

# Evaluation of H3A for Determination of Plant Available P vs. FeAlO Strips

Richard L. Haney<sup>1</sup>, Elizabeth B. Haney<sup>2</sup>, R. Daren Harmel<sup>1</sup>, Douglas R. Smith<sup>1</sup>, Mike J. White<sup>1</sup>

<sup>1</sup>Grassland, Soil and Water Research Laboratory, USDA-ARS, Temple, TX, USA

<sup>2</sup>Texas Agri Life Research and Extension Center, Texas A&M University, Temple, TX, USA

Email: rick.haney@ars.usda.gov

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## Abstract

Phosphorus is an essential nutrient for plant growth but in excess is a source of environmental pollution. Fertilizer additions of P are recommended based on soil tests; however, the commonly applied P extractants are often applied outside of their design criteria (specifically soil pH). As a result, soil tests can produce inaccurate estimates of plant available P in the soil, which either increases P loss in runoff, contributing to eutrophication, or decreases crop production contributing to economic loss. In this study, 200 diverse soils from across the US were extracted with Mehlich 3, water, H3A-3, and FeAlO strips. Comparison with FeAlO was critical, as this method is accepted as the “gold standard” for plant-available P, but it is rarely used in commercial labs because of time and financial constraints. H3A-3 produced mean, median, standard deviations that are very similar to FeAlO strip results and low relative errors (<10%), as well as highly correlated regression relationships ( $r^2 > 0.96$  with slopes 0.95 - 0.98). Although Mehlich 3 and water were correlated with FeAlO, Mehlich 3 (strongly acidic) extracted much more P than FeAlO, and water (low buffering capacity) extracted much less P across the range of soil pH values. Thus, H3A-3 provides an improved methodology to accurately determine plant-available P by mimicking root exudate action in the soil, while avoiding the time-consuming and costly FeAlO procedure. In the face of high-profile water quality impairments with enormous economic costs, such advancements are critical to balance agronomic production with environmental concerns.

## Keywords

Soil Testing, Soil Extraction, Soil Test Phosphorus

## 1. Introduction

Phosphorus is essential for plant growth and is applied to agricultural crops, sometimes

in excess of agronomic needs quantities. Eutrophication of surface waters is a major environmental concern and is commonly attributed to orthophosphate-P in agricultural runoff [1]. In the US, soil-test recommendations by universities and private laboratories are typically based on a few extractants including Mehlich 3 [2] and Olsen [3]. Mehlich 3 (M3) is a multi-nutrient extractant developed for use in neutral to acid soils, while Olsen was developed for extraction of P in calcareous soils; however, these soil extractants are often applied outside their intended pH range because of the soil-testing laboratory need for uniform procedures and rapid analysis. Extracting soils irrespective of soil pH with strong acid (M3, pH ~ 2.5) or alkaline (Olsen, pH ~ 8.5) extractants can produce inaccurate predictions of plant-available P because of the influence of soil pH on soil-solution chemistry [4] [5]. Soil pH directly affects P solubility [6] [7]; therefore, when a soil is extracted with a strong acid or base which alters soil pH, forms of P that are typically insoluble are solubilized. For instance, Mehlich 3 releases significantly more P than other extractants [8] [9] [10] by dissolving relatively insoluble  $\text{Ca}^-$ ,  $\text{Al}^-$  and Fe-bound P [4] [11]. Thus, the soil buffering capacity and extractant pH should be considered when interpreting soil-test P results [12].

Several alternative soil-test P methods are available. Water soluble P correlates well with P plant uptake [13] [14] [15] [16]; however, water extractable P may be inadequate to accurately reflect P solubility over time. Anion exchange and Fe-oxide [7] or Fe-Al oxide (FeAlO) [17] [18] impregnated strips are nondestructive methods and simulate plant-root extraction of dissolved P from soil solution [12]. Vadas and White [19] indicate that the amount of P absorbed in an anion exchange resin [20] is equivalent to labile or plant-available P. Lin *et al.* [21] found that anion-exchange resin and Fe-oxide strips correlated well with P plant uptake in wheat seedlings. Dils and Heathwaite [22] indicate that Fe oxide-impregnated strips are accurate for the determination of bio-available P. Van der Zee *et al.* [23] found that FeAlO strips could be used during routine soil analysis to determine available P but that Mehlich and Olsen were not as reliable. Sharpley [8] found that the amount of P extracted using FeAlO strips and the Olsen extractant was correlated in calcareous soil but not acidic soil, and the opposite was true for soils extracted with M3. Sharpley [8] also found that FeAlO-extractable P was closely correlated to resin-extractable P, which is accepted as plant-available P, for all soils studied and concluded that FeAlO-P is highly correlated with plant-available P in a wide range of soils. Thus, FeAlO is the commonly accepted method for determination of plant available P in a research setting [7] [8].

Menon *et al.* [5] noted that while deionized-water and the anion exchange-resin methods do not experience the limitations common in other extracting solutions, such as over-extracting P, they are not widely applied in a laboratory setting, due to analytical difficulties with water extraction (*i.e.*, cloudy solutions) and the time-consuming nature of resin or oxide exchange methods. Therefore, few, if any, commercial labs offer them for large-scale routine soil analysis.

The benefits of using a soil extractant that extracts plant nutrients near the ambient soil pH, along with the additional benefit of simultaneous N and P extraction, prompt-

ed development of the H3A-1 extractant [24] [25]. H3A-1 and its subsequent modification H3A-2 were developed to mimic the plant root environment by using plant exuded organic acids [26] [27] [28] to extract nutrients at a solution pH similar to that of the ambient soil pH. Three organic acids exuded by corn, wheat, and sorghum roots are malic, citric, and oxalic acid [29]. These organic acids have a low buffering capacity, which allows the soil pH to dominate the pH of the extractant solution in soil [24]. Mimicking the soil-root environment is important since plants utilize root exudates to overcome P, Fe, Zn, and Mn deficiencies [2] [30] [31]. Ion toxicity and pathogen attack can also stimulate an exudate response from plants [32] [33] [34]. 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 that release ligand-bound P to the soil solution [35].

The original H3A-1 formulation and processing methods were modified to address the rare occurrence (~5%) of soil extracts that did not have a visually clear supernatant [25]. In addition to a cloudy supernatant, these samples also had elevated Fe, Al, and P concentrations when analyzed using other well-known soil extractants. To resolve this issue, H3A-2 was developed and tested on 60 soils from the NAPT (North American Proficiency Testing) program with varying pH, organic C, and clay content. The adjustments, which included eliminating EDTA and DTPA and reducing the shaking time from 30 min to 5 min, reduced the extractable Fe and Al concentrations and improved the relationships for extractable NO<sub>3</sub>, NH<sub>4</sub>, PO<sub>4</sub>, total P, K, Ca, and Zn with the Olsen, KCl, water, Mehlich 3, Bray 1, and NH<sub>4</sub>OAc extractants [25].

While these modifications improved the H3A extractant and its relationships with other well-known soil extractants, further analysis was needed to determine which extractant(s) is/are best able to represent plant-available P in soils. Ideally, a single extractant capable of determining plant-available P across the pH range of soils would be available and capable of accurate, simultaneous multi-nutrient extraction. With this in mind, the objective of this study was to analyze the relationships between FeAlO-extractable P, which is the commonly accepted method for determination of plant-available P in a wide range of soils [7] [8], with H3A, Mehlich 3, and water extractable P.

## 2. Methods

To conduct this evaluation 201 soils were analyzed. Sixty of the soils analyzed were from the NAPT program, and the other 140 were from surface soils (approximately 2.5 cm) gathered from throughout the contiguous US. The remaining sample was an organic fertilizer (4-4-4) that was utilized to extend the upper analysis range. The samples were collected throughout the year of 2014. The soils analyzed had a wide array of soil pH (4.5 - 8.6), soil test P levels, clay content, tillage regimes, and management and climatic conditions.

FeAlO-impregnated filter paper strips were prepared by submerging hardened filter

paper circles (15 cm diam., Whatman no. 541) in a solution containing 10 g  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$  and 5 g  $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$  in 100 mL of distilled water [18]. The paper circles were dried and exposed to  $\text{NH}_3$  vapor, to convert  $\text{FeCl}_3$  and  $\text{AlCl}_3$  into the oxide form, by placing the paper on an evaporating dish containing a 2 cm depth of magnetically stirred 5  $\text{MnH}_4\text{OH}$  solution. The circles were cut into strips 2 cm by 12.5 cm, of which 1.25 cm was removed from each end giving a reactive surface of 2 cm by 10 cm. Phosphorus was extracted by shaking a 1-g soil sample and one paper strip in 40 mL of 0.01 M  $\text{CaCl}_2$  end-over-end for 16 h at 25°C. The strip was then removed, rinsed free of adhering soil particles, and dried. Phosphorus retained on the strip was removed by shaking the strip end-over-end with 40 mL of 0.1 M  $\text{H}_2\text{SO}_4$  for 1 h, and then total P was measured on an axial flow Varian ICP-OES.

Soil-test P was determined by H3A-1, H3A-2, H3A-3, M3, and water extractants. The H3A-3 extractant, which is a weakly buffered solution with a pH of 4, is a modification of H3A-2 [25]. The formula for H3A-3 is 2 L DI water, 2.6 g lithium citrate, 1.2 g malic acid, 1.0 g citric acid, and 0.6 g oxalic acid.

All H3A extractants were evaluated by shaking 4 g of soil with 40 ml of H3A for 10 min. Mehlich 3 was evaluated by shaking 4 g of soil with 40 mL of M3 (0.2 M  $\text{CH}_3\text{COOH}$ , 0.25 M  $\text{NH}_4\text{NO}_3$ , 0.015 M  $\text{NH}_4\text{F}$ , 0.013 M  $\text{HNO}_3$ , and 0.001 M EDTA) for 5 mins. described by Mehlich [2]. The water extract was evaluated by shaking 4 g of soil with 40 ml of DI water for 10 min. Following extraction, all samples were centrifuged for 5 min at 3500 rpm and filtered through Whatman 2V filter paper prior to analysis for total P on an axial flow Varian ICP-OES and orthophosphate P on an OI Analytical rapid flow analyzer (RFA).

Paired t-tests for means and Mann-Whitney tests for medians were used to determine significant differences between extractants and “true” value as determined with FeAlO strips. Minitab [36] was used for these statistical tests with an a priori  $\alpha = 0.05$  significance level. Linear regression analysis using Sigma Plot ver. 12 [37] was used to analyze correlations in P concentrations between each extractant and FeAlO strips.

### 3. Results

#### 3.1. Comparison of Extractants with FeAlO Strips

Results in **Table 1** show that the M3 extractant tended to overestimate plant available P in the soil, which is attributed to its strong acid composition that dissolves insoluble P forms. Means and medians for M3 were significantly greater than FeAlO values of plant available P determined with RFA and ICP analysis, and the relative error exceeded 100%. The standard deviations of M3 results were also substantially larger than for FeAlO. Means and medians for water extraction were significantly lower than for FeAlO strip results, as water tended to under predict plant available P (**Table 1**).

In contrast to M3 and water, the results for the H3A-3 extractant were quite close to FeAlO strip results (**Table 1**), which indicates that this extractant may be preferable for estimation of plant-available P. Mean and median RFA values were not significantly

different between H3A-3 and FeAlO-P, and neither were the median ICP values for these two methods. Only the mean for ICP analysis was significantly different, and relative errors were <10%. The standard deviations were also quite similar between H3A-3 and FeAlO strips. The close relationship between H3A-3 and FeAlO extractable P is interesting, as these two methods use quite different mechanisms to estimate plant-available P. The H3A-3 solution uses small chain organic acids to extract P from soil through ligand exchange, whereas the FeAlO method uses Fe and Al oxides, which tightly bind P, to remove P from the soil.

The correlation coefficients between P concentrations determined by RFA for each extractant are shown in **Table 2**. H3A-3 extractable P was highly correlated with P extracted using FeAlO strips ( $r^2 = 0.98$ ,  $p < 0.0001$ , **Figure 1**). The relationship remained strong even when the organic fertilizer sample was removed ( $r^2 = 0.96$ ,  $p < 0.0001$ , **Figure 2**). Similar amounts of P were extracted by H3A-3 and FeAlO strips as indicated by the 0.95 - 0.98 slopes, which provides further support that H3A-3 is able to accurately determine plant-available P in the soil, as it closely mimics the FeAlO strips benchmark of plant-available P.

Mehlich 3 was also highly correlated to FeAlO strip results with the organic fertilizer sample included ( $r^2 = 0.92$ ,  $p < 0.001$ , **Figure 3**) and excluded ( $r^2 = 0.83$ ,  $p < 0.001$ , **Figure 4**). However, the slopes of these lines indicate that M3 extracts 1.5 - 2.3 times the

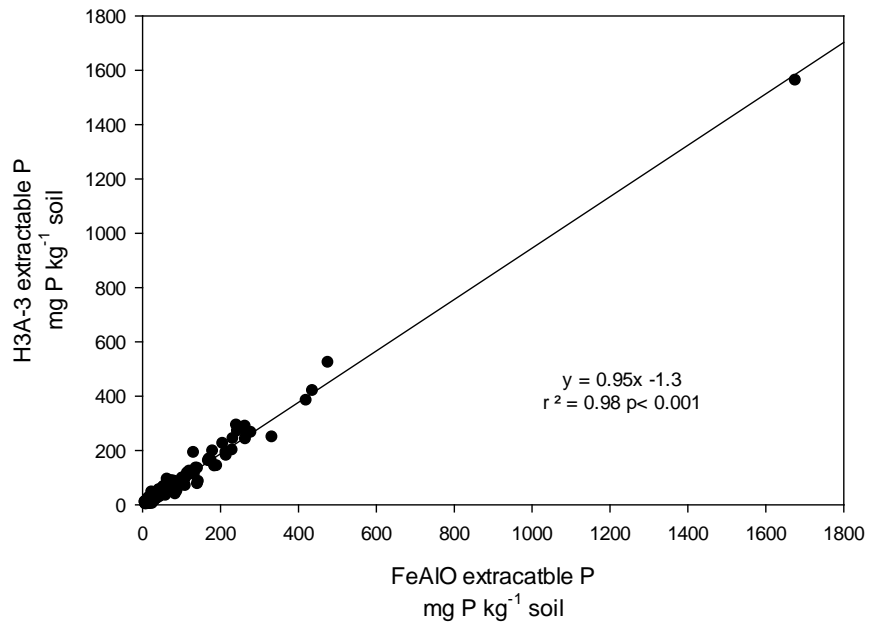
**Table 1.** Summary statistics for the plant available P estimates from the H3A-3, M3, and water extractants and FeAlO strips (n = 201).

	Average	Median	StDev	Rel Error vs. FeAlO	Average	Median	StDev	% Error vs. FeAlO
	RFA (mg P/kg soil)			(%)	ICP (mg P/kg soil)			(%)
<b>FeAlO</b>	58.2	31.9	103.6	-	74.7	39.4	137.2	-
<b>H3A-3</b>	58.1	32.6	104.3	-0.2%	69.4*	38.7	130.9	-7%
<b>M3</b>	142.4*	84.8*	315.4	145%	155.9*	96.6*	330.9	109%
<b>Water</b>	10.8*	23.4*	31.9	-81%	14.9*	6.8*	42.4	-44%

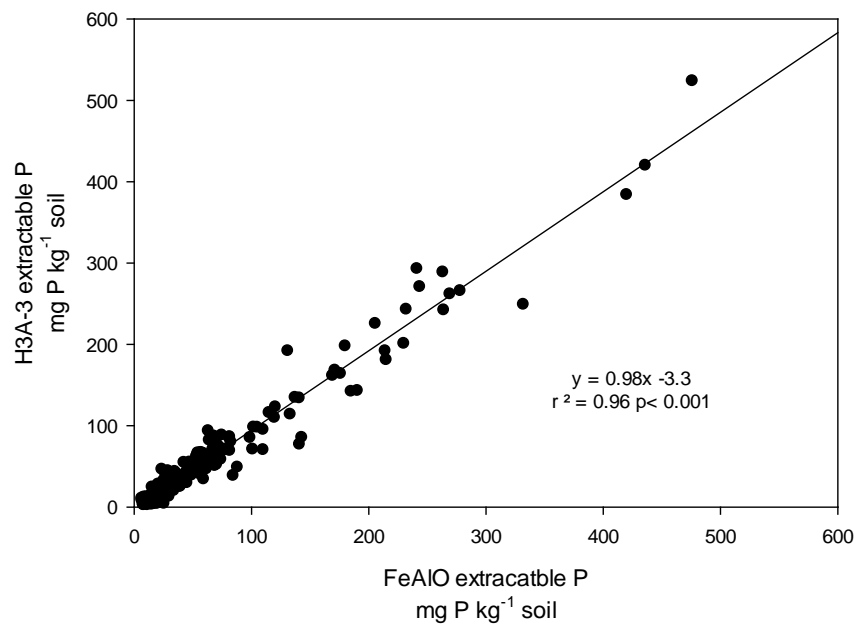
\*Significant differences in means and medians compared to FeAlO strips ( $\alpha = 0.05$ ).

**Table 2.** Correlation matrix for extractable P (determined with the colorimetric RFA method, n = 200). Correlation coefficients are presented (all P values <0.0001). Results exclude the organic fertilizer sample.

	H3A-3	M3	Water	H3A-1	H3A-2
<b>FeAlO strips</b>	0.977	0.903	0.868	0.951	0.946
<b>H3A-3</b>		0.885	0.879	0.963	0.957
<b>M3</b>			0.744	0.855	0.854
<b>Water</b>				0.846	0.862
<b>H3A-1</b>					0.984

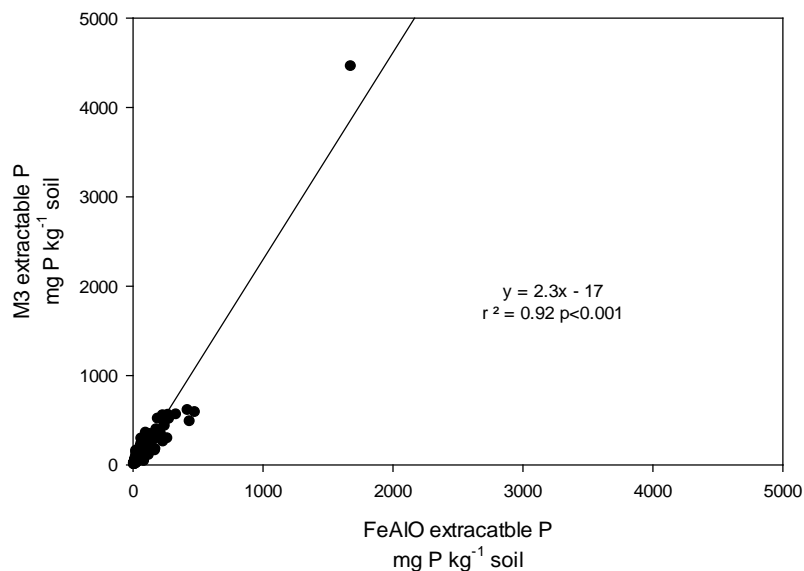


**Figure 1.** Linear relationship between P extracted with H3A and FeAlO strips and ICP analysis, including organic fertilizer sample.

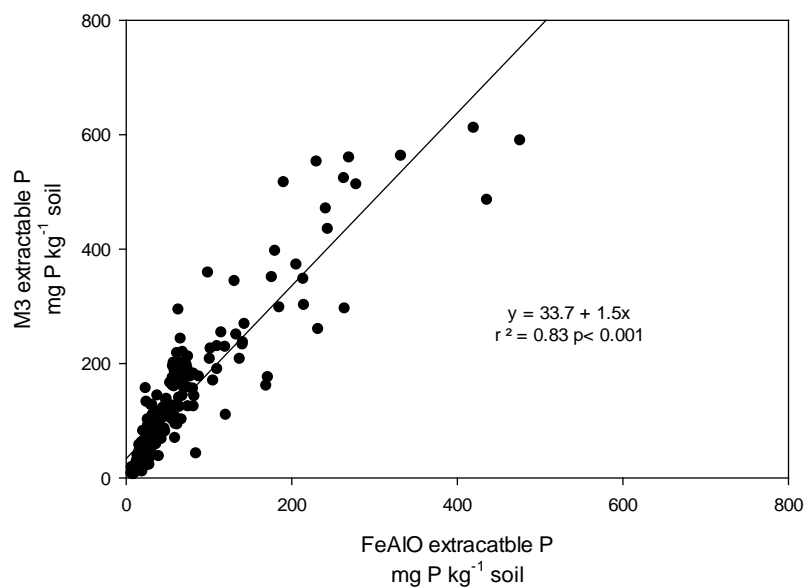


**Figure 2.** Linear relationship between P extracted with H3A and FeAlO strips and ICP analysis, excluding organic fertilizer sample.

amount of P than does FeAlO strips. Below 100 mg P/kg soil, the slope appears to be even steeper (**Figure 4**) likely because M3 releases insoluble P due to its highly buffered acidic nature. Water was also relatively well correlated to FeAlO strips (**Figure 5, Figure 6**), although the slopes (<0.3) again indicated the inability of water to extract P from the soil due to its lack of organic root exudates.



**Figure 3.** Linear relationship between P extracted with Mehlich 3 and FeAlO strips and ICP analysis, including organic fertilizer sample.

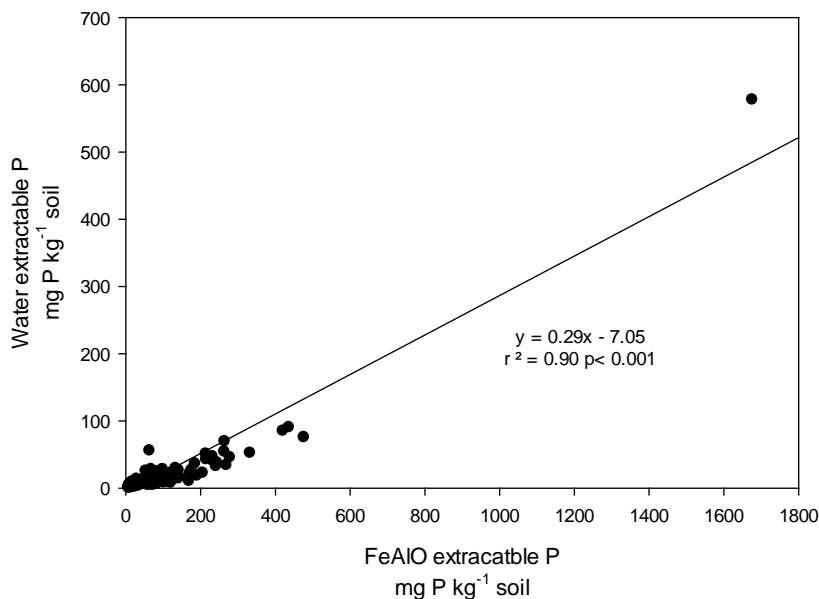


**Figure 4.** Linear relationship between P extracted with Mehlich 3 and FeAlO strips and ICP analysis, excluding organic fertilizer sample.

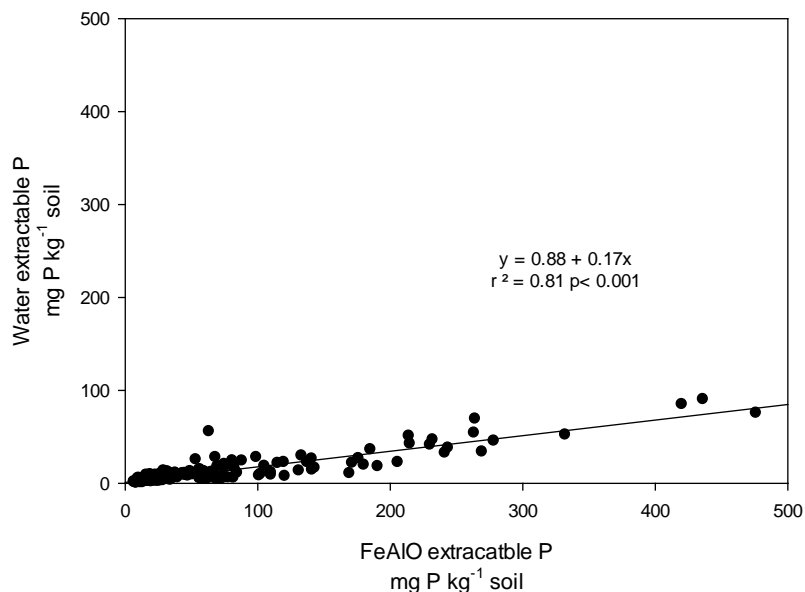
### 3.2. Progression of H3A

The ability of H3A-1 and H3A-2 to extract P near the ambient soil pH level and to correlate well with accepted extractants was documented in Haney *et al.* [24] [25]. In fact these two initial formulae better reproduced plant available P values, as determined by comparison with FeAlO results, than did M3 or water (data not shown). On average, H3A-1 was more aggressive in extracting P (97 mg P/kg soil) than was H3A-2 (77 mg P/kg soil), or H3A-3 (69 mg P/kg soil) based on ICP analysis. Colorimetric analysis

produced the same trend: H3A-1 (73 mg P/kg soil), H3A-2 (69 mg P/kg soil), and H3A-3 (58 mg P/kg soil). In spite of H3A-1 and H3A-2 better estimating plant-available P, somewhat subtle changes were made to better mimic natural soil solutions. Comparative results in the development of the H3A-3 extractant are shown in **Figure 7**. It is quite remarkable that soil extractable P can be altered so easily by using a natural process (organic acids as a soil extractant) as the basis of our understanding of the amount of P we are extracting from soil solutions.

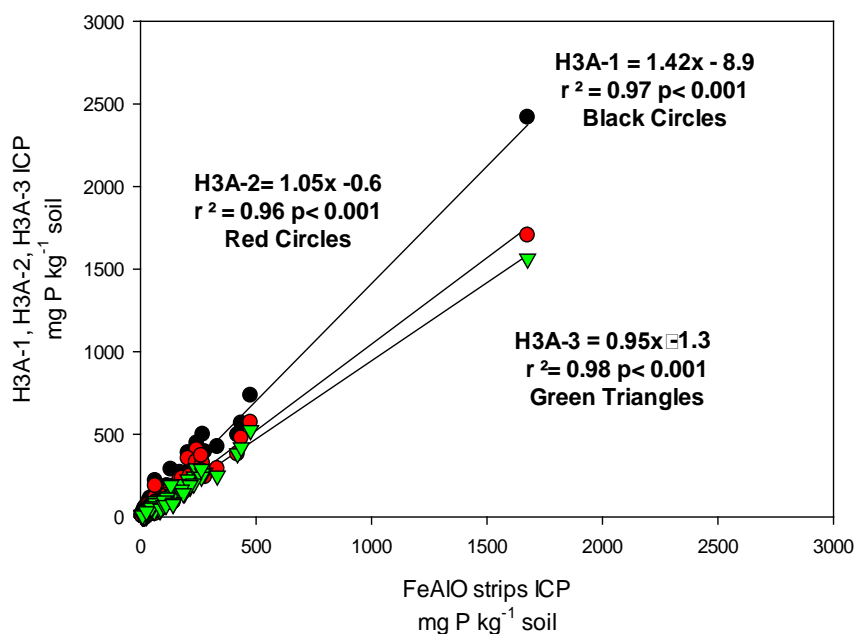


**Figure 5.** Linear relationship between P extracted with water and FeAlO strips and ICP analysis, including organic fertilizer sample.



**Figure 6.** Linear relationship between P extracted with water and FeAlO strips and ICP analysis, excluding organic fertilizer sample.





**Figure 7.** Comparison of the H3A modifications to FeAlO strips for extractable P.

### 3.3. Extractable Organic P (ICP versus RFA)

The extractable organic P (difference between ICP and RFA) results were interesting because all of the extractants and FeAlO strips estimated similar amounts. In contrast to inorganic P (RFA) and total P (ICP) results, which were drastically different (**Table 1**), average extractable organic P results were all within 4 - 24 mg P/kg soil. These data suggest that all the extractants are pulling P from a similar pool yet have large differences in inorganic plant-available P, due to their strong regression relationships. The differences in inorganic P appear to be closely related to the extractants' pH and buffering capacity. M3 is the most acidic and buffered, thereby releasing the most inorganic P. Water is by far the least aggressive since it has virtually no buffering strength.

## 4. Discussion

It is difficult for highly buffered soil extractants to be capable of accurate multi-nutrient extraction, including plant-available P, across a wide range of soil pH. As a result, either different extractants are applied for different soil pH ranges, or a single extractant is utilized regardless of whether the soil pH meets the design criteria. Most often, commercial labs prefer to use a single extractant, even though soil pH and P solubility are highly interrelated [6] [7] and soil pH has a strong influence on soil-solution chemistry [4]. The misapplication of the commonly used extractants (e.g., Mehlich 3, Olsen) results from pressure on commercial labs to utilize uniform procedures and perform rapid analysis. Additionally, the soil science community generally supports the views that soil tests that are insensitive to soil type are preferred and that FeAlO is the best method for determination of plant-available P [7] [8].

With these challenges in mind, the H3A extractant was developed and since modified

to provide an accurate estimate of plant-available P that was cost-effective and rapid, which has facilitated adoption by commercial soil testing labs. Previous research by Somenahally *et al.* [10] indicated that H3A better estimates plant-available nutrients than M3 because it mimics the soil-root microenvironment and preserves ambient soil pH levels. However, further evaluation of the ability of H3A to estimate plant-available P was warranted. Thus, the current study analyzed the relationships between FeAlO extractable P, as the “gold standard” for plant-available P, with three extractants: H3A-3, Mehlich 3, and water.

Statistical and regression analyses clearly indicated that H3A-3 accurately estimated plant-available P. Specifically, H3A-3 produced mean, median, standard deviations that are very similar to FeAlO strips with low relative errors (<10%), as well as highly correlated regression relationships ( $r^2 > 0.96$ ,  $p < 0.001$ ) with slopes 0.95 - 0.98). These results, along with those published in Haney *et al.* [24] [25], establish H3A-3 as a capable and accurate soil test P determination across the pH range of agricultural soils.

In the face of high-profile water quality impairments with enormous economic costs (e.g., Gulf of Mexico, Lake Erie, Chesapeake Bay), it is critical that scientists improve soil test P fertilizer recommendations to assist consultants, fertilizer dealers, and producers better match P application with crop needs [1]. H3A-3 provides this improved methodology. It accurately determines plant-available P by mimicking the action of root exudates in the soil, while avoiding the time-consuming and costly FeAlO procedure.

## 5. Conclusion

With pressure to produce economically viable, environmentally sustainable crops to feed the world, farmers need agronomic advice that guides fertilizer recommendations for both crop productivity and to protect water quality. Perhaps judicious P applications are of the utmost importance with this in mind, as declining non-renewable sources of P coupled with its role in aquatic eutrophication have highlighted these risks for many farmers, who have come under public scrutiny where P-associated water quality problems are prevalent. In this study, we compared the “gold standard” for assessment of plant-available P in soil (FeAlO strips) to M3, water, and H3A-3. We observed that H3A-3 offered the most comparable extractable P results when 200 soil samples collected from across the US were compared to FeAlO strips. The use of root exudates and the relatively unbuffered nature of H3A-3 allows soil to control P release to the solution. In contrast, other extractants such as M3 are highly acidic and well buffered and are known to dissolve recalcitrant forms of P that are unavailable to plants under normal physiochemical conditions. Thus, strong correlation to the FeAlO method is most accepted by the soil science community, and H3A-3 may be an alternative to the currently offered soil extractants to evaluate plant-available P in soils.

## Consent

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