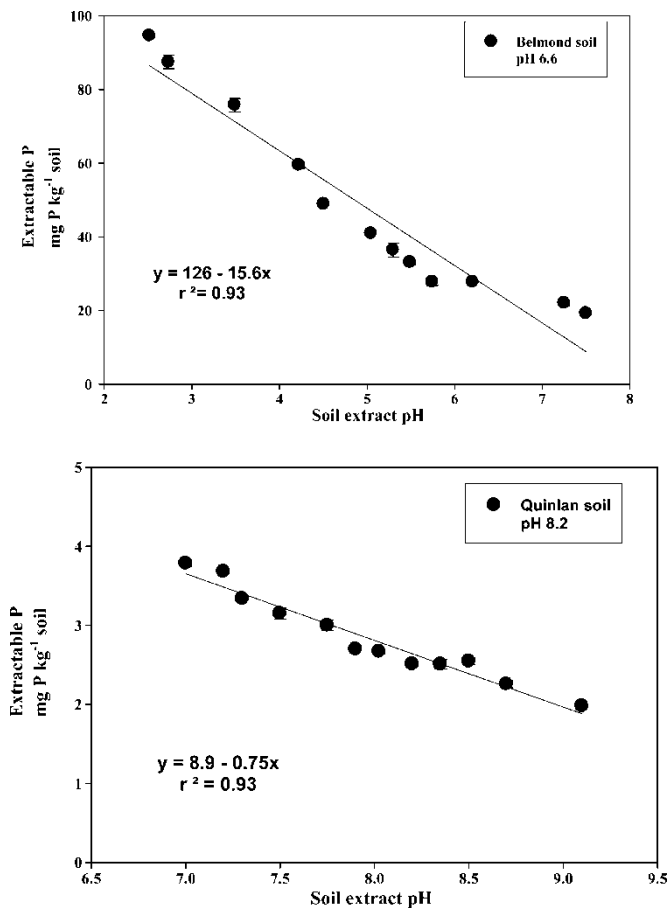


Figure 2. Effect of changing extract pH on extractable P from a low- and high-pH soil (changes described in Table 1). Error bars indicate one standard deviation.

accurately representing field conditions) of the soil would increase the accuracy of estimating plant-available phosphorus.

The soil extractants we tested differed in their ability to extract soil P depending upon the soil pH and the pH of the extractant. The effect of extractant pH on extractable P is marked by the acidity of the extractant. For example, Mehlich 3 releases significantly more P than the other three extractants we tested because of its ability to dissolve iron, aluminum, and calcium phosphates (Nelson, Mehlich, and Winters 1953). The range of extractable P from Mehlich 3 was 0–80 ppm with a mean of 34.3, H³A 0–40 ppm with a mean of 16.6, Olsen 0–20 ppm with a mean of 9.9, and water 0–10 ppm with a mean of 3.3 on the same soils. Using 3D graphing and comparing soil pH, soil extract pH, and extractable P reveals an interesting picture of the interaction

between the three components (Figure 3). Mehlich 3 extracted soil P in the 2.9–4.2 pH range (mean = 3.4) regardless of the soil pH value and extracted more than twice as much P as H^3A , almost four times that of Olsen and eight times as much as water. H^3A and water extracted P in the 5.0–8.5 pH range (H^3A mean = 6.2, water mean = 6.7); however, H^3A tended to produce soil extracts from acid to neutral soils in the 5.0–5.5 range until soil pH increased above 7.5 where soil extracts had higher pH values (6.5–8.0), demonstrating an increased sensitivity to soil pH. The pH of the water extract followed the soil pH very closely, as we would expect. The Olsen soil extract pH range was 8.3–9.0 (mean = 8.6) regardless of soil pH and was completely opposite Mehlich 3 in extract pH (Figure 3). Interestingly, H^3A is almost exactly between Mehlich 3 and Olsen in soil extract pH and extractable P. In the soils we tested, water would be the ideal extractant to extract nutrients near the soil pH; however, the water-extractable P among

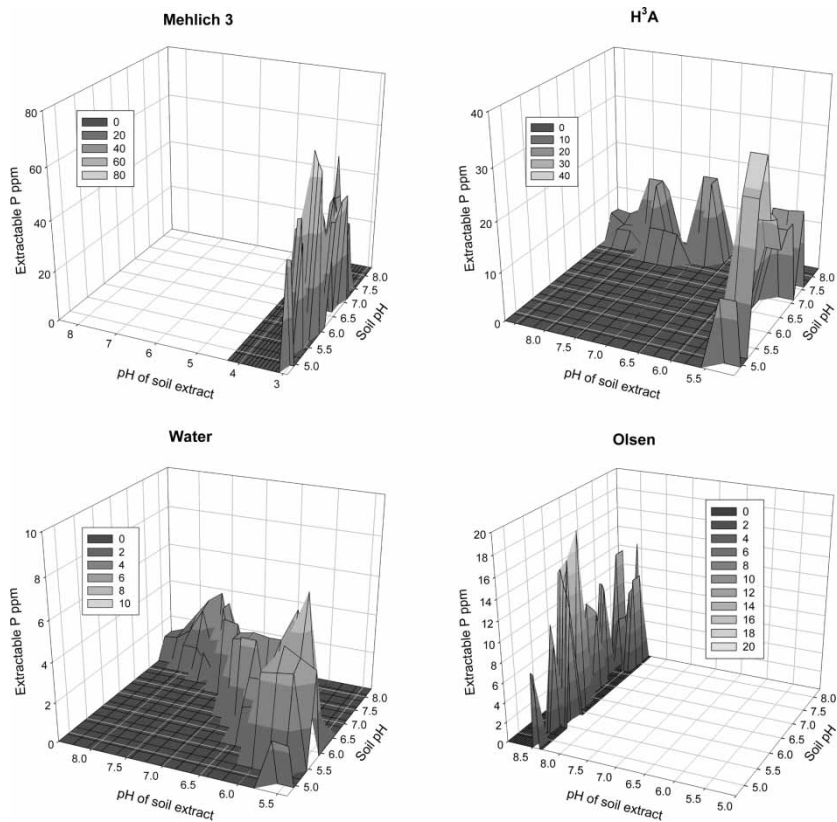


Figure 3. Interaction of soil pH and pH of soil extract on soil phosphorus from the four different extractants.

different soils is usually quite low and only moderately correlated with Olsen ($r = 0.63$) and Mehlich 3 ($r = 0.52$) extractable P (Table 2).

Water-extractable P was significantly correlated with Mehlich 3, Olsen, and H³A (Table 3), strongest with H³A and weakest with Mehlich 3. Mehlich 3 and Olsen were better correlated with each other than Mehlich 3 and H³A, although the differences were slight ($r = 0.84$ vs. $r = 0.83$). H³A extracted roughly half the P as Mehlich 3. Olsen extracted roughly one-fifth the P as that of M3. Olsen extractable P had the best relationship with H³A followed by Mehlich 3 and water. Although good correlations are observed between all four extractants, the concentration of extractable P varies greatly with each extractant.

Based on the chemical composition of Mehlich 3, H³A, and Olsen, soil-extractable P may be defined based on acidity or alkalinity of the extractant and the ability of the extractant to respond to soil conditions. Using Mehlich 3 on calcareous soils may overestimate available P, and using Olsen on acid soils may underestimate available P because of the buffering capacity of the soil. Water-extractable P does not take into account the action of plant root exudates upon soil P, whereas H³A better simulates the soil solution when plants are present without artificially driving the extract pH too low (Mehlich 3) or too high (Olsen). The data in Figure 4 illustrate the effect of extract pH to extractable P. Figure 4a shows the proximity of the soil extractant pH to the actual soil pH (0-line). The soil pH becomes more alkaline as the samples move from left to right (Figure 4a). As the soil pH increases, the Mehlich 3 extract pH deviates further from the original soil pH. The average of soil extract pH deviation for the 32 soils we tested using Mehlich 3 was 3.4pH units away from the soil pH. Olsen is just the opposite; soil extract pH is over 3pH units from the soil pH for low pH soils and slowly becomes more similar to the soil pH for high pH soils, with an average of 1.8pH units from the soil pH. Water and H³A extract pH tend to be close to the soil pH; however, H³A deviates as much as 1.5 units at soil pH of 7.5. H³A contains both dilute organic

Table 3. Correlation matrix for extractable P by various solutions and their associated regression equations

	Olsen (y)	Water (y)	H ³ A (y)
Mehlich 3 (x)	0.84*** $y = 2.9 + 0.2x$	0.52*** $y = 1.2 + 0.06x$	0.83*** $y = 1.1 + 0.45x$
Olsen (x)		0.63*** $y = 0.14 + 0.33x$	0.84*** $y = -2.3 + 1.9x$
Water (x)			0.71*** $y = 6.2 + 3.2x$

***Indicates $p < 0.001$.

Notes: (x) and (y) are for the regression equations. N = 64 (32 soils, 2 reps).

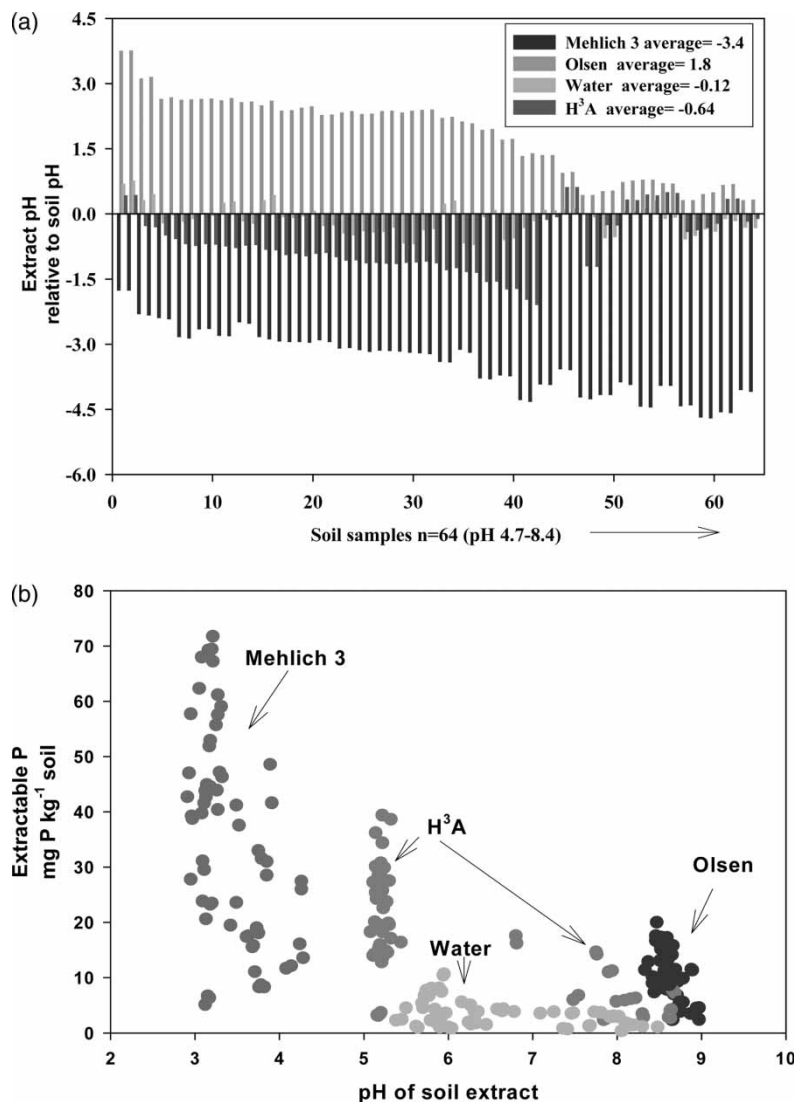


Figure 4. Proximity of extractant pH to soil pH and impact on extractable P. (a) Soil extract proximity to soil pH; (b) Soil phosphorus study soil pH 4.7–8.5, 32 soils, 4 extractants, and 2 reps.

acids and lithium citrate. Lithium citrate alone (5 g) has an extractant pH of 8.4; however, when 1.5 g of acid are added in addition to the lithium citrate, the pH falls to 5.5 (extractant 7, Table 1). Therefore, the acids dominate the extract pH until soil pH reaches 7.5 and above, where the alkalinity of the soil overwhelms the organic acid concentration and the extract pH in soil

increases sharply and is similar to the soil pH. Conversely, the lithium citrate appears to keep H³A from acidifying the soil as strong as Mehlich 3 does, because the organic acids alone in H³A have an extractant pH of 2.6 (extractant 11, Table 1). These factors make H³A more flexible in extracting P near the soil pH, but extractable P is much higher than for water. The average proximity of extract pH to soil pH is 0.64 for H³A and 0.12 for water (Figure 4a). H³A extract pH was within one pH unit of the soil pH for 86% of the 32 soils, water 100%, Olsen 23% and Mehlich 30%.

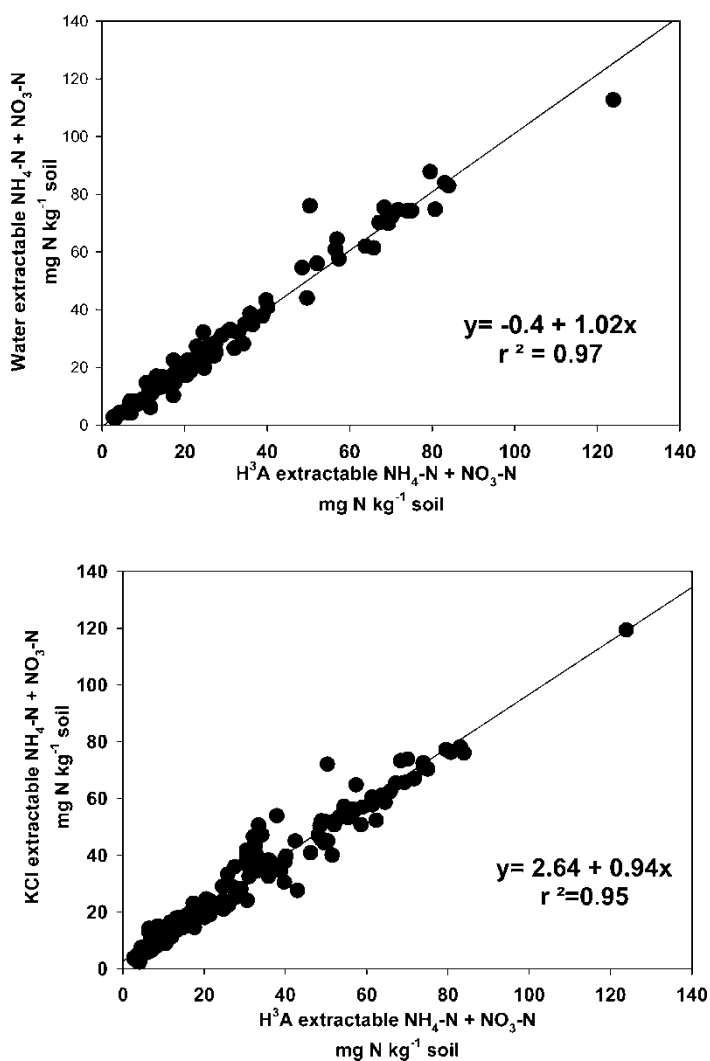


Figure 5. Relationships for soil extractable NH₄ plus NO₃ from H³A compared to 1 M KCl and water.

Soil-extractable ammonium and nitrate were highly correlated between H³A and water and H³A and 1 M KCl ($r^2 = 0.97$ and 0.95 , respectively) (Figure 5). H³A and water consistently extracted 5–10% more nitrate than KCl; however, KCl usually extracted more ammonium than H³A or water. When added together, the extractable NH₄ + NO₃ was nearly identical for the three extractants, with H³A extracting slightly more nitrogen than water but slightly less than KCl (Figure 5). The pH of the soil extractant using KCl, water, and H³A had no significant effect on the extracting ability of soil NH₄ and NO₂/NO₃ (data not shown).

CONCLUSIONS

The soils used for this preliminary test of a new soil extractant based on organic acid root exudates had a wide range of soil pH, organic C, and clay content. H³A was highly correlated with soil-extractable inorganic N from both water and 1 M KCl, as well as being highly correlated with water, Mehlich 3, and Olsen extractable P. These results indicate that H³A may be used as a limited multinutrient (inorganic N and P) extractant, which would eliminate the need for two extractants to test for plant-available NH₄, NO₃, and P. Because soil-extractable P is highly influenced by soil pH and pH of the soil extract, extracting soil within one unit of the soil pH would be a desirable attribute of a soil extractant. On average, H³A extract pH was within one pH unit of the soil pH for 86% of the 32 soils, water 100%, Olsen 23%, and Mehlich 30%. Based on this data, extracting near the soil pH could provide a more reliable estimate of plant = available inorganic N and P without overestimating soil P on calcareous soils and underestimating P on acid soils.

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