

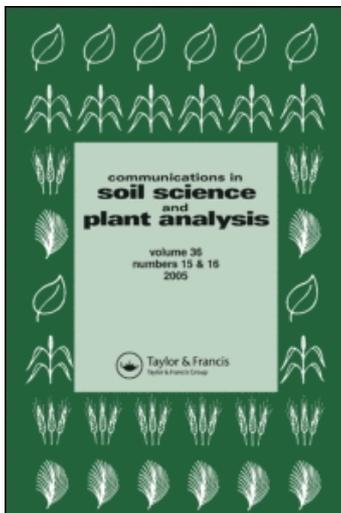
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Modifications to the New Soil Extractant H3A-1: A Multinutrient Extractant

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A new soil extractant (H3A-1) with the ability to extract ammonium (NH₄) nitrogen (N), nitrate (NO₃) N, and phosphorus (P) from soil was originally developed and tested against 32 soils (Haney et al. 2006). H3A-1 is composed of organic acid root exudates, which are naturally occurring, and lithium citrate. In this study, H3A-1 was modified to reduce the extractable iron and aluminum and improve the nutrient extracting relationships with other well-known soil extractants. Correlations show improved relationships with NO₃, NH₄, PO₄, P, potassium, calcium, and zinc when compared to the original H3A-1 as well as standard soil-test methods [Olsen, potassium chloride (KCl), water, Mehlich 3, Bray 1, ammonium acetate (NH₄ OAc), and diethylenetriaminepentaacetic acid (DTPA)]. The modifications were tested against the results from the North American Proficiency Testing (NAPT) database, which included 60 soils, varying in pH, organic carbon, and clay content.

Keywords Organic acids, pH, root exudates, soil extractant

Introduction

Because of the recent increase in both fertilizer prices and environmental concerns, the need for efficient use of fertilizer has increased. Soil-testing methods that more accurately determine available nutrients can provide the means for more efficient use of fertilizers by increasing the accuracy of fertilizer recommendations. For example, nitrogen (N) analysis and N recommendations can include both ammonium (NH₄) N and nitrate (NO₃) N, instead of simply determining NO₃-N, because both forms of N are available to plants. Commonly used soil extractants for N include potassium chloride (KCl) and water. Greater concentrations of NH₄-N are observed using the KCl extract when compared to the water extract because of the ability of potassium (K⁺) to replace NH₄-N on soil exchange sites.

Few of the soil extractants currently available are capable of accurate multinutrient extraction without sacrificing accuracy for one compound or another (Holford 1980). In the case of soil phosphorus (P), many different extractants are used to determine

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plant-available P, depending upon the nature of the soil being tested. For example, Mehlich 3 is a widely used multinutrient extractant because of its ability to extract a number of nutrients with the exceptions of $\text{NH}_4\text{-N}$, $(\text{NO}_2)\text{N}$, and $\text{NO}_3\text{-N}$, and was primarily developed for neutral to acidic soils (Mehlich 1984). Mehlich 3, however, is commonly used on soils with a wide range of pH values (basic to acidic). The effect of extractant pH on extractable P is marked by the acidity of the extractant; therefore, Mehlich 3 releases significantly more P than other extractants because of its ability to dissolve iron (Fe-), aluminum (Al-), and calcium (Ca)-associated phosphates (Nelson, Mehlich, and Winters 1953). The Olsen extractant was developed primarily to extract P from calcareous soils (Olsen et al. 1954).

The results from the Mehlich 3 and Olsen extractants are incorporated into a P index (from low to very high) to determine the probability of a response to P fertilizer, in lieu of actually determining the available P directly. The results of these extractants are presented in parts per million of P and are accompanied by a fertilizer recommendation, which is often handed directly to a fertilizer dealer. No interpretation of how the fertilizer P recommendation was determined is normally provided to the producer. It would be beneficial to the producer if he or she could calculate his or her own fertilizer recommendations based on soil-test P, crop, and desired yield. This process would give the producer a better understanding of the quantities of fertilizer that are needed because he or she will be given an estimate of P and N from soil-test data and can relate this information to his or her own intimate knowledge of specific field conditions.

Soil phosphate is a difficult nutrient to estimate given that soil pH and P solubility are highly interrelated (Golterman 1998; Sharply 1993). Because soil pH has a strong impact on soil-solution chemistry, extracting soils with highly buffered soil extractants such as M3 or Olsen seems unrealistic. In other words, driving the soil extractant solution chemistry to roughly pH 3 (Mehlich 3) or pH 8.5 (Olsen) regardless of soil pH may not represent a true estimate of soil-test P. In the case of Mehlich 3, the strong acid solution can solubilize relatively insoluble fractions of Ca-, Al-, and Fe-associated P (Ketterings et al. 2002). The need for rapid analysis in soil-testing laboratories has resulted in the use of acidic and alkaline extractants applied to soil regardless of soil pH. This practice renders false-positive P values when insoluble P compounds are dissolved.

The development of a soil extractant that can extract plant nutrients near the soil pH is a desirable trait, especially if the extractant utilizes organic acids that are naturally exuded from plant roots to the soil solution to acquire necessary nutrients (Rengel 2002; Baudoin, Benizri, and Guckert 2003). Plants have the ability to increase production of these root exudates to overcome nutrient deficiencies such as P, Fe, zinc (Zn), and manganese (Mn) (Azaizeh et al. 1995; Rengal 1997, 2002; 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). The mechanism for increased P availability from plant-root exudates is mediated by a decrease in soil pH at the plant root-soil interface, which induces ligand exchange, dissolution, and binding to exchange sites by organic acids exudates that release ligand-bound P to the soil solution (Gerke 1992). Many organic acids exuded by plant roots have been identified. The three common to many plant species such as corn, wheat, and sorghum are malic, citric, and oxalic acid (Hinsinger 2001).

H3A was developed to mimic the plant root environment by using organic acids that are exuded by plants to acquire nutrients at a solution pH similar to that of the soil pH. These organic acids are easily neutralized by calcareous soils, which allow the soil pH to dominate the pH of the extractant solution in soil (Haney et al. 2006).

Because the findings of the original paper were published in 2006 (Haney et al.), some soil extracts (roughly 5%), for reasons not yet understood, did not have a visually clear supernatant (H3A-1). These few samples also showed elevated Fe, Al, and P concentrations as compared to other soil extractants when the H3A-1 extracts were analyzed using inductively coupled plasma (ICP). To resolve or clarify this issue, during the course of 2 years, the extractant has been modified by comparing and selecting more appropriate combinations of chemicals, shaking times, centrifuge times, and filter papers. Hundreds of soil samples were analyzed using various combinations and compared to the results obtained using standard published soil-test methods along with results from the North American Proficiency Testing (NAPT) database. The final result of these many modifications is presented in this article.

Our objective was to test modifications to the H3A soil extractant to improve the relationships with currently used soil extractants and possibly eliminate interferences caused by soil particles remaining in solution after extractions. Eliminating the suspended soil particles in the supernatant should effectively remove elevated readings of available Al, Fe, and P from slightly dirty extracts because these soil particles are destroyed during ICP analysis. The results of modifications to the H3A extract were compared to the original H3A extractant and against nutrient values obtained from NAPT database from 60 soils.

Materials and Methods

Soils were collected from the NAPT database using soil ID 101-120 from years 2005, 2006, and 2007 for a total of 60 soils samples from the continental United States. The soils had a wide range in soil pH (4.5–8.6), extractable Ca (148–7199 mg Ca kg⁻¹ soil), organic carbon (C) (0.38–7.51%), and clay content (1.6–32.8%). The tables consist of the results from the NAPT database for Mehlich 3, Olsen, water, diethylenetriaminepentaacetic acid (DTPA), and ammonium acetate (NH₄Oac).

The three organic acids (oxalic, malic, and citric) that plants most commonly use to overcome deficiencies of various nutrients (Rengel 2002; Baudoin, Benizri, and Guckert 2003; Shenker, Hadar, and Chen 1999) are contained in the H3A extract. The acidic nature of the extract is balanced by the lithium citrate, which acts as a weak buffer. Lithium citrate can work alone for the determination of extractable P in calcareous soils (similar to Olsen). 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. The aim was to create a soil extractant based on organic acids (citric and oxalic acid are chelators) and lithium citrate that would extract nutrients near the soil pH; therefore, the final combination of chemicals was based on extractant pH. The new extractant was designated “H3A” (Haney et al. 2006). The original (H3A-1) extractant contained the following:

- 5 g/L lithium citrate
- 0.5 g/L citric acid
- 0.5 g/L malic acid
- 0.5 g/L oxalic acid
- 0.25 g/L EDTA
- 0.25 g/L DTPA

H3A-1 was modified to eliminate the ethylenediaminetetraacetic acid (EDTA) and DTPA, reduce the concentrations of lithium citrate, malic and oxalic acids, and increase

the concentration of citric acid. The resulting extractant (H3A-2) is composed of the following:

- 2 g/L lithium citrate
- 0.6 g/L citric acid
- 0.4 g/L malic acid
- 0.4 g/L oxalic acid

The result of these changes is a clean supernatant after centrifugation and filtration and a reduction in the pH of the extractant from 5.0 to 4.4.

Four g of soil were placed into 50-mL conical-bottom centrifuge tubes. The samples were extracted with 40 ml of H3A-1 or H3A-2. The samples were shaken for 30 min (H3A-1) and 5 min (H3A-2), centrifuged for 5 min, and filtered through Whatman 2V fluted filter paper (Whatman, Kent, U.K.). The samples were analyzed using an OI Analytical rapid flow analyzer (RFA, colorimetric) for $\text{NH}_4\text{-N}$, $\text{NO}_3\text{-N}$, and phosphate ($\text{PO}_4\text{-P}$) and a Varian Vista-MPX axial flow ICP (Varian Inc., Palo Alto, Calif.) for Ca, P, K, Zn, Fe, and Al. The results of these analyses were compared to the $\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$ concentrations from the NAPT database for 2 M KCl. Potassium data from extraction with H3A-1 and H3A-2 were compared to the K concentrations from the NAPT database for ammonium acetate ($\text{NH}_4\text{-OAc}$). Phosphorus results from H3A-1 and H3A-2 were compared to P concentrations from the NAPT database for water, Bray 1, Mehlich 3, and Olsen. Mehlich 3-, H3A-1-, and H3A-2-extractable Ca were also compared. DTPA (Lindsey and Norvell 1978) concentrations from the NAPT database for Zn and compared to Zn concentrations in H3A-1 and H3A-2 extracts.

Results and Discussion

The modified extractant (H3A-2) averaged 12% more inorganic N (NH_4 and NO_3) than the original (H3A-1) based on the 60 NAPT samples. The average of the combined inorganic N was $30.03 \text{ mg N kg}^{-1}$ soil for H3A-1 (original) and $33.92 \text{ mg N kg}^{-1}$ for H3A-2 (modified). Linear regression showed a strong relationship between the extractants with a coefficient of determination (r^2) of 0.97 (Figure 1). Inorganic N values from the H3A-2 extract had a slightly better relationship ($r^2 = 0.97$) to KCl-extractable inorganic N than H3A-1 with an r^2 value of 0.94 (Figures 2 and 3).

H3A-2 extracted 20% more K than water, and the results were highly related ($r^2 = 0.83$, data not shown). H3A-1 extracted 25% more K than water but had a weaker relationship with water-extractable K ($r^2 = 0.68$, data not shown). The correlation between $\text{NH}_4\text{-OAc}$ -extractable K and H3A-1-extractable K ($r^2 = 0.69$, Figure 4) was improved with the modified extract, H3A-2 ($r^2 = 0.95$, Figure 5). Both H3A-1 and H3A-2 extract roughly three times less K than $\text{NH}_4\text{-OAc}$. This low extractable-K concentration indicates that H3A-2 may extract the K present in the soil solution and extracts only small portions of exchangeable K from cation exchange sites due to the activity of H^+ and Li^+ and most likely no K from within the clay lattice. Therefore, if H3A-2 were to be used for K fertilizer recommendations, the K extracted should only be considered as a fraction of K that is comparable to currently used soil extractants.

The pH of the H3A-2 supernatant is highly correlated with the amount of H3A-2 extractable Ca (Figure 6). The linear increase in extractable Ca and extractant pH is an indication that the extracting power of H3A-2 is directly related to the amount of Ca, which explains H3A-2's sensitivity to soil pH. Because H3A-2 is weakly buffered by its

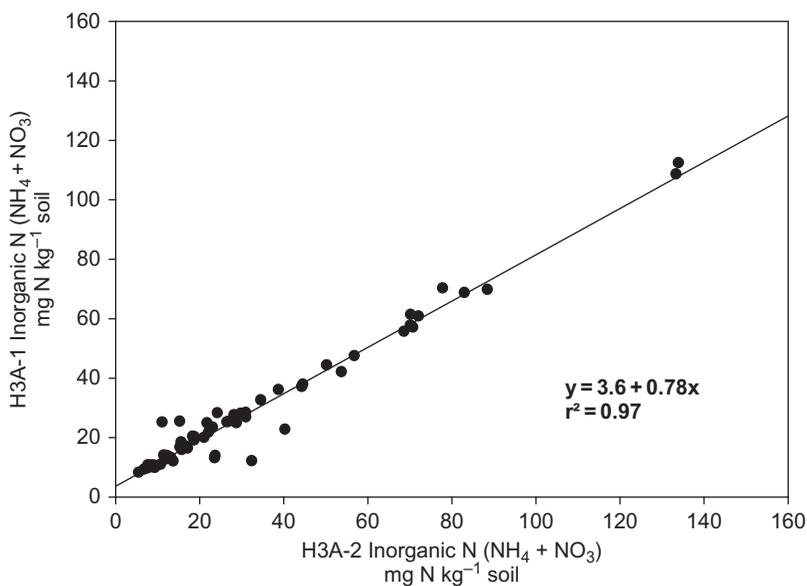


Figure 1. Relationship between inorganic N using the H3A-2 (modified) and H3A-1 (original) extractants from 60 NAPT samples.

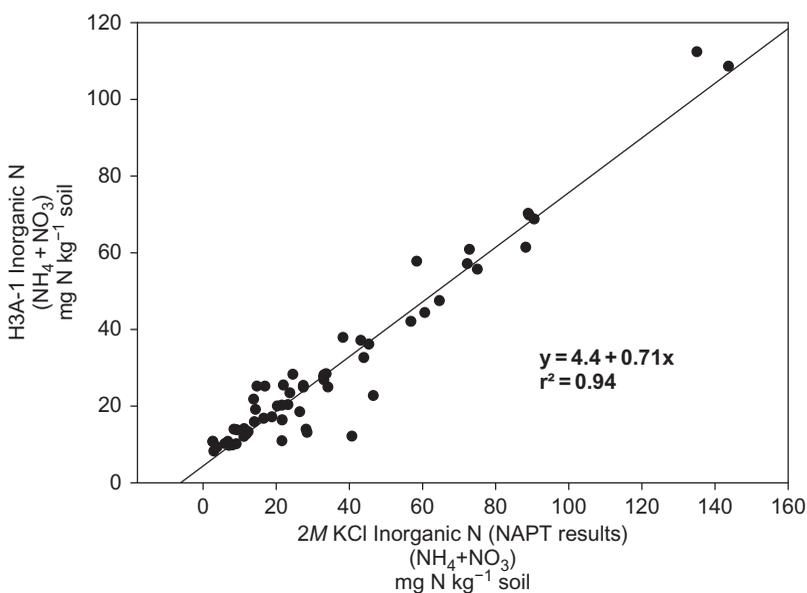


Figure 2. Relationship between 2 M KCl-extractable inorganic N and H3A-1-extractable inorganic N from 60 NAPT samples.

organic acids, when free CaCO₃ is present, the supernatant pH increases to near the soil pH and therefore should not overestimate extractable P.

The amount of P extracted from soil is dependent upon soil pH, clay content, and concentrations of Ca, Fe, and Al (Cox 2001). Extractable P is strongly influenced by soil

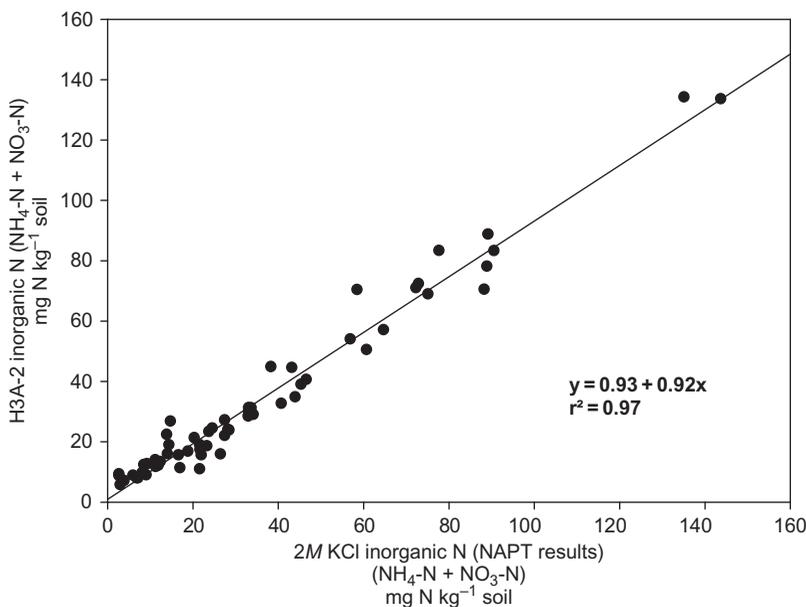


Figure 3. Relationship between 2 M KCl-extractable inorganic N and H3A-2-extractable inorganic N from 60 NAPT samples.

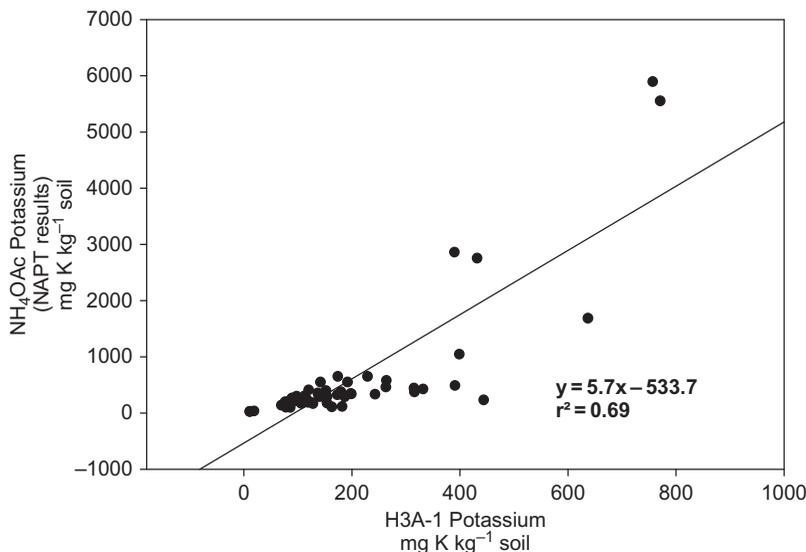


Figure 4. Relationship between H3A-1- and NH₄-OAc-extractable K from 60 NAPT samples.

pH but is also highly influenced by the pH of the soil extractant (Golterman 1998). In the article by Haney et al. (2006), this effect was demonstrated by varying the extractant (H3A-1) pH and analyzing extractable P. Organic acid concentrations were increased to change the extractant pH from 2.6 incrementally to 9.0. Extractable P increased as the extractant pH decreased from 15 mg P kg⁻¹ soil (at pH 9) to 90 mg P kg⁻¹ soil (at pH 2.6) (Haney et al. 2006).

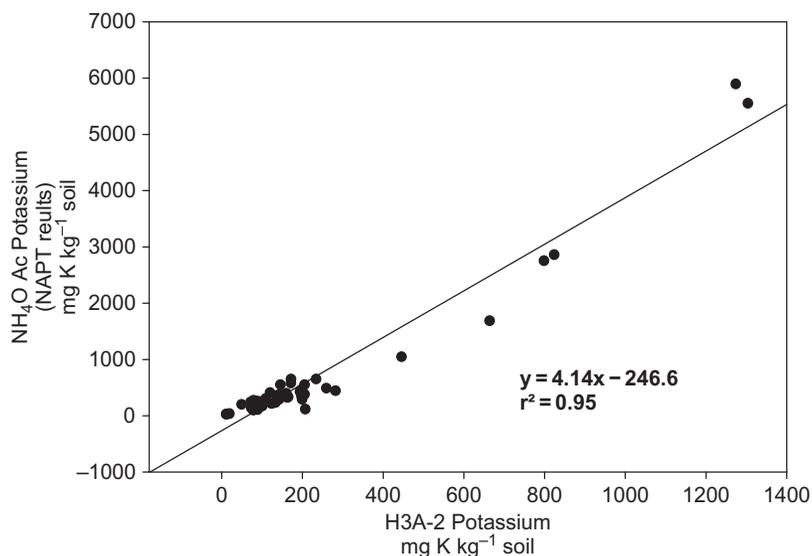


Figure 5. Relationship between H3A-2- and NH₄-OAc-extractable K from 60 NAPT samples.

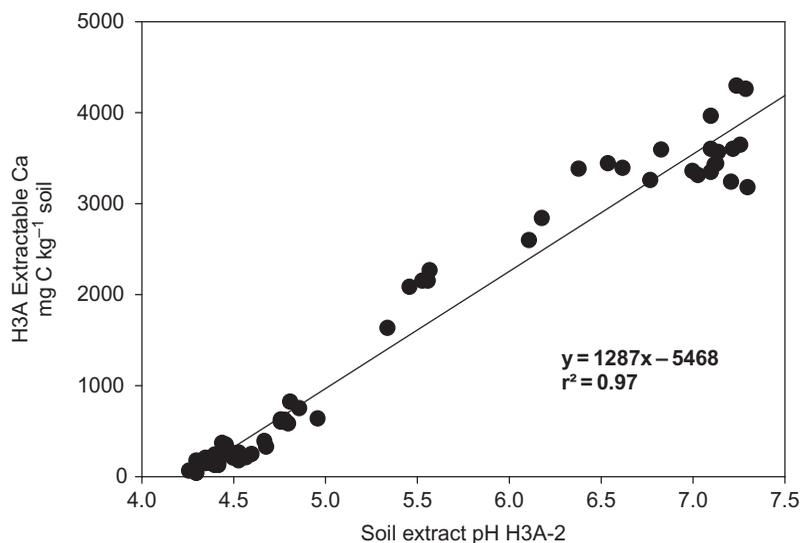


Figure 6. Relationship between the pH of H3A-2 after extraction from 60 NAPT soils compared to H3A-2-extractable Ca.

Based on the chemistry of P availability at varying pH values, it is logical to assume extracting P near the soil pH (more accurately representing field conditions) may increase the accuracy of estimating soil-test P. A soil extractant based on naturally occurring organic acids lowers the extractant pH 1 to 2 units from the soil pH in an acid soil, mimicking the rhizosphere. The organic acids in H3A-2 are neutralized in calcareous soils, driving the extractant pH near the soil pH and mimicking the natural release of organic acids.

H3A-2-extractable P was more strongly correlated with Olsen, Bray 1, Mehlich 3, and water-extractable P than H3A-1-extractable P. H3A-2-extractable P was most strongly

correlated with water-extractable P, followed by Olsen, Mehlich 3, and Bray 1 (Table 1). Averaged across all 60 samples, H3A-2 extracted 86% more P than water and 20% more P than Olsen. H3A-2 extracted 49% less P than Mehlich 3, 35% less P than Bray 1, and 26% less P than H3A-1.

H3A-2 extracted 45% less Al, 53% less Fe, and 26% less P than H3A-1 (data not shown). These results are most likely due to the increased clarity of the H3A-2 supernatant as compared to the H3A-1 supernatant. Soil-test P from ICP analysis of H3A-1 and H3A-2 extracts (Table 2) were virtually identical in their relationship to Mehlich 3 when analyzed on ICP ($r^2 = 0.79$ and 0.80 , respectively). H3A-1 extracted 18% more P than H3A-2. H3A-2 extracted 45% less P than Mehlich 3 as determined using ICP analysis, which is similar to the RFA (colorimetric) analysis results (49% less).

Table 1

Correlation matrix for soil-extractable $\text{PO}_4\text{-P}$ (colorimetric rapid flow analyzer (RFA)) by various extractants from NAPT results (N = 60). Correlation Coefficient, P Value, and Number of Samples are stated.

	Bray 1	Water	Mehlich 3	H3A-2	H3A-1
Olsen	0.83 3.43E-016 60	0.71 0.00000000020 60	0.94 1.42E-029 60	0.82 9.07E-016 60	0.74 0.000000000021 60
Bray 1		0.43 0.00053 60	0.85 6.42E-018 60	0.64 0.000000027 60	0.60 0.00000048 60
Water			0.69 0.0000000013 60	0.86 2.27E-018 60	0.72 0.0000000013 60
Mehlich 3				0.81 7.17E-015 60	0.78 2.21E-013 60
H3A-2					0.92 4.97E-026 60

Average value for 60 samples: Mehlich 3: 71.24, Bray 1: 56.28, H3A-1: 48.9, H3A-2, 36.3, Olsen: 29.16; water: 5.00.

Table 2

Correlation matrix for soil-extractable P (ICP) by various extractants (NAPT samples N = 60). Correlation Coefficient, P Value, and Number of Samples are stated.

	P H3A-2	P H3A-1
P M3	0.80 2.56E-014 60	0.79 3.44E-014 60
P H3A-2		0.98 5.57E-045 60

Average value for 60 samples: Mehlich 3: 78.5 ppm, H3A-1: 52.73, H3A-2: 43.49.

Table 3

Correlation matrix for soil extractable Ca (ICP) by various extractants (NAPT samples N = 60). Correlation Coefficient, P Value, and Number of Samples are stated.

	Ca M3	Ca H3A-2	Ca H3A-1
Ca NH ₄ -OAc	0.95 2.11E-030 60	0.91 1.51E-023 60	0.91 2.87E-023 60
Ca M3		0.93 6.84E-027 60	0.93 9.31E-027 60
Ca H3A-2			0.99 5.04E-058 60

Average value for 60 samples: Mehlich 3: 3414 ppm, NH₄-OAc: 2608 ppm, H3A-1: 2090 ppm, H3A-2: 1547 ppm.

Because H3A-2 extracts 55% less Ca, 63% less Al (data not shown), and 45 to 49% less P than Mehlich 3, it appears that H3A-2 is not dissolving as much Ca-P or Al-P as Mehlich 3. Interestingly, H3A-2 on average extracted 3% more Fe than Mehlich 3 yet 49% less P, which further suggests that the increase in Mehlich 3-extractable P above H3A-2 P is most likely from Al- and Ca-bound phosphates.

The matrix (Table 3) indicates NH₄-OAc Ca and Mehlich 3-extractable Ca are strongly correlated. H3A-1 and H3A-2 are identically correlated to NH₄-OAc Ca or Mehlich 3 Ca, although H3A-2, on average ($n = 60$), extracted 26% less Ca than H3A-1. H3A-2 extracted 41% less Ca than NH₄-OAc, but these parameters were strongly related ($r^2 = 0.91$). H3A-2 extracted 55% less Ca than Mehlich 3, but Ca from these extractants were also highly related ($r^2 = 0.91$).

Extractable Zn from both H3A-1 and H3A-2 was highly correlated to DTPA-extractable Zn (Table 4); however, H3A-1 extracted 2.7 times more Zn than DTPA with

Table 4

Correlation matrix for soil-extractable Zn (ICP) by various extractants (NAPT samples N = 60). Correlation Coefficient, P Value, and Number of Samples are stated.

	Zn H3A-2	Zn H3A-1
Zn DTPA	0.93 3.09E-027 60	0.87 1.19E-019 60
Zn H3A-2		0.84 3.14E-017 60

Average value for 60 samples: DTPA: 1.83 ppm, H3A-1: 4.90 ppm, H3A-2: 1.88 ppm.

an average of 4.9 mg Zn kg⁻¹. The average value of DTPA-extractable Zn was 1.83 mg Zn kg⁻¹ soil and 1.88 mg Zn kg⁻¹ for H3A-2. The greater Zn results from H3A-1 are most likely due to interference from suspended solids in the extract during ICP analysis as well as the greater pH of the extractant (5.0 for H3A-1 as compared to 4.4 for H3A-2).

Conclusions

The 60 soil samples used to test the new modifications to the soil extractant had a wide range of soil pH, organic C, and clay content. The data indicate that H3A-2 is an improvement compared to H3A-1 when used for the estimation of plant-available NH₄-N, NO₃-N, and P, as well as for K, Ca, and Zn. The H3A-2 extractant is visually much cleaner, which resulted in lower Fe, Al, Ca, and P values, which were thought to have been erroneously inflated in some soil samples when using H3A-1. H3A-2-extractable inorganic N (NH₄-N, NO₃-N) is highly correlated with 2 M KCl-extractable inorganic N. H3A-2-extractable P is also highly correlated with water, Mehlich 3, Bray 1, and Olsen-extractable P. These results indicate that H3A-2 may be used as a multinutrient extractant to simultaneously determine inorganic N and P, K, Ca, and Zn. The use of H3A-2 also effectively eliminates the need for two extractants to test for NH₄-N, NO₃-N, and P. The results indicate that H3A-2 soil extractant using naturally occurring organic root exudates may be offered as an alternative to current soil extractants or used in conjunction with them for comparative purposes. This new extractant should be a more reliable estimate of inorganic N and soil test P, without overestimating soil P with a strongly acidic extractant on calcareous soils and underestimating P with an alkaline extractant on acidic soils.

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