

Removal of Lithium Citrate from H3A for Determination of Plant Available P

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Abstract

The soil extractant, H3A, has undergone several iterations to extract calcium (Ca), iron (Fe), aluminum (Al), potassium (K), phosphorus (P), ammonium (NH₄-N) and nitrate (NO₃-N) under ambient soil conditions. Few soil extractants currently used by commercial and university soil testing laboratories can perform multi-nutrient extraction without over- or under-estimating at least one nutrient. Soil pH and plant root exudates have a strong influence on nutrient availability and H3A was developed to mimic soil conditions. Lithium citrate was previously used in the H3A formulation, but resulted in a cloudy supernatant in some samples, complicating laboratory analyses. In this study, we removed lithium citrate and compared the nutrients extracted from the modified (H3A-4) to the established (H3A-3) solutions. We found that the new extractant, H3A-4, produced a clear supernatant even in soils with low pH and high iron and aluminum concentrations. H3A-4 accurately predicts plant available nutrients and is a viable choice for commercial and laboratory settings due to its ease of use.

Keywords

Phosphate, Soil Extraction, Lithium Citrate

1. Introduction

Few currently available soil extractants are capable of multi-nutrient extraction without sacrificing accuracy for one compound or another [1]. Plant-available soil P has been difficult to accurately assess across the naturally occurring pH range of soils (calcareous to acid) with a single extractant because soil pH and P solubility are highly interrelated [2] [3] and soil pH has a strong influence on soil-solution chemistry [4]. Thus multiple extractants have been developed to

determine “plant available” P depending upon the pH of the soil being analyzed. Mehlich 3 [5] was developed for use in neutral to acid soils, while the Olsen extractant was developed primarily to extract P from calcareous soils [6]. Their development demonstrates early awareness of the difficulty of accurately extracting plant available P across such a wide range in natural conditions. Despite its development for neutral to acid soils, Mehlich 3 is commonly used without regard to soil pH. In addition, Mehlich 3 cannot be used for $\text{NH}_4\text{-N}$ and $\text{NO}_2\text{-N}/\text{NO}_3\text{-N}$ analysis.

The need for rapid analysis in commercial and university soil-testing labs contributed to use of soil extractants outside of the soil conditions for which they were developed. Using strong acid extractants such as Mehlich 3 (pH ~3) or alkaline extractants such as Olsen (pH ~8.5) without regard to soil pH can result in over-estimation of plant-available P. As Menon *et al.* (1988) stated “a major disadvantage in using extracting solutions; however, is that they may mobilize not only the phosphate available for plant use but also some otherwise stable and non-mobile soil components.” For instance, Mehlich 3 releases significantly more P than other extractants [7] [8] [9] by dissolving relatively insoluble fractions of Ca-, Al- and Fe-associated P [10]. Menon *et al.* (1988) suggest that deionized water and the anion exchange resin method (FeAlO exchange) do not experience the limitations common with other extracting solutions. While FeAlO exchange is an accurate method for the measurement of plant available P [3] [7], it is slow and cumbersome. In addition, there are well known analytical issues with water extraction, such as cloudy extract resulting in instrument interference. Therefore, few, if any, commercial labs offer FeAlO and water extractions for large scale routine soil analysis.

H3A-1 [11] and its subsequent modifications, H3A-2 and H3A-3 [12], were developed to mimic the plant root environment by utilizing organic acid plant exudates [13] [14] to extract nutrients at ambient soil pH, with the additional benefit of simultaneous N, P and K extraction. Many organic acids exuded by plant roots have been identified. Three exuded by plants species such as corn, wheat, and sorghum are malic, citric, and oxalic acid [15]. These organic acids are used in H3A and have a low buffering capacity, which allows the soil pH to dominate the pH of the extractant solution in soil [11]. The effort to mimic the soil-root environment is important since plants utilize root exudates to overcome P, Fe, Zn, and Mn deficiencies [13] [16] [17]. Ion toxicity and pathogen attack can also stimulate an exudate response from plants [18] [19] [20]. 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 [21]. As the pH of the soil increases, the acids struggle to extract P as the effects of soil calcium take effect, much like in the Texas Houston Black (fine, smectitic, thermic UdicHaplusterts) soils (pH 8.3) of the Blackland Prairie. In the Blackland Prairie, agriculture producers

generally see a crop response to P fertilizer additions. H3A has been able to accurately reflect limited availability of P in these soils as the extracting power of H3A decreases in proportion to increases in soil pH [22]. H3A begins to extract less soil P around pH 7.7 where free CaO_3 is abundant [12].

The original H3A formulation and processing methods were altered to address the occurrence (roughly 5%) of soil extracts that did not have a visually clear supernatant with H3A-1 (Haney *et al.*, 2010). Samples with a cloudy supernatant also had elevated Fe, Al, and P concentrations when analyzed using other well-known soil extractants. To resolve this issue, H3A-1 was modified and tested on 60 soils from the NAPT (North American Proficiency Testing) program with varying pH, organic C, and clay content. The modifications deemed H3A-2 included eliminating the use of EDTA and DTPA and reducing the shaking time from 30 min to 5 min. H3A-2 reduced the extractable Fe and Al concentrations and improved the relationships for extractable NO_3 , NH_4 , PO_4 , total P, K, Ca and Zn with the Olsen, KCl, water, Mehlich 3, Bray 1, NH_4OAc extractants [12].

H3A-2 contained lithium citrate (Li-citrate). The concentration of Li citrate was reduced resulting in the formulation of H3A-3. Because analysis with FeAlO strips are considered to best represent plant available P in soils [3] [7], we compared the relationships between H3A-2 and H3A-3 extractable P with FeAlO P. H3A-3 extractable P was strongly correlated with P from FeAlO strip extraction results ($r^2 > 0.96$, $P < 0.001$) [22].

As we continually endeavor to mimic nature in the lab, it is logical to remove Li citrate since it is not a naturally occurring organic compound. In the past, we made the mistake of synthetically forcing a natural process to conform to a lab method rather than allowing the natural process to drive lab methodology. Lithium citrate buffered organic acids in H3A to stabilize extractant pH and extractable P across a range of soils; however, this process does not occur in the field. Plants control their response to P deficiency by changing the organic acid strength or type of acid they exude into the soil [23]. The objective of this study was to compare extractable P from H3A-3 to extractable P from H3A-3 without Li-citrate (H3A-4). We believe that the removal of Li citrate is a necessary change in inching us ever closer to extracting solutions that mimic plant root and soil interactions.

2. Methods

Extractable P from a total of 106 soils were analyzed with H3A-3 and H3A-3 without Li citrate (H3A-4). Thirty of the soils analyzed are from the North American Proficiency Testing (NAPT) program (<http://www.naptprogram.org>), and the other 76 are from surface soils (approximately 10 cm) gathered in 2015 from throughout the contiguous United States (18 states). The soils analyzed have a wide range of soil pH, clay content, tillage regimes, management and climatic conditions.

The H3A extractant is slightly modified since the published formula included in Haney *et al.* (2016) and is termed H3A-4. The modified formula for H3A-4 is as follows.

3. Liters Deionized Water

- 1.1 grams Malic acid
- 0.7 grams Citric acid and
- 0.45 grams Oxalic acid.

The pH of the resulting extractant is 3.75, and is weakly buffered.

The 106 soils were extracted with H3A-3 and H3A-4 by shaking 4-g samples on a Eberbach shaker with 40-ml extractant for 10 minutes at 120 back and forth movements per minute. All samples were centrifuged for 5 minutes at 3500 rpm at room temperature and filtered through What man 2V filter paper prior to analysis for calcium (Ca), iron (Fe), aluminum (Al), potassium (K) and phosphorus (P) on an axial flow Agilent MP-4200 as well as a Seal Analytical rapid flow analyzer (RFA) for phosphate ($\text{PO}_4\text{-P}$), ammonium ($\text{NH}_4\text{-N}$) and nitrate ($\text{NO}_3\text{-N}$). Data analysis was completed using SigmaPlot ver. 13 (Systat, Inc.) for regression analysis to determine the relationships between H3A-3 and H3A-4 extractable nutrients.

4. Results and Discussion

Soil pH values range from 4.2 to 8.5. Averaged across all 106 samples from ICP analysis, H3A-3 extractable P ranges from 1.0 mg P kg^{-1} soil to 215 mg P kg^{-1} soil with a mean of 36.3 mg P kg^{-1} soil, while H3A-4 extractable P ranges from 1.0 mg P kg^{-1} soil to 227 mg P kg^{-1} soil with a mean of 34.6 mg P kg^{-1} soil. H3A-4 extracted 95.4% of that extracted with H3A-3 from 106 samples (3672 mg P kg^{-1} soil compared to 3849 mg P kg^{-1} soil). H3A-4 extractable P was highly correlated with H3A-3 extractable P ($r^2 = 0.98$, $p < 0.001$, **Figure 1**). The roughly one to one slope is a strong indicator that H3A-4 is an accurate soil extractant for P when compared to H3A-3 (slope = 1.08). These data indicate that H3A-4 is extracting P within roughly 5% of H3A-3.

Analysis of data from the rapid flow analyzer revealed that H3A-4 extractable PO_4 and H3A-3 extractable PO_4 were very similar (24.3 mg PO_4 kg^{-1} soil and 24.8 mg PO_4 kg^{-1} soil, respectively). Extractable PO_4 values were increased slightly when analyzed using the ICP (H3A-3, 36.3 mg P kg^{-1} soil vs H3A-4, 36.6 mg P kg^{-1} soil). Extractable PO_4 from the H3A-4 and H3A-3 extractants had a strong linear relationship ($r^2 = 0.99$, $p < 0.001$) when analyzed using the colorimetric method for $\text{PO}_4\text{-P}$ also (**Figure 2**). Note the slope is similar to that from **Figure 1** (1.08 vs 1.05) indicating strong correlation between both machines (extractable P from ICP vs. extractable PO_4 from RFA).

Extractable $\text{NO}_3\text{-N}$ was almost identical between the two extractants, which was expected since $\text{NO}_3\text{-N}$ is considered water soluble ($r^2 = 0.99$, $p < 0.001$, **Figure 3**). **Figure 4** depicts the strong relationship between $\text{NH}_4\text{-N}$ extracted from

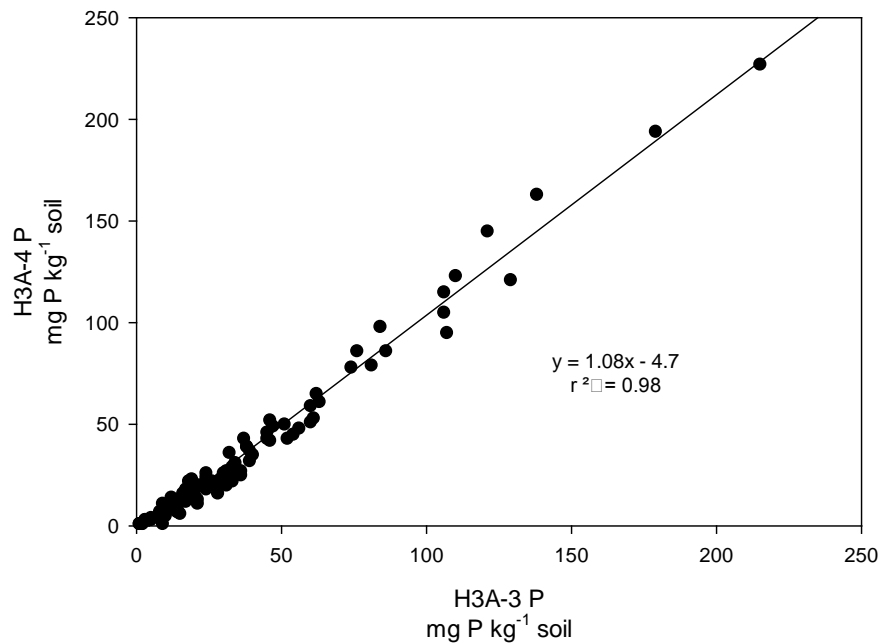


Figure 1. Linear relationship between P extracted with H3A-3 vs. H3A-4 from ICP analysis $P < 0.001$. The removal of licit rate slightly increased the amount of P.

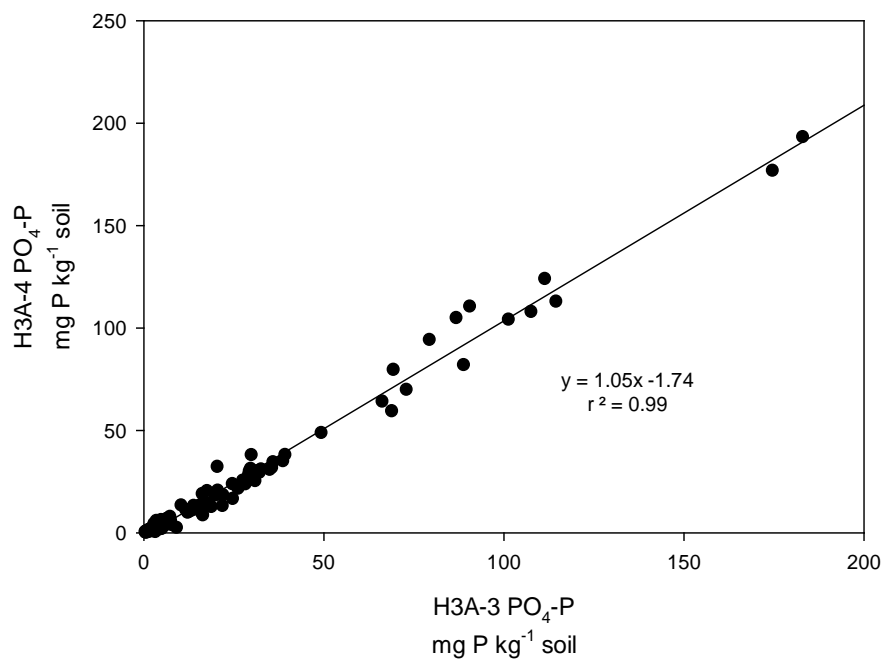


Figure 2. Linear relationship between PO_4 extracted with H3A-3 and H3A-4 from colorimetric method $P < 0.001$. Note the slope is similar to that from **Figure 1** (1.08 vs 1.05) indicating strong correlation between both machines (ICP vs. colorimetric).

H3A-3 and H3A-4 ($r^2 = 0.99$ $p < 0.001$). **Figure 5** illustrates the same data with the highest $\text{NH}_4\text{-N}$ value removed, demonstrating that the correlation holds well at lower levels of extractable $\text{NH}_4\text{-N}$ ($r^2 = 0.99$ $p < 0.001$). These data also indicate a slight increase in extractable $\text{NH}_4\text{-N}$ with H3A-4 compared to H3A-3.

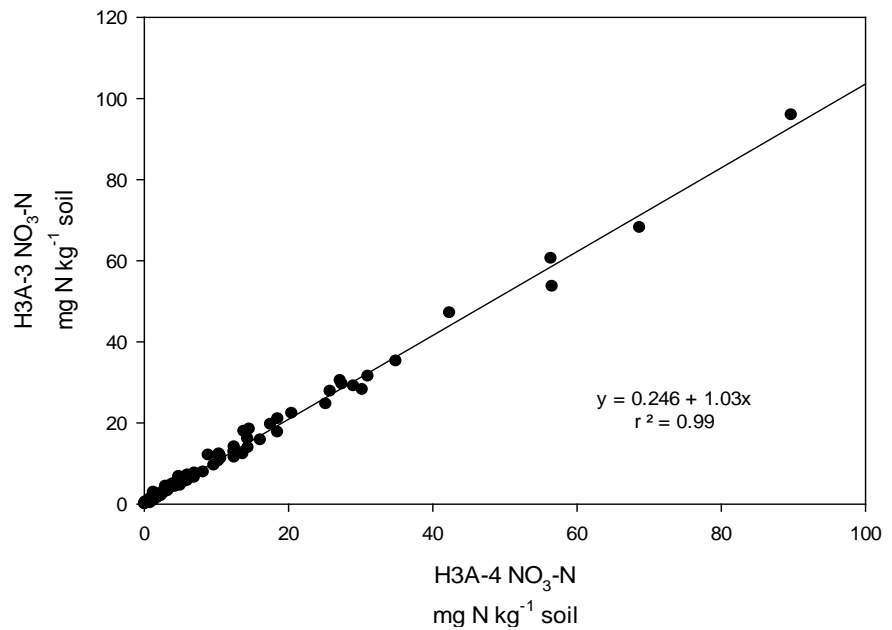


Figure 3. Linear relationship between $\text{NO}_3\text{-N}$ extracted with H3A-4 and H3A-3P < 0.001.

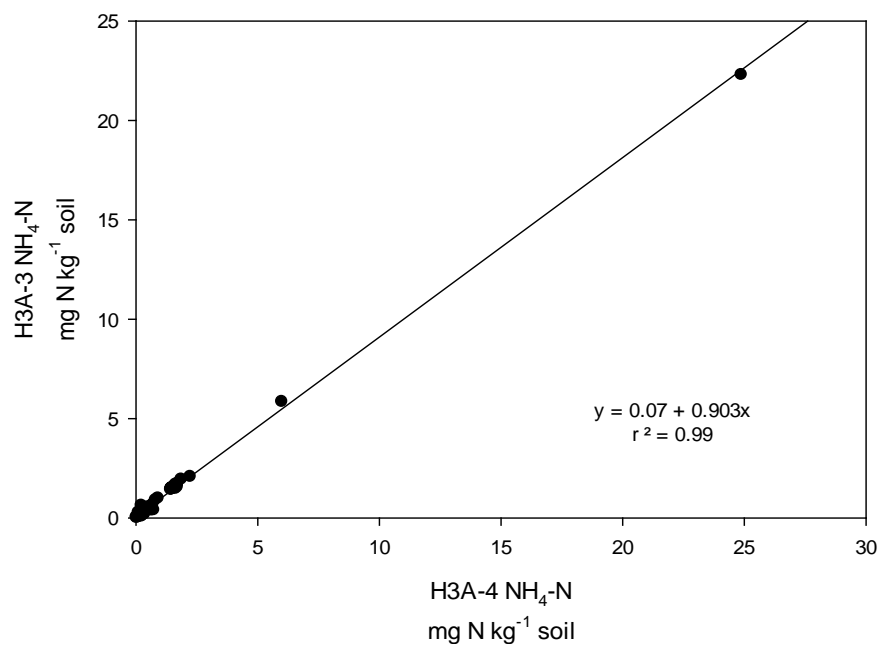


Figure 4. Linear relationship between $\text{NH}_4\text{-N}$ extracted with H3A-4 and H3A-3P < 0.001.

Two samples had distinctly increased Al values when extracted with H3A-3 compared to H3A-4. H3A-4 greatly reduced the extractable Al of the two samples from 1420 mg Al kg^{-1} soil to 160 mg Al kg^{-1} soil (point 6a, **Figure 6**) and from 1300 mg Al kg^{-1} soil down to 200 mg Al kg^{-1} soil (point 6b, **Figure 6**). With the outlier samples removed, regression analysis indicates that H3A-3 is displacing twice the amount Al compared to H3A-4 ($y = 1.8x - 9.5$). The correlation between H3A-3 and H3A-4 extractable Al was improved from $r^2 = 0.42$ to $r^2 = 0.80$ with the two extreme samples removed. The relationship between H3A-3

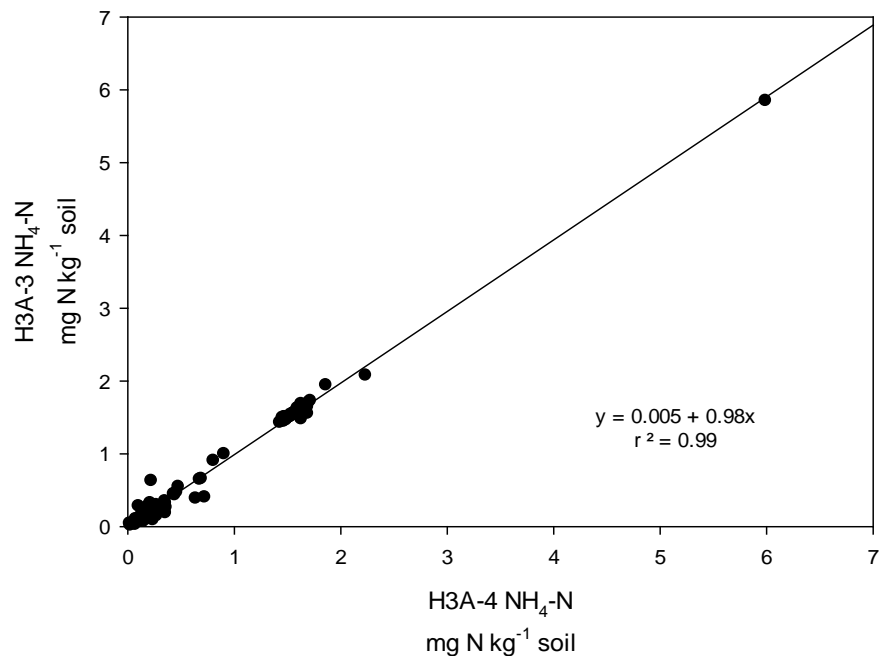


Figure 5. Linear relationship between ammonium extracted with H3A-3 and H3A-4P < 0.001 with the high sample removed from **Figure 4**. This figure demonstrates that the correlation holds even with the high sample eliminated from the relationship.

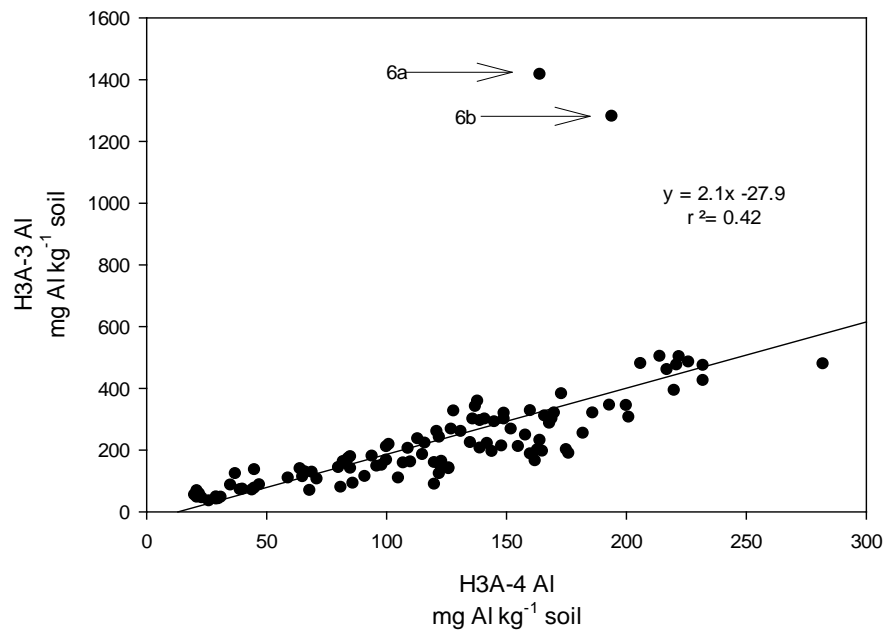


Figure 6. Linear relationship between Al extracted with H3A-3 vs. H3A-4 from ICP analysis $P < 0.001$. The arrows point to the 2 samples that cleared after the removal of lithium citrate (**Figure 8**).

and H3A-4 extractable Fe was very similar to extractable Al ($r^2 = 0.45$ $p < 0.001$, **Figure 7**). When the two highest Fe values were removed from the sample set, regression analysis indicates that H3A-4 extracts roughly half of the Fe compared to H3A-3 ($y = 15 + 1.8x$, $r^2 = 0.81$). Extractable Fe from the two samples

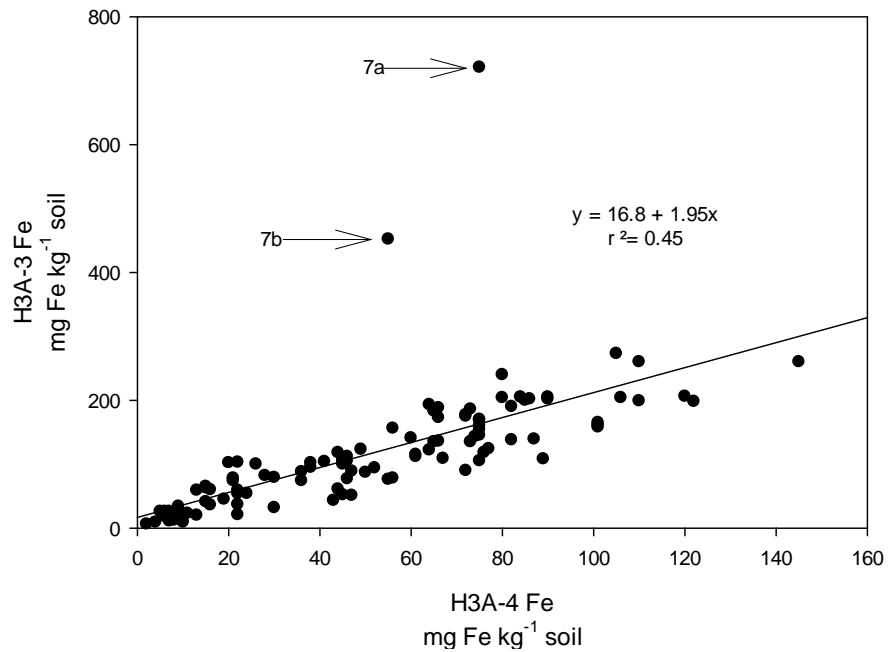


Figure 7. Linear relationship between Fe extracted with H3A-4 vs. H3A-3 from ICP analysis $P < 0.001$. The arrows point to the 2 samples that cleared as a result of the removal of lithium citrate (**Figure 8**).

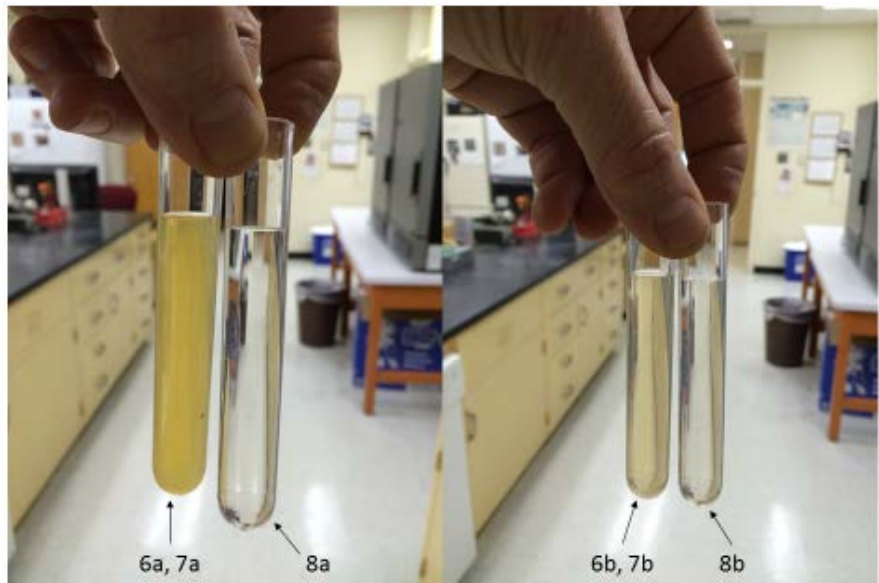


Figure 8. The visual difference between 2 of the soil extracts with lithium citrate and without lithium citrate (left side is soil 1, right side is soil 2), 6a and 6b correspond with outliers for extractable Al, from **Figure 6**. **Figure 7(a)** and **Figure 7(b)** correspond with outliers for extractable Fe from (**Figure 7**). The supernatant from H3A-4 (8a and 8b) was much clearer resulting in lower aluminum and iron results.

decreased from 722 mg Fe kg⁻¹ soil to 78 mg Fe kg⁻¹ (point 7a, **Figure 7**) soil and from 480 mg Fe kg⁻¹ soil to 55 mg Fe kg⁻¹ soil (point 7b, **Figure 7**).

Removing Li-citrate from the extractant when applied to the two highly wea-

thered, low pH, soils (vials 6a, b and 7a, b; **Figure 8**) cleared the supernatant considerably (vials 8a and 8b, **Figure 8**). The two soils with higher H3A-3 Al and Fe had low pH values, indicative of highly weathered soils. We speculate that the Li in Li citrate keeps Fe and Al in suspension due to its small size and ability to displace larger atoms, artificially inflating Al values. Exceptional values such as these are part of the reason Li citrate was ultimately removed from the H3A-3. From the thousands of samples we have analyzed over the years in the lab, we can predict, with relative accuracy, the extractable Al and Fe extracted from the soil based on color and cloudiness of the supernatant. Supernatant from soils with higher values of Al and Fe normally appear visually similar to the images in **Figure 8**.

Potassium was mostly unaffected by the removal of Li citrate except for one outlier shown in **Figure 9**, which is one of the samples that cleared because of the removal of Li citrate as seen in the picture from **Figure 8**. Regression analysis shows that the relationship between H3A-3 and H3A-4 extractable K can be described as $y = 8.2 + 0.97x$ ($r^2 = 0.97$ $p < 0.001$) with the outlier removed. The removal of Li citrate had negligible effect on extractable Ca (**Figure 10**). The slope of the regression line H3A-4 vs. H3A-3 was nearly 1:1 with a slight decrease in extractable Ca without Li citrate.

The most notable difference between H3A-3 and H3A-4 was the increase in percent P saturation ($\%P \text{ sat} = (\text{extractable P}/(\text{Al} + \text{Fe})) * 100$) with H3A-4 (**Figure 11**). The rise in $\%P \text{ sat}$ is due to the approximately two-fold decrease in Al and Fe extracted with H3A-4 and relatively no change to extractable P. Phosphorus saturation percentages over 15 commonly indicate that P fertilizer has been applied or excessive P is available. Averaged across all samples, $\%P \text{ sat}$

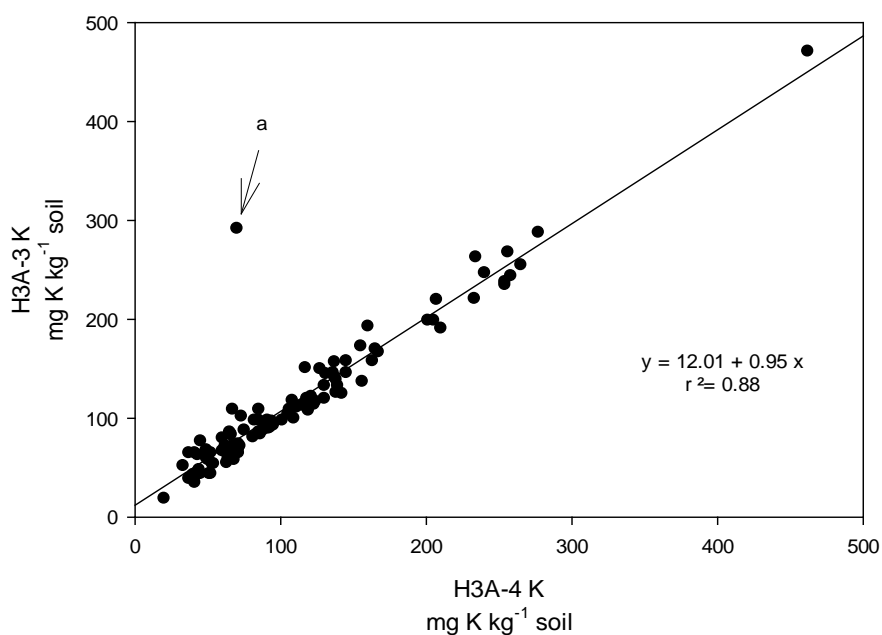


Figure 9. Linear relationship between K extracted with H3A-4 vs. H3A-3 from ICP analysis $P < 0.001$. The outlier (a) is from **Figure 8** and is tube 6a from soil 1.

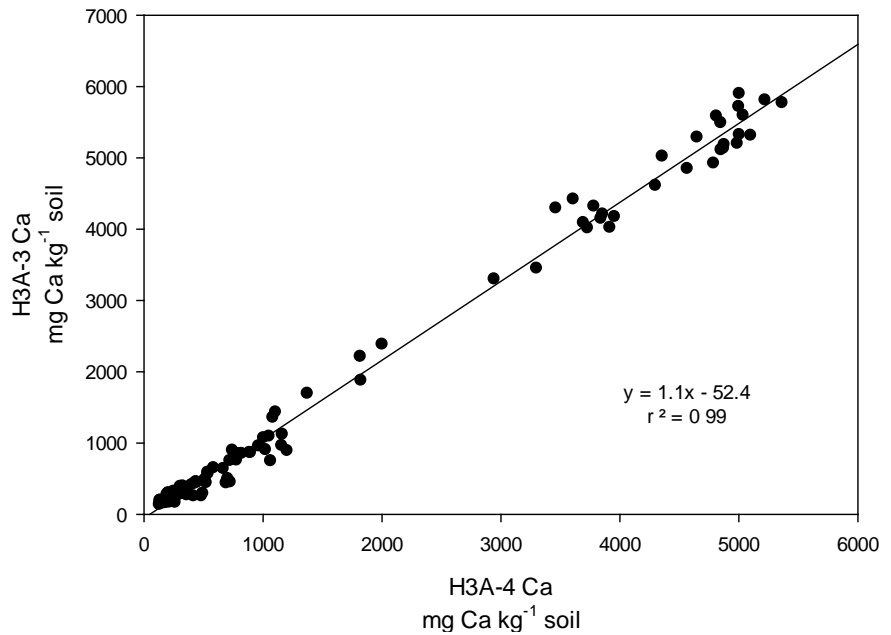


Figure 10. Linear relationship between Ca extracted with H3A-4 vs. H3A-3 from ICP analysis $P < 0.001$. The removal of Li citrate had very little effect upon extractable Ca.

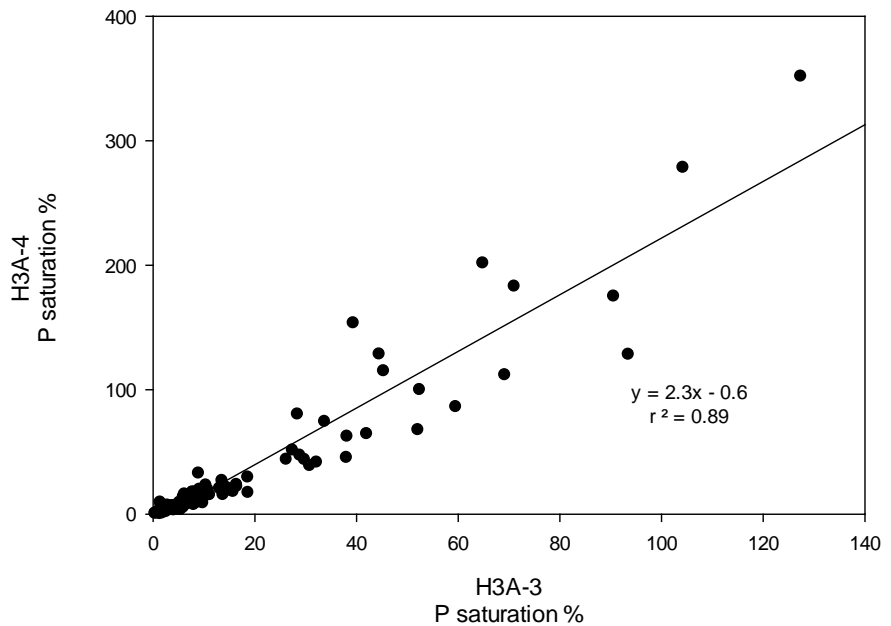


Figure 11. Percent phosphate saturation from H3A-3 vs. H3A-4 $P < 0.001$.

is 17.3 for H3A-3 and 33.5 for H3A-4, thereby doubling the scale with H3A-4. Therefore, the new ratio would indicate adequate P at 30% P sat and excessive P above 30%. Percent P saturation is an excellent indicator of excessive P when we receive samples from fields to which no P fertilizer has been recently applied.

Phosphate recommendations are known to be inherently unreliable over a wide range of soils [24] [25] [26]; therefore our approach to soil testing differs from traditional soil tests for plant available P. Laboratories using Bray, Mehlich

and Olsen find a critical value for extractable P and rate soils as having a low, medium or high P response probability. P recommendations are therefore based on P response curves to added P fertilizer from field trials of a few sample soils. This approach does not account for the plants ability to naturally extract P through root exudates under varying climatic conditions as well as variations in management such as no-till versus conventional-till. Additionally, fertilizer response curves are known to be highly variable year to year [27], decreasing the likelihood that P recommendations will be on target with crop needs. The best soil tests are those that are insensitive to soil type [28] and thereby viable over a wide range of soils. Menon [29] stated at the time, only the water and anion exchange resin methods can be considered insensitive to the soil types.

Fertilizer recommendations from soil-testing with H3A-4 are dynamic because we account for the inherent soil chemistry and natural plant biochemistry, as has been shown by Somenahally [8] and Haney [11]. Somenahally [8] indicate that H3A is much improved over Mehlich 3 for plant-available nutrient determination because H3A mimics the soil-root microenvironment and preserves ambient soil pH levels. The amount of P extracted is subtracted from the P needed for the given crop based on yield goal. This innovative approach is more versatile because it assesses the soil in its natural state as opposed to an artificially induced chemical state. The artificial extraction environment was based on the lack of understanding of the complex and dynamic living soil system. For example, if Mehlich extracts 50 mg P kg⁻¹ soil, a fertilizer response curve indicates that no fertilizer P is needed. On the same soil, when H3A extracts 21 mg P kg⁻¹ soil, we convert the value to P₂O₅ resulting in 58 kg·ha⁻¹ of available P₂O₅. If the yield goal is 12.56 Mg/ha corn, which requires 84 kg P₂O₅ ha⁻¹, then we recommend applying 26 kg P₂O₅ ha⁻¹. Mehlich 3 recommendations may have resulted in under application of P. While critical values are an understandable and broad approach, all soils within a region are treated the same regardless of inherent soil properties. The only flexibility in Mehlich recommendations comes from different regions of the US. Lobell [30] found that within-field soil variability is greater than variability in weather when water availability is not a limiting factor so we must attempt to account for variations in soil in our fertilizer recommendations versus regional blanket approaches.

5. Conclusions

H3A-4 was developed to overcome the inherent over- or under-estimations of plant available nutrients when soil extractants are too harsh (*i.e.* very high or low pH) or too weak (water). Fertilizer recommendations based on response curves are outdated and ignore the inherently variable soil and climatic factors. While it is not ideal to forgo field studies for every nutrient extractant, there is a shortage of time, funding, and labor to conduct the far-reaching research that would be needed to test over the vast gamut of soils and climatic conditions. Coupled with the fact that farmers need updated nutrient management information now, and

not in another 20 to 40 years, it is critically important to advance our soil-testing techniques so we can give producers the best data available without extensive multi-year greenhouse or research plot experiments. A move toward on-farm research could promote advances in soil analyses, ultimately benefiting producers as they endeavor to yield high quality feed and fiber efficiently and economically.

We found it remarkable that extractable P values can be altered so easily by using a natural process as the foundation of development. The variations in the H3A extractant over time have been somewhat subtle, yet have effectively improved extractable P capitalizing on a biomimicry approach. The first iteration (H3A-1) was a little more aggressive in extracting P than was H3A-2. Transition to H3A-3 resulted in a very strong relationship with FeAlO extractable P (Haney *et al.*, 2016). The modifications to H3A-3, although slight, have cleared the supernatant and appear to have eliminated excess Al and Fe from highly weathered low pH soils. The relationships between H3A-3 extractable P, K and Ca and H3A-4 are only slightly changed and therefore appear to maintain the relationship previously found with FeAlO extractable (Haney *et al.* 2016). The H3A-4 soil extractant demonstrates that our endeavor to mimic the natural processes in the lab were successful. The results will undoubtedly improve our P fertilizer recommendations for producers.

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