

Article

# A Discussion on Mehlich-3 Phosphorus Extraction from the Perspective of Governing Chemical Reactions and Phases: Impact of Soil pH

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**Abstract:** Mehlich-3 (M3) is one of the most common agronomic and environmental phosphorus (P) extractants for determining P fertilizer requirements and the potential for non-point source pollution. Understanding how soil properties impact M3 extractability can improve our ability to properly use this soil test. The objectives of this study were to investigate the impact of soil pH on P extractability by M3 and water in different soils containing equal total P, and to ascertain information about mechanisms of M3-P extraction. Soil pH at four field sites was previously adjusted to a range of approximately 4.5–7.5. Soils (Grant, Dale, Teller, Easpor) were characterized, and P was extracted with M3 and water. Extraction of Mehlich-3 P decreased 40% to 55% with increasing pH, which was potentially due to changing P forms, partial neutralization of extractant pH, and consumption of extractant fluoride (F<sup>-</sup>) by non P-containing calcium (Ca) minerals. Water-soluble P (WSP) increased with increasing pH up to pH 6–7. Mehlich-3 P and WSP were not positively correlated except for one soil type. Mehlich-3 P is best utilized with WSP as indicators of quantity and intensity, respectively. Use of M3-P alone at pH < 5.5 may overestimate solubility. Further research should examine the suitability of M3-P at pH > 7.

**Keywords:** Mehlich-3; phosphorus; soil pH; phosphorus forms; phosphorus solubility; phosphorus extractions; phosphorus testing; soil testing

## 1. Introduction

The Mehlich-3 (M3) extraction has been extensively used as an agronomic soil test since the 1980's in the Southern, Mid-Atlantic, and Mid-Western United States. Since then it has also been adopted in several other countries, for example, Estonia, Czech Republic, and Canada. Mehlich-3 is integral to making fertilizer recommendations for phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), and certain trace elements to achieve optimum yields. The intended purpose of this test with respect to P is to extract portions of several different P pools that are correlated with the amount of P that will be available to plants over the growing season [1]. While not the original intent, M3-P has also been widely used for predicting the potential for non-point loss of dissolved and total P in runoff, leaching, and tile drainage to surface waters [2], as well as identifying locations that may be vulnerable to P loss (e.g., P indices; [3]). Understanding the factors that may influence M3-P is therefore critical for both agronomic and environmental applications.

Previous research and current experience indicate that M3 is well-suited as a general indicator of soil P fertility and P solubility, as it continues to be utilized by many states and soil testing laboratories. However, its relationship with optimum plant P requirements and with water solubility can be influenced by soil type, mineralogy, clay content, and pH [2,4–12]. Much of this research has focused on the effect of soil type, clay content, and mineralogy on M3-P, with few studies considering the impact of pH. For example, Lins and Cox [5] found that the M3-P critical level for soybean varied as a function of clay content and clay type. Specifically, soils with higher clay content required a lower M3-P level to achieve optimum yield, and soils rich in gibbsite also required less M3-P.

Water-soluble P (WSP) is a direct measure of the ability of a soil to provide dissolved P instantly (not prolonged) to runoff and leachate, and it also provides a snapshot of crop P availability, as P must first be desorbed or dissolved from the solid soil phase into the solution to be taken up by the plant root [13]. Although soil WSP cannot provide a complete assessment of long-term plant availability, it does provide an instantaneous measure of a critical component, solution P concentration. The relationship between M3-P and WSP has largely been studied since the 1990's for this reason. Fuhrman et al. [8] observed that soil pH may influence the relationship between M3-P and WSP. The authors found three different relationships between M3-P and WSP based on grouping soils by pH: <5.5, 5.5–7.0, and >7.0. Many studies examining the impact of soil properties on M3-P extractability, including the study by Fuhrman et al. [8], are often complicated by the use of non-equivalent soil total P concentrations among treatments. Alternatively, an experimental approach where soil total P is maintained and changes in M3-P extractability are observed as soil properties such as pH are varied, would enhance interpretation of study results.

The objective of this study was to investigate the impact of soil pH on P extractability of M3 and water in different soils containing constant total P. We also sought to ascertain information about appropriate use of M3 and mechanisms of M3-P extraction in soils of varying pH using speciation equilibrium modeling conducted on soil water extracts, analysis of M3 extracts in light of the governing reactions, and a priori and a posteriori reasoning.

## 2. Materials and Methods

Soils from four locations in Oklahoma, USA were collected from field plots with the same fertilizer management and pH adjusted from 4.0 to 7.0. Soils were a Grant silt loam (fine-silty, mixed, superactive, thermic Udic Argiustolls) collected from the North Central Agronomy Research Station near Lahoma, OK, USA (hereby referred to as “Lahoma”); an Easpor silt loam (fine-loamy, mixed, superactive, thermic Fluventic Haplustolls) collected from the Stillwater-Efaw Agronomy Research Station in Stillwater, OK, USA (“Efaw”); a Dale silt loam (fine-silty, mixed, superactive, thermic Pachic Haplustolls) collected from the South Central Agronomy Research Station in Chickasha, OK, USA (“Chickasha”); and a Teller fine sandy loam (fine-loamy, mixed, active, thermic Udic Argiustolls) collected from the Cimarron Valley Research Station located near Perkins, OK, USA (“Perkins”). Original pH values before adjustment for Lahoma, Efaw, Chickasha, and Perkins, were 5.5, 5.2, 6.2, and 4.9, respectively. General soil information is listed in Table 1.

**Table 1.** General soil characteristics among the four locations used in the study. Sand, silt, clay, and organic matter (OM) values are means. Values shown for pH, total P, water-soluble P (WSP) and Mehlich-3 (M3) extractable elements are the range, with means indicated in parentheses. DPS = degree of P saturation calculated on a molar basis as  $P/(Al + Fe) \times 100$  for M3 and ammonium oxalate extracts (DPS<sub>M3</sub> and DPS<sub>Ox</sub>, respectively).

Location	Soil Series	Sand	Silt	Clay	OM	pH	Total P	WSP	M3-P	M3-Fe	M3-Al	M3-Ca	DPS <sub>M3</sub>	DPS <sub>Ox</sub>
		g 100 g <sup>-1</sup>		g kg <sup>-1</sup>					mg kg <sup>-1</sup>				%	
Chickasha	Dale	40	45	15	14.5	4.6–7.6 (5.9)	283–336 (320)	0.46–7.61 (2.29)	15–34 (25)	67–103 (82)	458–1001 (643)	762–1997 (1212)	2.1–3.9 (3.1)	5.9–7.7 (6.9)
Efaw	Easpur	60	27.5	12.5	7.6	4.3–7.6 (5.64)	174–210 (186)	0.27–6.91 (3.48)	16–58 (40)	74–139 (107)	370–704 (514)	461–2016 (949)	2.5–9.9 (6.9)	12.8–16.9 (14.1)
Lahoma	Grant	30	50	20	16.9	4.8–7.1 (5.6)	246–302 (278)	0.35–3.38 (1.91)	26–64 (48)	46–100 (81)	590–1070 (842)	760–2289 (1232)	2.4–6.4 (5.0)	6.1–12.6 (9.5)
Perkins	Teller	65	25	10	9.6	4.6–7.1 (5.2)	139–183 (163)	0.68–2.22 (1.31)	23–51 (36)	70–120 (92)	600–950 (754)	180–1100 (496)	2.9–5.2 (4.1)	5.9–10.5 (7.7)

Relationships between soil pH and M3-P and between soil pH and WSP were assessed. Soil pH trials were established in 2009 to estimate the critical soil pH for grain sorghum (*Sorghum bicolor* L.) [14] and sunflower (*Helianthus annuus* L.) [15]. During these field trials, all plots received the same fertilizer regiment (no P applied) and management, and pH was originally adjusted (20 cm depth) from 4.0 to 7.0 (in six increments at Chickasha and Efav locations and twelve increments at Perkins and Lahoma locations), as described in Butchee et al. [14], Sutradhar et al. [15], and Lollato [16]. Briefly, the necessary equivalents of acid and base required for achieving each target pH was determined by automated pH titration (Metrohm; Herisaau, Appenzell Ausserrhoden, Switzerland) using 0.01 M HCl and NaOH and 1 g of soil suspended in 10 mL of deionized water while stirring. Each of the pH-adjusted treatments were replicated four times. After the 2009–2010 growing season, the field plots were planted with either winter wheat or canola and no additional fertilizer or lime was applied. This allowed for several years of dissolution of any residual carbonates that may have been present through pH adjustment; calcimeter measurements indicated that there were no residual carbonates in the collected soils. From the time of amendment application to the collection of soils several years later, the pH increased from the original target range of 4.0 to 7.0, to about 4.5 to 7.5.

A post-hoc observational study was conducted by soil sampling the original four sites [14–16], which at the time of sampling, had a range in pH from 4.5 to 7.5. At Chickasha and Efav, in June of 2014 and 2015 (post winter wheat harvest), respectively, soil probes (1.2 cm diameter) were used to collect 15–20 cores per plot to a depth of 0–15 cm to create one composite sample per plot. Using the same sampling method, soils were collected at Perkins and Lahoma in June 2016. Soils were dried at 60 °C overnight and passed through a 2 mm sieve prior to analysis.

### 2.1. Laboratory Analysis

Total P was determined by acid-digestion (3050B [17] utilizing HNO<sub>3</sub>, HCl, and hydrogen peroxide with heating). Soil pH was measured with a glass pH electrode (1:1 soil:deionized water, 30 min equilibration time). Sand, silt, and clay were measured by the hydrometer method [18]. Total C was measured with a LECO Truspec (LECO, St. Joseph, MI, USA) dry combustion analyzer [19]. Soils were extracted with Mehlich-3 (M3) solution [1] at a 1:10 soil:solution ratio: 0.2 M CH<sub>3</sub>COOH + 0.25 M NH<sub>4</sub>NO<sub>3</sub> + 0.015 M NH<sub>4</sub>F + 0.13 M HNO<sub>3</sub> + 0.001 M EDTA, 5 min reaction time, filtration with Whatman #42 paper (GE Healthcare, Chicago, IL, USA). Solution pH was measured on a subset of filtered M3 extracts with a glass electrode. Water extracts were conducted at a 1:10 soil:deionized water ratio with 1 h reaction time, followed by centrifugation (1700 RCF for 10 min) and filtration with 0.45 µm Millipore membrane (Merck KGaA, Darmstadt, Germany). Ammonium oxalate extractions were performed at a 1:20 soil:solution ratio for 2 h in the dark according to the method of Schoumans [20]. Soil Mehlich-3 and water extracts were analyzed for P, Ca, Mg, Fe, Al, and Mn by inductively coupled plasma atomic emission spectroscopy (ICP-AES). Ammonium oxalate extracts were analyzed for P, Al, and Fe by ICP-AES. Degree of P saturation was calculated on a molar basis as  $P/(Al + Fe) \times 100$  for M3 (DPS<sub>M3</sub>) and ammonium oxalate extracts (DPS<sub>ox</sub>) [2]. The MINTEQA2 speciation model [21] was used to investigate solution species that are potentially present in a reacted M3 extract, assuming the initial presence of strengite, variscite, dolomite, calcite, gibbsite, goethite, hydroxyapatite, kaolinite, vermiculite, and quartz. The data from water extracts, including pH, were used to investigate the potential presence of solid soil mineral phases at equilibrium as determined by the MINTEQA2 speciation model; the results of this speciation are not intended to represent the absolute presence of specific minerals, only as an indicator of the potential changes in the types of P forms that may be occurring with variable pH. All equilibrium reactions listed throughout the paper are taken from Lindsay [22].

### 2.2. Statistical Analysis

Data collected over the four sites were analyzed using R Version 3.4.0 [23]. Mehlich-3 P, WSP, and TP data were separated by location, and ordered by extraction method and soil pH. Data normality

was tested using the Shapiro-Wilk normality test and no transformations were applied prior to analysis. Pearson correlation tests were used to evaluate the relationship between total P and soil pH within each site using non-averaged data (i.e., composite samples were treated as experimental units). Second-order linear regression models were used to investigate the relationship between soil pH and M3-P, and between soil pH and WSP; if the second order term was not significant, then it was removed from the model. Single-degree-of-freedom contrasts using Statistical Analysis System (SAS) software [24] were used to compare P concentrations among three different pH categories: <5.5, 5.5–7.0, and >7.0. The identification and location of significant changes in the slope of the relationship (i.e., “breakpoint”) between soil pH and WSP were determined using the PROC NLIN procedure of SAS [24].

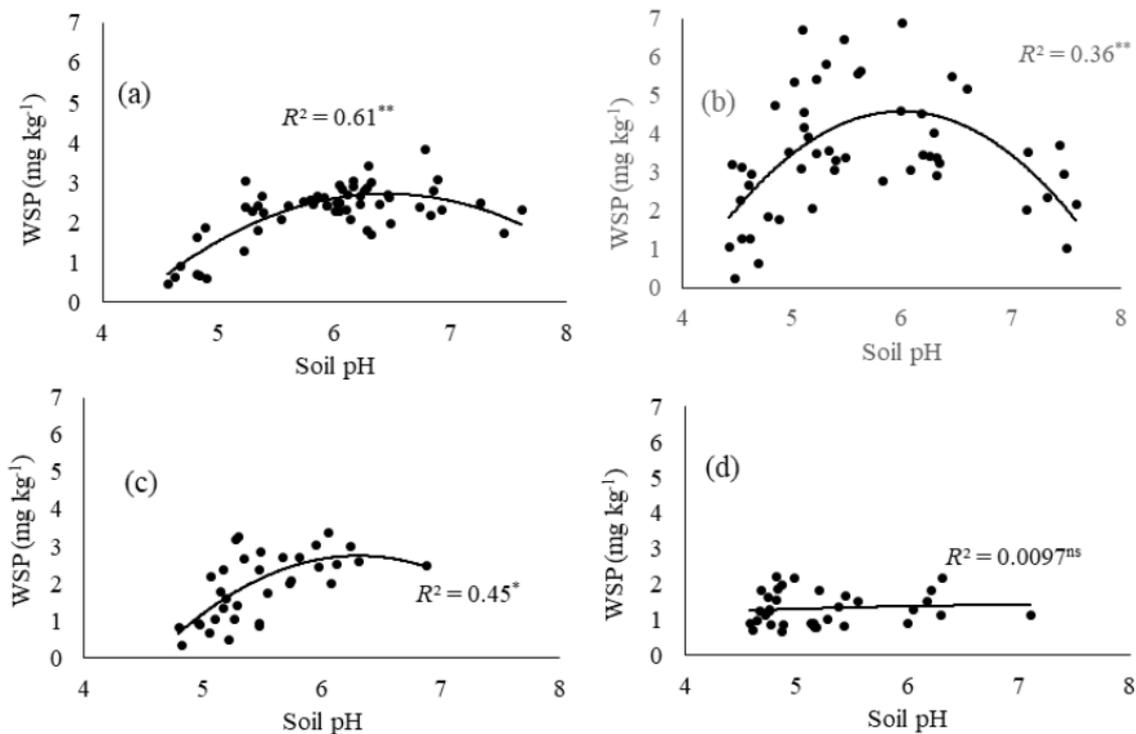
### 3. Results and Discussion

#### 3.1. General Soil Characteristics

All soils were similar based on taxonomical description, with the exception of the Perkins location that contained an argillic horizon and generally possessed lower cation exchange capacity (i.e., “active” vs. “superactive” mineralogy [25]; Table 1). The two soils with the highest sand content (Perkins and Efaw) also contained the least soil organic matter (SOM). Total soil P varied from 163 to 320 mg kg<sup>-1</sup> among soils, but within each location, total soil P did not differ with pH ( $p < 0.05$ ; Table 1). This allowed us to directly evaluate the impact of pH on soil P extractability without the confounding effect of differences in total soil P concentration. Values of soil DPS, calculated as either DPS<sub>M3</sub> and DPS<sub>ox</sub>, were also statistically equal within each location; values were considered low with regard to risk of P losses to surface waters [2]. Total P was highest for soils from Chickasha and Lahoma, located in western OK where soils naturally tend to have a higher pH due to less rainfall. Mehlich-3 Ca was also higher for these locations, compared to Efaw and Perkins. The Perkins location was expected to possess lower pH and Ca due to its sandy texture, lower cation exchange capacity, and higher rainfall, than the other locations. Soil M3-P averaged 25 to 48 mg kg<sup>-1</sup> across locations (Table 1). Although recommendations vary with region, an M3-P value of ~30 mg kg<sup>-1</sup> is considered “optimum” for crop growth [2,26]. Water-soluble P at the four locations averaged 1.31 to 3.48 mg kg<sup>-1</sup>.

#### 3.2. Impact of Soil pH on Water-Soluble Phosphorus

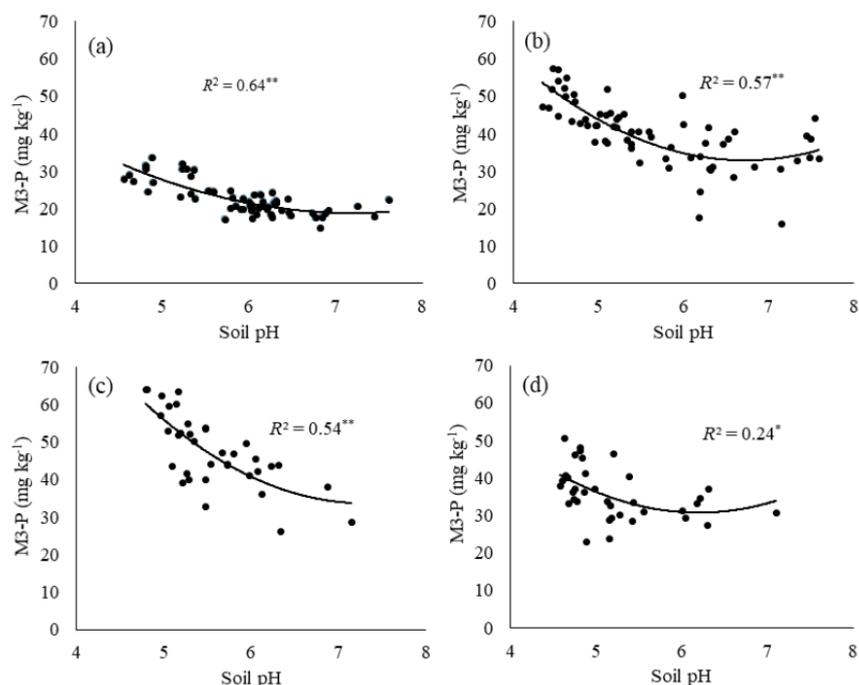
For all locations combined, there was no significant relationship between soil pH and WSP. When data were separated by location, significant curvilinear relationships were found at three of four locations (Figure 1), with relationships varying by location. Water-soluble P at Chickasha, Efaw, and Lahoma increased with increasing pH up to a pH of 5.0 to 6.0. The linear-linear and linear-plateau method revealed that the pH “breakpoint” where the pH-WSP relationship significantly changed was 5.4, 5.1, and 5.9 for Chickasha, Efaw, and Lahoma, respectively. After the pH breakpoint, the slope for the relationship turned from positive to slightly negative, indicating that further increases in pH resulted in a slope of near zero and then a decrease in WSP. For Perkins, there was no significant relationship between pH and WSP (Figure 1). The presence of two different relationships (i.e., a positive and a negative relationship) between pH and WSP, within a single soil, was expected due to the solubility patterns of various P minerals. It is generally understood that for most soils at “pseudo-equilibrium”, maximum soil P solubility will occur at a pH around 6.5 [22]. As pH decreases below 6.5, Fe and Al-P minerals precipitate. In contrast, as pH increases above 6.5, Ca-P minerals precipitate. This will vary as a function of soil mineralogy and size of soluble Ca, Al, and Fe pools. Soil organic P as Al, Fe, and Ca-phytate shows similar patterns in solubility with changes in pH, as their inorganic P mineral (and adsorbed phases) counterparts [27]. It is important to note that in addition to precipitated Al and Fe-P minerals, P adsorbed onto the surface of variable charged Al and Fe oxides/hydroxides will also tend to desorb less at low pH due to decreased competition with hydroxide [28].



**Figure 1.** Relationship between soil pH and water-soluble phosphorus (WSP) for each location: (a) Chickasha, (b) Efaw, (c) Lahoma, and (d) Perkins. Total soil P was constant within each location. \*\*, \*: indicates significance at  $p < 0.01$  and  $0.05$ , respectively, for the second-order linear regression model. ns: not significant.

### 3.3. Impact of Soil pH on Mehlich-3 Extractable Phosphorus

Since soil total P was constant within each location, measurements of M3-P additionally represent M3 extraction efficiency for P. For all locations combined, the relationship between pH and M3-P was significant and negative ( $R^2 = 0.27$ ;  $p < 0.001$ ). However, better relationships between pH and M3-P were observed when the data were separated by location (Figure 2). Previous studies have shown for extractants such as Bray-1 or M3 that decreasing soil pH can have no effect on [29,30] or can increase extractable soil test P concentrations [31]. As an extractant for P, Bray-1 and Mehlich-3 are similar in that they are both dilute strong acid solutions containing ammonium fluoride. Sharpley et al. [31] characterized Bray-1 P in 78 soils obtained from the U.S. National Soil Characterization Laboratory, and separated the soils into three different groups based on their degree of weathering. The highly weathered, slightly weathered, and calcareous soils had a median pH of 5.6, 6.3, and 7.7, respectively, which corresponded to Bray-1 P concentrations of 47, 21, and 11  $\text{mg kg}^{-1}$ . Although the study by Sharpley et al. [31] was not definitive evidence of the effect of pH on Bray-1 P because total soil P was not constant, it suggests that Bray-1 was more effective at extracting P at low pH. Since soil total P within each location was constant in the current study, results clearly illustrate that P extraction by M3 decreases with increasing soil pH. Visual examination of the data by location shows that there was a large decrease in M3-P with increasing pH until about 5.5 (Figure 2). At a pH of approximately 5.5, the change in M3-P with pH (i.e., slope) decreases, and then reaches a slope near zero at pH  $\sim 7.0$ . Perkins displayed the weakest relationship between soil pH and M3-P, although the pattern was the same for the other locations.



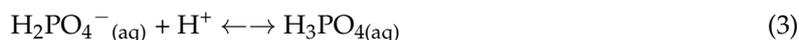
**Figure 2.** Relationship between soil pH and Mehlich-3 phosphorus (M3-P) for each location: (a) Chickasha, (b) Efaw, (c) Lahoma, and (d) Perkins. Total soil P was constant within each location. \*\*, \*: indicates significance at  $p < 0.01$  and  $0.05$ , respectively, for the second-order linear regression model. ns: not significant.

### 3.3.1. Mehlich-3 Phosphorus Extraction in Low pH Soils (<5.5)

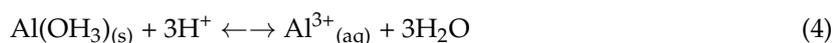
Close examination of several reactions that are occurring in the M3 extractant support the empirical observation that M3 was more effective at extracting P in low pH soils. As previously discussed, P forms in low pH soils are generally dominated by Al and Fe-P phosphates, P adsorbed onto the surfaces of Al and Fe oxides/hydroxides, and Al and Fe-phytate. Consider that the pH of the M3 solution is 2.5, and after reaction with the soil per extraction protocol, the pH of the solution will increase. In the current study, the final reacted M3 solution pH varied from 2.7 to 3.3. The acid pH level promotes the desorption of P from Al and Fe mineral surfaces as well as the dissolution of Al and Fe phosphates:



At such an acid pH level found in the M3 solution, extracted  $\text{H}_2\text{PO}_4^-$  will partly protonate according to the reaction:



and Al and Fe hydroxide minerals will partly dissolve to produce  $\text{Al}^{3+}$  and  $\text{Fe}^{3+}$  thereby increasing pH through consumption of solution protons:



where we use the common shorthand notion of  $\text{Al}^{3+}$  and  $\text{Fe}^{3+}$  to symbolize  $\text{Al}(\text{OH})_2\text{O}^+_{(aq)}$  and  $\text{Fe}(\text{OH})_2\text{O}^+_{(aq)}$ , respectively. Thus, higher clay content and Fe and Al oxides/hydroxides will contribute to neutralization of the M3 solution [32], which could reduce the amount of P extracted. However,

M3 is considered a poor extractant of Fe compared to Al [12]. This is partly due to Fe oxides and oxyhydroxide minerals such as goethite and hematite that will remain partly insoluble at pH ~3.0, while Al minerals such as gibbsite will dissolve [22]. Further, the  $\text{Al}^{3+}$  and  $\text{Fe}^{3+}$  produced from reactions 4 and 5 can supply reactions 1 and 2, which can inhibit the dissolution of Al and Fe-P or even promote precipitation of them (i.e., reactions 1 and 2 moving backwards). This is why the solubility of Fe and Al phosphate minerals in soils is dependent on the solubility of Al and Fe minerals that supply  $\text{Al}^{3+}$  and  $\text{Fe}^{3+}$ . On the other hand, any P adsorbed onto the edges of Al and Fe oxides/hydroxides will be released to solution as reactions 4 and 5 proceed under acid conditions. This is in agreement with our empirical observation of the M3 solution pH increasing from 2.5, up to 3.2.

Protons are an integral part of each of the listed reactions. At the acid pH levels of the M3 solution, the thermodynamic drive is for solution protons to be consumed, which will allow the solution pH to slightly increase. Depending on the solution composition and soil mineralogy, the solution protons may be consumed through protonation of  $\text{H}_2\text{PO}_4^-$  and  $\text{HPO}_4^{2-}$  (reaction 3), dissolution of Fe and Al-P minerals (reactions 1 and 2), or dissolution of Al and Fe oxides/hydroxides (reactions 4 and 5). The dynamics of these reactions with each other will therefore dictate the amount of P extracted by M3 in acid soils. Based on the trends shown in Figure 2 (i.e., M3-P increasing with decreasing pH), protons in the M3 extract are predominantly being consumed via reactions 1–5, with reaction products from 4 and 5 minimally inhibiting Al and Fe-P dissolution (reactions 1 and 2) at low soil pH levels, otherwise, P would not be extracted. The consumption of protons in reactions 4 and 5 will, however, increase the pH of the solution, thereby decreasing its efficiency to extract P.

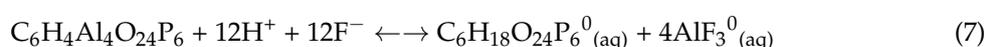
An extremely important component of the M3 (and Bray-1) extract is fluoride ( $\text{F}^-$ ). Fluoride has a strong ability to complex Al in solution, thereby dissolving Al-containing minerals by releasing any Al-associated P to solution:



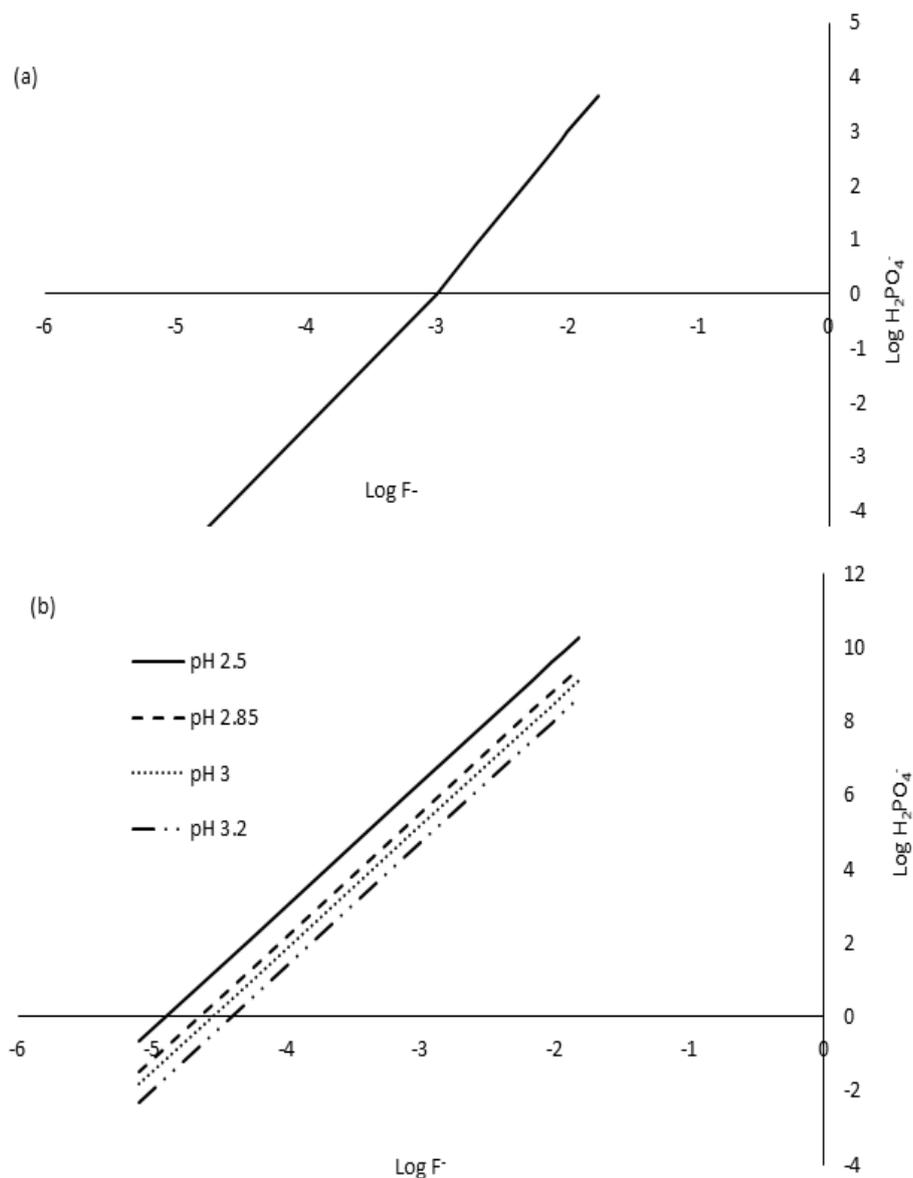
Solution complexation of Al by  $\text{F}^-$  thermodynamically promotes dissolution of Al minerals by consuming the products from reactions 1 and 4 (i.e.,  $\text{Al}^{3+}$  and  $\text{Fe}^{3+}$ ), thereby increasing the solubility of those minerals. This will directly dissolve Al-P minerals and release P adsorbed to the surface of Al hydroxides. Simulation of a M3 extraction using MINTEQA2 and assuming initial presence of soil strengite, variscite, dolomite, calcite, gibbsite, goethite, hydroxyapatite, kaolinite, vermiculite, and quartz, predicted that nearly 100% of the Al in solution was some type of Al-F solution complex. For this reason, Bray-1 was developed to include  $\text{NH}_4\text{F}$  and Mehlich-1 was improved to Mehlich-2 through addition of  $\text{NH}_4\text{F}$  [33]. However,  $\text{F}^-$  does not readily form complexes with Fe as it does with Al. For example, Swenson et al. [34] found that P release from pure Fe phosphate was small compared to Al-P. Similar results were noted by Chang and Jackson [35].

The solubility diagram in Figure 3a highlights the effectiveness of  $\text{F}^-$  in M3 (and Bray) solution to release P from Al-P minerals such as variscite. In this example, variscite equilibrium was supported by gibbsite as the Al source, and soluble Al was further speciated into F and hydroxide complexes. Clearly, increased solution  $\text{F}^-$  will promote dissolution of Al-P minerals. The pH range for this demonstration was representative of values measured in reacted M3 solutions.

Regarding organic P, phytate is considered to be the largest soil organic P pool [36]. Aluminum and Fe-phytate extraction with M3 will behave similar to inorganic P bound with Fe and Al under acid conditions. Specifically, phytate will mostly exist as Al and Fe-phytate in low pH soils, making it insoluble in water and mostly non-available to plants [37–39]. The extreme acid conditions of M3 solution along with added EDTA and  $\text{F}^-$  will remove Al and some Fe [38] from the phytate ring (IP6) and convert it to phytic acid as it protonates at low pH. An example reaction of Al-phytate with  $\text{F}^-$  in acid solution is shown below:



Phytic acid (i.e., the product of reaction 7), is soluble in water at low pH. It is important to note that extraction reactions that solubilize IP6, such as reaction 7, will not necessarily hydrolyze P from the IP6 molecule; this lack of hydrolysis makes IP6 mostly undetectable with colorimetric methods. However, non-hydrolyzed IP6 will be detected if solution P concentrations are measured with an atomic emission spectroscopy (AES) technique [40]. Thus, M3 extraction of Al and Fe-phytate followed by ICP-AES analysis will over-estimate P plant availability and solubility. Both strong acid and base solutions have been shown to extract phytic acid from soils and manure [41]. Shang et al. [42] sorbed IP6 to several clay minerals at different saturation levels, followed by extraction with M3. They found that M3 was able to remove 50–100% of IP6 from gibbsite, kaolinite, and montmorillonite, but less than 10% from goethite.

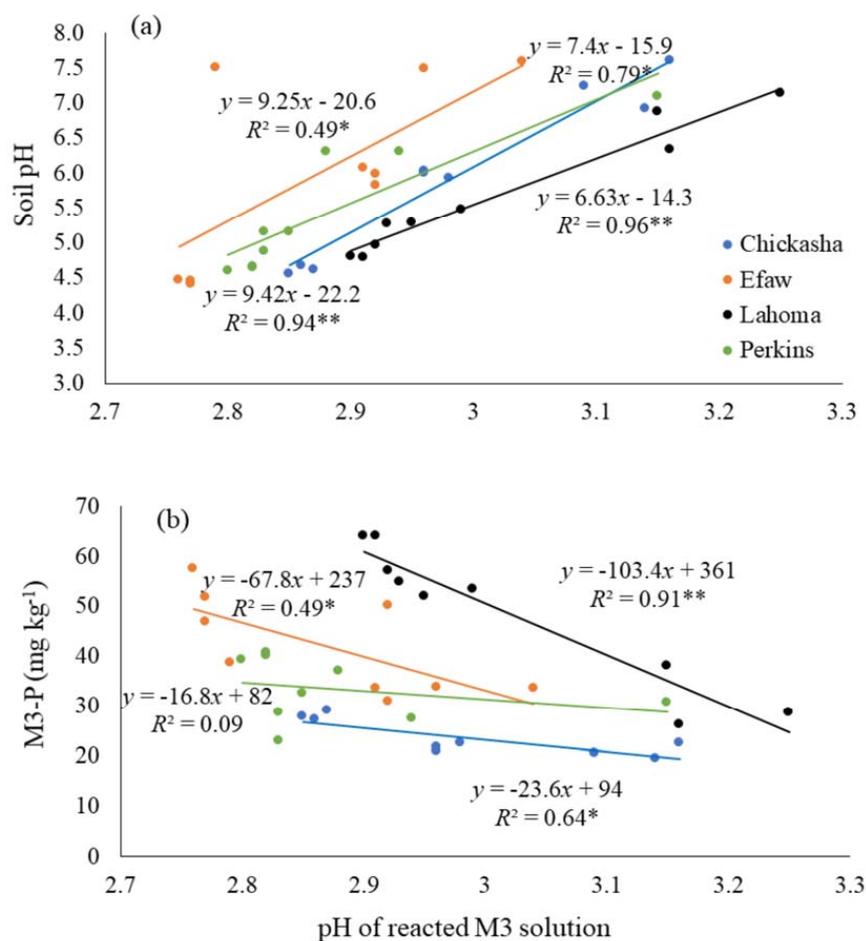


**Figure 3.** Solubility diagrams for (a) variscite and (b) hydroxyapatite (HAP) as a function of solution fluoride ( $F^-$ ) concentration. Variscite-solution equilibria were calculated with consideration of Al-hydroxide and Al-F solution complex formation. All equilibrium constants were obtained from Lindsay [21].

Acetate from M3 solution can also extract some P from soil, although it is considered relatively minor compared to the strong acids and  $F^-$  addition [32]. Briefly, acetate remains dominantly de-protonated at a pH above 4.76, and is able to compete with phosphate for adsorption sites onto valence un-satisfied hydroxide groups, thereby displacing P into solution.

### 3.3.2. Mehlich-3 Phosphorus Extraction in Medium pH Soils (5.5–7.0)

While Al-bound P is extracted by M3 in acid soils, the discussion above does not explain why P extraction by M3 decreases with increasing soil pH. Possible reasons include decreased pH of extracting solution and a shift in Al related P from Al-P minerals to P adsorbed to the surface of Al oxides/hydroxides. First, increased soil pH will result in an increased M3 extractant solution pH after soil reaction. Increased pH of the M3 solution would decrease the degree in which reactions 1, 2, 4, and 5 occur. This would result in less direct Al and Fe-P mineral dissolution, and less P release from dissolving Al and Fe oxide/hydroxides where P is adsorbed to the surface. There is evidence for this as there was a significant ( $p < 0.01$ ) and positive relationship between the reacted M3 solution and soil pH: reacted M3 pH =  $0.0807 \times \text{soil pH} + 2.4633$  ( $R^2 = 0.49$ ). Figure 4a shows the relationship between soil pH and reacted M3 solution for each soil. In essence, increasing soil pH will increase the pH of the reacted M3 extract. An increased extractant pH could also reduce the ability of the fluoride to extract P [33]. The importance of the pH of reacted M3 extracts is indicated by the fact that M3-P was inversely related to the pH of the reacted M3 extract for three of four soils (Figure 4b).



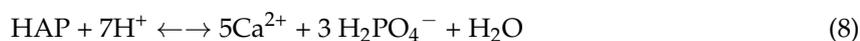
**Figure 4.** Relationship between the final pH of reacted Mehlich-3 (M3) solution and soil pH (a) and M3 phosphorus (M3-P) concentration (b). \*,\*\* indicate statistical significance at  $p < 0.05$  and 0.01, respectively.

Second, at this soil pH range, Al is generally less soluble and exists more as Al oxide and hydroxide minerals than as Al-P minerals. This is due to the fact that Al hydrolyzes rapidly as pH increases above 5, with hydroxyl becoming a better competitor for Al than P [43]. This will also shift the P forms associated with Al from Al-P minerals to P bound onto the surface of Al oxide/hydroxide minerals via ligand exchange [28,44,45]. Consider that for many Al-P minerals, the Al:P molar ratio is less than 2:1 and approaches 1:1 when pH is low enough to prevent extensive Al hydrolysis [45]. For P bound onto Al mineral surfaces after Al hydrolysis, the Al:P molar ratio will be much greater, often near 6:1. This is due to the fact that only the surface Al-OH groups are able to react with solution P, specifically only those which are valence unsatisfied functional groups. The result is that for each mole of Al-P mineral dissolved in M3 solution, more P will be released to solution compared to dissolution of a mole of Al hydroxide mineral with P bound to its surface. In this regard, the decreased P extracted at medium pH is partly due to how the soil P is distributed at a medium pH level compared to low pH, since the total P remains the same.

### 3.3.3. Mehlich-3 Phosphorus Extraction in High pH Soils (>7.0)

With further increase in pH (>7.0), soil P further shifts from Al and Fe related P into Ca-P, including Ca-phytate [37,41], although Ca-P is not relegated to soils with pH > 7.0. Since Ca-P is highly soluble in acid solutions, it is expected that M3 extraction of P would increase and potentially overestimate plant available P at pH > 7.0. While M3 may or may not be overestimating plant available P in these high pH soils, P extraction efficiency (the proportion of total P extracted by M3) did not dramatically change with pH increasing above 7.0, although the extraction of P is clearly depressed compared to pH < 5.5 (Figure 2). It should be noted that the number of soils in this high pH range (13) was somewhat limited in the current study.

There are two potential mechanisms for M3 extraction of Ca-P: dissolution via decreased pH and precipitation of fluorite (CaF<sub>2</sub>). Consider the dissolution of a standard soil Ca-P mineral, hydroxyapatite (HAP):



The addition of acid promotes formation of the dissolution products. Another possible reaction with M3 and Bray-1 solution is the formation of fluorite:



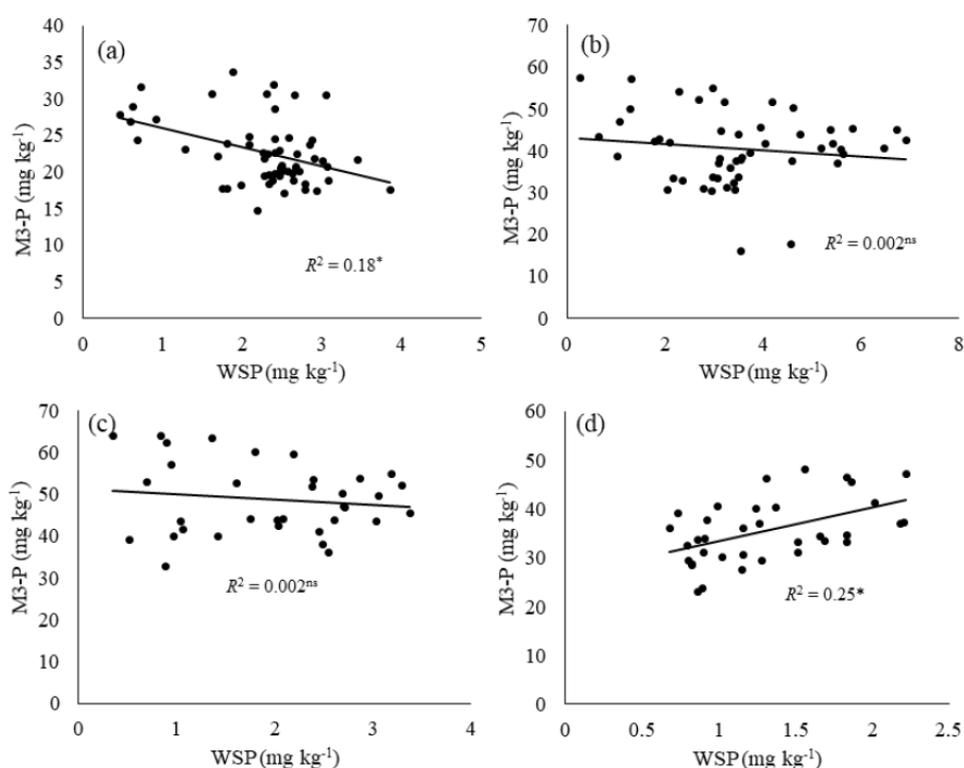
The consumption of Ca<sup>2+</sup> in Ca-P by added F<sup>-</sup> from the extracting solution (reactions 8 and 9) will promote dissolution of Ca-P. Figure 3b illustrates theoretical HAP dissolution with increasing solution F<sup>-</sup> concentration. A similar reaction would occur with Ca-phytate, releasing phytic acid into solution where it would be measured through solution analysis by AES methods. The formation of fluorite can indeed occur during the short 5 min extraction period for M3 [33] and Bray-1 [46]. For example, Mehlich [33] extracted commercial dibasic Ca phosphate (DCP) and tribasic Ca phosphate (TCP) with Bray and Mehlich-2 (same as M3 except no EDTA). He showed that although 70–80% of P was extracted, only 21% to 47% of Ca was dissolved in solution, indicating formation of another Ca mineral. Fluoride is less effective at dissolving more basic and stable Ca-P such as HAP and fluoroapatite at neutral pH, but will solubilize these minerals if the F<sup>-</sup> solution is acidified [32,33,35]. Consumption of Ca by F<sup>-</sup> will not only dissolve Ca-P, but also non-P minerals that contain Ca. Therefore, higher amounts of soil Ca not associated with P will reduce the ability of M3 to extract P [47]. For the current study, this is supported by the fact that water-soluble Ca and M3-Ca were significantly and positively correlated to soil pH ( $R^2 = 0.60$  and  $0.58$ , respectively), which may have decreased M3-P extraction efficiency for soils with higher pH levels.

Figure 3b also illustrates decreased Ca-P solubility with greater reacted extractant pH, as evident from reaction 8. Mehlich [33] also emphasized the importance of solution acidity in the ability to extract Ca-P. This suggests the possibility that higher pH soils containing Ca-P will have less P extracted

by M3 due to increased pH of the reacted extraction solution, as was previously noted in this study. Soils with higher clay contents, CEC, and Fe/Al oxides/hydroxides will further enhance this effect by buffering the soil pH more effectively than sandy soils. As previously discussed, there was a significant positive correlation between soil pH and the reacted M3 solution pH. The pH of the M3 extractant did not remain below the recommended level of 2.9 for proper P extraction [33] for many of the soils in this study (Figure 4); pH of reacted M3 extracts in this study ranged from 2.7 to 3.3, with a mean of 2.9. Similarly, Ebeling et al. [48] showed that the Bray-1 extractant could be partly neutralized by certain soils, which reduced its capacity to dissolve soil P. Mallarino [49] found that at pH < 7.4, Bray-1 extracted nearly the same amount of P as M3, but at pH > 7.4, soils with Ca carbonate content > 4% extracted much less P with Bray-1 than M3. This was attributed to a greater buffering capacity of M3 compared to Bray-1. For this reason, M3 is generally recommended over Bray-1 in high pH soils.

### 3.4. Relationship between Mehlich-3 and Water-Soluble Phosphorus

Unlike previous studies [2,8–10,12], there was no significant positive relationship observed between soil WSP and M3-P. McDowell and Sharpley [9], however, showed that the WSP vs. M3-P relationship improved when separating data based on soil types. Separation by location for the current study only resulted in a significant positive correlation for the Perkins location (Figure 5). The significant negative relationship for the Chickasha location suggests that M3-P extraction efficiency is decreasing due to either consumption of  $F^-$  by Ca in minerals that do not contain Ca-P and/or neutralization of the extraction solution (reaction 8 and Figures 3b and 4).



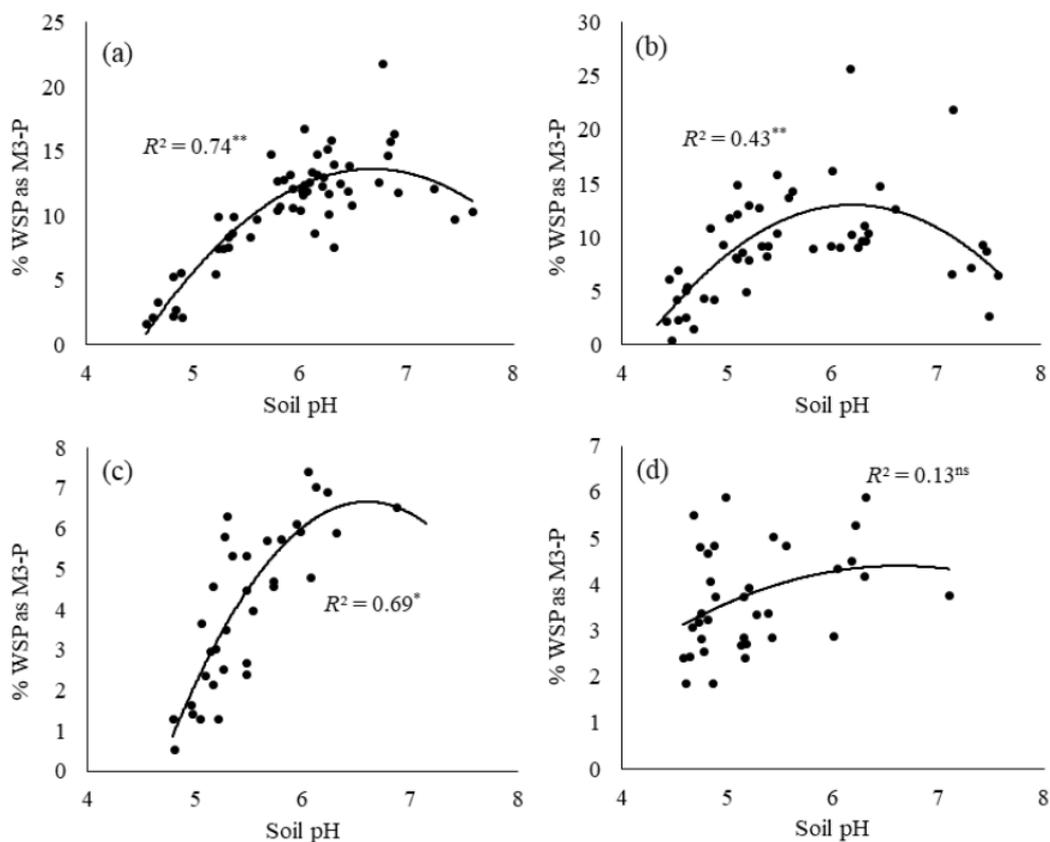
**Figure 5.** Relationship between soil water-soluble phosphorus (WSP) and Mehlich-3 phosphorus (M3-P) for each location: (a) Chickasha, (b) Efaw, (c) Lahoma, and (d) Perkins. Total soil P was constant within each location. \*: indicates significance at  $p < 0.05$ , for the second-order linear regression model. ns: not significant.

Using the pH point where the WSP vs. pH relationship changed (Figure 1), the M3-P vs. WSP in Figure 5 was further examined by separating the data at each location (except Perkins) according

to each respective “break-point” pH. Fuhrman et al. [8] improved the relationship between M3-P and WSP by separating their soils into three groups based on pH: <5.5, 5.5–7, and >7. However, no appreciable improvement was observed in this study when separating data based on change-point pH. In contradiction to the current study, previous studies showing significant correlations between M3-P and WSP did not utilize soils with the same total P contents, which suggests that while pH clearly has an impact on P solubility in M3 solution and water (Figures 1 and 2), total soil P has a more substantial effect. Thus, results from the current study offer greater insight into the relationship between M3-P and WSP, and how it is affected by pH compared to previous studies.

To further explore the relationship between WSP and M3-P, WSP was expressed as a percentage of M3-P, and plotted against soil pH. In general, a lesser percentage of M3-P was soluble in water for Lahoma and Perkins compared to Chickasha and Efaw (Figure 6). This appears to be due to a lower water solubility in Lahoma and Perkins, which may be the result of greater M3-Al levels (Table 1), as Al will react with P at acid to neutral pH levels. The results in Figure 6 are similar to Figure 1: maximum P solubility expressed as a percentage of M3-P occurs in the pH range of 6.0 to 7.0, with decreasing solubility below this range. Above pH 7.0, %WSP as M3-P somewhat decreased for Chickasha and Efaw, and appears to mostly plateau for Lahoma and Perkins (Figure 6). At low pH, P solubility in water is low and P is highly extracted by M3, resulting in the minimum level of %WSP as M3-P. At the middle pH value of about 6.5, P solubility in water is at a maximum (Figure 1) while P extraction by M3 is approaching a minimum (Figure 2), causing the maximum in %WSP as M3-P (Figure 6). Although the trend for the Lahoma and Perkins locations is not as distinct as Chickasha and Efaw, this is likely due to the fact that the soil pH levels for these soils did not extend as high as Chickasha and Efaw (maximum pH for Chickasha, Efaw, Lahoma, and Perkins was 7.6, 7.6, 7.1, and 7.1, respectively). Nevertheless, observation of the data in Figure 6 suggests that the general patterns were similar among locations. Similarly, Fuhrman et al. [8] found that the highest slope for the WSP vs. M3-P relationship (indicating a larger value of WSP per unit of M3-P) was for the soil group with pH 5.5–7.0, followed by pH < 5.5, and finally pH > 7.0. The pH range where %WSP as M3-P is fairly constant may indicate the ideal range for appropriate M3 use. This pH breakpoint was estimated using the linear-linear and linear-plateau models (see methods). The resulting values were 5.87, 5.29, 6.0, and 4.68 for Chickasha, Efaw, Lahoma, and Perkins, respectively ( $p < 0.05$ ). Based on the overall average of 5.5, this suggest that soils with pH < 5.5, use of M3-P should be exercised with caution. These data also suggest that excessively high pH may interfere with M3-P extraction, although it is difficult to assess pH > 7.0 using the current dataset because that pH range contained the least number of data points.

It is probably not a coincidence that the Perkins location was the anomaly (Figure 5) given that its general soil characteristics were somewhat different from the other locations (Table 1). Specifically, while Perkins possessed a similar range in M3-P, its range in WSP was limited compared to other locations. Perkins also contained the least amount of M3-Ca. Mehlich-3 P decreased less with increasing pH compared to other locations, while WSP was relatively constant (Figures 1 and 2). The result was a slightly positive correlation between the two (Figure 5) and a relatively constant %WSP as M3-P (Figure 6). We hypothesize that the lower soil Ca at Perkins prevented extreme reductions in the ability of M3 to extract P with increasing pH that occurs with complexation of  $F^-$  with Ca from non-Ca-P minerals (reaction 9) compared to other soils. Recall that M3-Ca and WS-Ca were positively and significantly correlated with pH for all soils. In fact, for any given extractant pH level, Perkins had the lowest M3-Ca. Additionally, the poor pH buffering capacity of Perkins due to its high sand content and lower CEC than the other soils (“active vs. superactive”: Table 1) would also prevent great reductions in the ability of M3 to extract P. This is supported by the relationship between soil pH and extraction pH (Figure 4a); the locations with the highest sand content, Perkins and Efaw, had the lowest extractant pH for any given soil pH, compared to Chickasha and Lahoma.



**Figure 6.** Impact of soil pH on the amount of water-soluble phosphorus expressed as a percentage of Mehlich-3 phosphorus (%WSP as M3-P) for each location: (a) Chickasha, (b) Efaw, (c) Lahoma, and (d) Perkins. Total soil P was constant within each location. \*\*, \*: indicates significance at  $p < 0.01$  and  $0.05$ , respectively, for the second-order linear regression model. ns: not significant.

### 3.5. Soil Phosphorus Forms with Changes in pH

Speciation of soil water extracts can provide valuable information on what solid P forms may be present, or expected to precipitate at equilibrium, and therefore help to explain the observed trends of WSP and M3-P with changing pH at constant total P. It is important to keep in mind that such predictions based on solution analysis and thermodynamic modelling are not meant to be understood as absolute in this case, but only to provide some insight into the type of potential minerals and patterns that may be influencing P solubility. For example, while HAP is often predicted by such thermodynamic techniques, it may truly be a meta-stable Ca-P mineral that is present and controlling solubility. This is more likely since our extraction method used a relatively low soil:water solution ratio (1:10) and a short equilibration time (1 h). Likewise, strengite and variscite may not truly be present, but we utilize such predictions as indicators of P associated with Fe and Al. Table 2 lists the results of the speciation of the water extract, indicating the predicted minerals present for each soil sample, as well as the percentage of Al, Ca, Fe, Mn, and P that is likely to be precipitated at equilibrium. Percent precipitated Mg was not shown as it was 0% for every sample.

For all locations, Al and Fe were mostly precipitated at all pH levels, while Ca and Mn precipitation increased with pH. Iron was predicted to be precipitated as hematite at all pH levels. No prediction of strengite was predicted, although it is likely that some amorphous Fe-P minerals were present as these minerals are not included in the MINTEQA2 database. Aluminum was predicted to precipitate as both gibbsite and variscite, with the former occurring at all pH levels and variscite only at acid pH levels (mostly below 6.0). With increasing pH, Al hydrolysis increases and forms Al hydroxides such as gibbsite (i.e., reverse of reaction 4), which also consumes the Al contained in variscite. Increasing

pH beyond that, any P adsorbed onto the surfaces of Al and Fe oxides/hydroxides becomes less stable. Calcium was predicted to precipitate as HAP as pH levels increased above 7.0, and Mn was predicted to precipitate as a Mn-phosphate mineral over much of the pH range of all soils in this study.

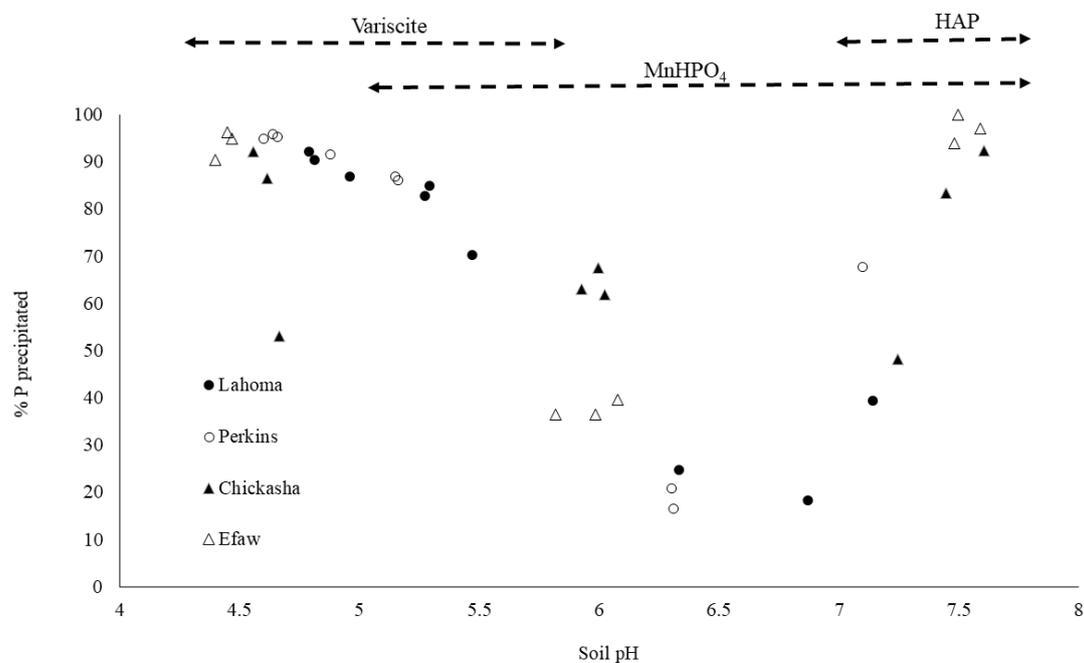
**Table 2.** Speciation model output of water soluble extracts indicating percent Al, Ca, Fe, Mn, and P precipitated and predicted solid minerals, as a function of soil pH and location. Minerals listed are not meant to explicitly indicate those specific minerals, only the general presence of Al, Mn, and Ca-related P and oxides/hydroxides. Gib = gibbsite, Var = variscite, Hem = hematite, HAP = hydroxyapatite.

Location	Soil pH	Percent Precipitated					Predicted Solid Minerals
		Al	Ca	Fe	Mn	P	
Lahoma	4.79	99.6	0	100	0	92.2	Var, Hem, Gib
Lahoma	4.81	99.6	0	100	0	90.5	Var, Hem, Gib
Lahoma	4.96	99.8	0	100	0	86.9	Var, Hem, Gib
Lahoma	5.27	100	0	100	39.8	82.9	Var, Hem, Gib, MnHPO <sub>4</sub>
Lahoma	5.29	100	0	100	55.1	85	Var, Hem, Gib, MnHPO <sub>4</sub>
Lahoma	5.47	100	0	100	75.1	70.3	Var, Hem, Gib, MnHPO <sub>4</sub>
Lahoma	6.33	100	0	100	94.1	24.9	Hem, Gib, MnHPO <sub>4</sub>
Lahoma	6.87	100	0	100	99.2	18.4	Hem, Gib, MnHPO <sub>4</sub>
Lahoma	7.14	100	1.5	100	97.3	39.4	Hem, Gib, MnHPO <sub>4</sub> , HAP
Perkins	4.66	99.9	0	100	0	95.3	Var, Hem, Gib
Perkins	4.64	99.9	0	100	0	95.8	Var, Hem, Gib
Perkins	4.6	99.9	0	100	0	94.8	Var, Hem, Gib
Perkins	4.88	99.9	0	100	0	91.6	Var, Hem, Gib
Perkins	5.15	100	0	100	1.4	86.8	Var, Hem, Gib, MnHPO <sub>4</sub>
Perkins	5.16	100	0	100	0	86.1	Var, Hem, Gib
Perkins	6.31	100	0	100	98.9	16.5	Hem, Gib, MnHPO <sub>4</sub>
Perkins	6.3	100	0	100	98.5	20.9	Hem, Gib, MnHPO <sub>4</sub>
Perkins	7.1	100	6.9	100	98.5	67.7	Hem, Gib, MnHPO <sub>4</sub> , HAP
Chickasha	4.56	99.2	0	100	1.1	92.1	Hem, Gib, MnHPO <sub>4</sub>
Chickasha	4.6	99.3	0	100	1.5	86.5	Var, Hem, Gib
Chickasha	4.67	99.5	0	100	0	53	Var, Hem, Gib
Chickasha	5.93	100	0	100	94.6	63	Hem, Gib, MnHPO <sub>4</sub>
Chickasha	5.99	100	0	100	94.5	67.6	Hem, Gib, MnHPO <sub>4</sub>
Chickasha	6.03	100	0	100	95.4	61.9	Hem, Gib, MnHPO <sub>4</sub>
Chickasha	7.25	100	1.7	100	99	48.1	Hem, Gib, MnHPO <sub>4</sub> , HAP
Chickasha	7.44	100	4.8	100	95.9	83.3	Hem, Gib MnHPO <sub>4</sub> , HAP
Chickasha	7.61	100	5.4	100	89.9	92.3	Hem, Gib MnHPO <sub>4</sub> , HAP
Efaw	4.40	13	0	100	4.4	65.3	Var, Hem, MnHPO <sub>4</sub>
Efaw	4.45	97.1	0	100	0	96.2	Var, Hem, Gib
Efaw	4.47	95.6	0	100	0	94.8	Var, Hem, Gib
Efaw	5.82	100	0	100	96.5	36.4	Var, Hem, Gib, MnHPO <sub>4</sub>
Efaw	5.99	100	0	100	98.4	36.4	Hem, Gib, MnHPO <sub>4</sub>
Efaw	6.08	100	0	100	97.4	39.5	Hem, Gib, MnHPO <sub>4</sub>
Efaw	7.50	98.2	0	100	8.2	99.9	Hem, Gib, MnHPO <sub>4</sub>
Efaw	7.48	100	6.5	100	95.8	93.9	Hem, Gib, MnHPO <sub>4</sub> , HAP
Efaw	7.59	100	4.5	100	87	97.1	Hem, Gib, MnHPO <sub>4</sub> , HAP

The data in Table 2 for predicted percentage of P precipitated and P minerals are shown as a function of pH in Figure 7. As expected, the minimum %P precipitated occurs at pH 6.0–7.0, with precipitation increasing below and above this pH range (Figure 6) which is in agreement with Figure 1. Notice that all soils generally fell on the same line, indicating the universality of P solubility predicted by P forms and pH, and that P forms and pH ultimately control soil P solubility. As expected, variscite and HAP dominate at low and high pH, respectively, and the minimum in P precipitation occurs in the absence of these two minerals. This re-enforces the common recommendation that maximum P solubility occurs at pH ~6.5, and supports observations in Figures 1 and 5. Penn and Bryant [50] showed similar results in soils with constant total P: as pH increased, WSP decreased

and HAP was predicted at equilibrium, as determined by plotting data on double-function solubility diagrams. Similarly, McDowell et al. [51] identified solid Ca-P and Al-P minerals in arable soils (pH ~7) from Rothamsted UK, using solid state nuclear magnetic resonance spectroscopy.

The presence of Mn-P was unexpected, although the  $\text{MnHPO}_4$  mineral appears to be stable over a broad pH range. Little is known or discussed about Mn-P; however, Mn phosphates can control solution Mn [52] and P [53] concentrations in some soils. Boyle and Lindsay [54] showed that HAP was less soluble (i.e., more stable) than  $\text{MnHPO}_4$  at a pH greater than 6.0, and Mn phosphates were often more stable than variscite and strengite. With a maximum in soil WSP (Figure 1), minimum in percent P precipitation (Figure 6) at pH 6.0–7.0, and knowing that  $\text{MnHPO}_4$  is the dominant mineral in this pH range (Table 2 and Figure 6), this suggests that  $\text{MnHPO}_4$  is soluble at around pH 6.5, supporting observations made by Boyle and Lindsay [54] and Schwab and Lindsay [52]. It is important to keep in mind that Mn solubility is strongly influenced by redox status [22]. The Mn mineral predictions in these soils are somewhat incomplete since *pe* was not measured, although all soils were well drained and highly oxidized. While the presence of Mn-P minerals was predicted to exist in these soils over a wide pH range, it is unknown how M3 might extract such minerals. Since appreciable Mn concentrations were measured in the M3 extracts, from about 15 to 180  $\text{mg kg}^{-1}$ , it is likely that Mn-P is also being extracted.



**Figure 7.** Percent phosphorus (%P) precipitated as a function of pH, and potential solid-phase P minerals as predicted by solution modelling. Minerals listed are not meant to explicitly indicate those specific minerals, only the general presence of Al, Mn, and Ca-related P.

#### 4. Conclusions and Implications

Soil pH had a dramatic influence on M3-P values when total P was constant. Depending on location, M3-P varied from 40 to 55% with changes in pH from approximately 4.5 to 7.5, which equates to variation from 25 to 35  $\text{mg kg}^{-1}$  M3-P. Considering that recommended agronomic optimum P is often around 30  $\text{mg kg}^{-1}$ , this degree of variation due to pH could result in incorrect fertilizer recommendations. For both agronomic crop production and environmental testing related to P losses in runoff, the quantity-intensity (Q-I) concept is often used to describe soil P availability. The WSP concentration, i.e., intensity, is of utmost immediate importance to a growing crop, as plant roots absorb P directly from the soil solution. Similarly, dissolved P losses in runoff and leaching represent

the P form that is immediately bioavailable to aquatic ecosystems, making it a potent eutrophication agent [55]. In crop production, the goal is to maintain a sufficient P intensity (i.e., WSP), which is replenished from various solid P pools in the soil, i.e., the quantity. Agronomic and environmental soil testing would be incomplete without a measurement of the quantity, or the long-term ability of the soil to replenish P to solution over time and the growing season. In this regard, M3 and other agronomic soil tests were designed to serve as quick indicators of the Q-I status of the soil [32,56]. Many studies have found that M3-P was well correlated to WSP [2,8–10,12] and that M3-P concentrations were highly influenced by soil total P concentrations. The relation of M3-P to both total and WSP is why it has been a useful indicator of plant-available P and potential for non-point P loss. However, the current study clearly shows that soil pH had a strong influence on M3-P for the four soil types examined, and therefore complicates interpretation of plant availability and the potential for non-point dissolved losses.

Rather than utilize M3-P as a sole indicator of Q-I status, the results of this study suggest that for these soils, M3-P and WSP together could serve as an improved measurement of the Q-I relationship, specifically with M3-P and WSP as indicators of Q and I, respectively. Mehlich-3 P was not correlated with WSP for three of the four locations, indicating that they were extracting different ratios of soil P pools. Because soil tests were purposely developed to indicate both quantity and intensity throughout the growing season [32], it follows that soil tests such as M3 should therefore be positively correlated with both quantity and intensity (such as WSP). At low pH, M3 is expected to be highly efficient at extracting Al-related P (Figure 2), likely due to the use of an acidic and fluoride-rich extraction solution, yet at low pH levels, Al-P is poorly water soluble [22], which will be reflected in plant availability [57], coinciding with the minimum water solubility (Figures 1, 6 and 7). This could help explain the common observation that potatoes often respond to P fertilizer, even under conditions where soil test P is optimum [58], as potatoes are mostly grown in fairly acidic soils. Mehlich-3 is generally a poor extractor of Fe compared to Al, partly due to the lack of complex formation with  $F^-$  and short reaction time. At pH 6.0–6.5, where soil P pools are generally shifting from Al/Fe phosphate minerals and Al/Fe-phytate to Mn-P and P adsorbed onto the surfaces of Al and Fe oxides/hydroxides that possess higher Al:P ratios, M3-P extraction greatly decreased. The 6.0–6.5 pH range was confirmed as the maximum WSP, which is traditionally considered to be due to the existence of the highest solubility coexistence point for P Al, Fe, and Ca-P minerals, as Fe and Al are forming oxides and hydroxides, and Ca-P solubility is not yet highly depressed compared to pH 7 and above (Figure 7; [22]). This expected shifting of P forms was also observed in this study (Figure 7 and Table 2). However, M3 was not able to reflect this maximum in P water solubility at around pH 6.5 (Figure 2). Mehlich-3 P extractions also did not capture decreasing WSP at higher pH levels (Figures 1 and 2) that occurred presumably due to formation of Ca-P and potentially, Mn-P [54] (Figures 1, 2 and 7). Other studies have shown the presence of Ca-P formation with increasing pH above 7, along with decreasing WSP ([50,51]). Partial neutralization of the M3 extractant and consumption of  $F^-$  by Ca from non Ca-P minerals with increasing soil pH levels may have reduced extraction of soil P. The pH of the M3 extractant did not remain below the recommended level of 2.9 for proper P extraction [33] for many of the soils in this study. Thus, the data from this study suggest that the reason for increased ability of M3 to extract P at acid pH levels is likely due to both the changes in P forms and the M3 solution, as influenced by soil properties and pH. It is difficult to separate these two factors because P forms and the final reacted M3 pH are both strongly impacted by soil pH.

In addition to the use of WSP as an indicator of P intensity, the results of this study suggest that caution should be exercised when utilizing M3-P for agronomic and environmental purposes at pH levels < 5.5, or perhaps M3 should be calibrated separately at this pH range. This recommendation is based on the Q-I perspective and evidence of a near constant %WSP as M3-P at pH greater than 5.5 (Figure 6), verified by linear-linear and linear-plateau analysis. Likewise, a contrast analysis confirmed that soil M3-P, WSP, and %WSP as M3-P was significantly different for pH group < 5.5, compared to soil pH groups 5.5–7.0 and > 7.0. However, there was no significant difference between the pH

groups 5.5–7.0 and > 7.0. It is difficult to make conclusions for the pH range > 7.0 in this study with so few data points, but it is possible that M3-P could be inefficient at pH values well above 7.0 because the %WSP as M3-P began to somewhat decrease at pH > 7.0. With total P constant at each location, M3-P extraction mostly decreased with increasing pH. For environmental P indices that rely on the relationship between M3-P and total soil P, this would impact the prediction of potential non-point total P losses. Overall, while this study provided some information about M3-P extraction mechanisms and potential shortfall that may occur in some soils, further research is needed to obtain a more complete understanding that can help lead to improved soil fertility recommendations using M3-P.

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