Phosphate fertilizer impacts on glyphosate sorption by soil

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Abstract
This research examined the impact of field-aged phosphate and cadmium (Cd) concentrations, and fresh phosphate co-applications, on glyphosate sorption by soil. Soil samples were collected in 2013 from research plots that had received, from 2002 to 2009, annual applications of monoammonium phosphate (MAP) at 20, 40 and 80 kg P ha⁻¹ and from products containing 0.4, 70 or 210 mg Cd kg⁻¹ as an impurity. A series of batch equilibrium experiments were carried out to quantify the glyphosate sorption distribution constant, Kd. Extractable Cd concentrations in soil had no significant effect on glyphosate sorption. Glyphosate Kd values significantly decreased with increasing Olsen-P concentrations in soil, regardless of the pH conditions studied. Experiments repeated with a commercially available glyphosate formulation showed statistically
similar results as the experiments performed with analytical-grade glyphosate. Co-applications of MAP with glyphosate also reduced the available sorption sites to retain glyphosate, but less so when soils already contain large amounts of phosphate. Glyphosate Kd values in soils ranged from 173 to 939 L kg\(^{-1}\) under very strong to strongly acidic condition but the Kd was always < 100 L kg\(^{-1}\) under moderately acidic to slightly alkaline conditions. The highest Olsen-P concentrations in soil reduced Kd values by 25-44% relative to control soils suggesting that, under moderately acidic to slightly alkaline conditions, glyphosate may become mobile by water in soils with high phosphate levels. Otherwise, glyphosate residues in agricultural soils are more likely to be transported off-site by wind and water-eroded sediments than by leaching or runoff.

**Keywords:** Glyphosate; Batch equilibrium experiment; Sorption distribution constant; Mono ammonium phosphate; Cadmium; Competitive effect.

1. **Introduction**

Glyphosate [N-(phosphonomethyl) glycine] is a broad spectrum, non-selective systemic, post-emergent herbicide introduced for agricultural use in the 1970s. Glyphosate-tolerant crops were commercialized in 1996 (Benbrook, 2012) and glyphosate now accounts for about one-fourth of global herbicide sales (GSBR, 2011). The glyphosate molecule contains a phosphonic acid moiety that facilitates molecule sorption by soil (Sprankle et al., 1975). With more than 40 million tons of phosphate fertilizers applied on agricultural land around the world (FAO, 2012), a wide range of studies have focused on phosphate and glyphosate interactions in soil (de Jonge et al., 2001, Gimsing and Borggaard, 2007; Gimsing et al., 2007; Kanissery et al., 2015). Glyphosate and inorganic phosphate have shown to compete for the same sorption sites (de Jonge and de Jonge,
1999) and hence phosphorus applications may influence the bioavailability and transport potential of glyphosate in soil (Gimsing and Borggaard, 2002a). Phosphate fertilizers contain cadmium (Cd) as an impurity and repeated application of phosphate fertilizer results in Cd accumulation in soil (Lambert et al., 2007). The addition of Cd to glyphosate solutions in the laboratory has been shown to enhance glyphosate sorption by soil because Cd and glyphosate form complexes that are retained on the surface of negatively-charged soil colloids (Zhou et al., 2004). The effect of impurities of Cd in phosphate fertilizers on glyphosate sorption in soil is not known.

The batch equilibrium procedure is a common method to examine the effect of phosphate additions on glyphosate sorption. All researchers, except de Jonge et al. (2001), added phosphate to soil in the laboratory prior to or during the batch equilibrium experiment, and hence exclusively studied the effect of “fresh” phosphate on glyphosate sorption. Phosphate did compete with glyphosate sorption in a wide range of soils (Sprankle et al., 1975; de Jonge and de Jonge 1999; de Jonge et al., 2001; Kanissery et al., 2015), but not in other soils (Gimsing et al., 2004). Some research demonstrates that phosphate is preferentially sorbed by clay minerals (Gimsing and Borggaard, 2001), for example because the phosphate molecule (0.25 nm) is smaller than the glyphosate molecule (0.43 nm) (Cáceres-jensen et al., 2009). The impact of phosphate on reducing glyphosate sorption was stronger for synthesized Fe and Al-oxides than for pure clay-minerals (Gimsing and Borggaard, 2002b). All batch equilibrium studies utilized analytical-grade glyphosate, while herbicide products applied on agricultural land contain other ingredients that could impact the sorption of the active ingredient by soil (Farenhorst and Bowman 1998). Hence, in evaluations of the impact of phosphate additions on glyphosate sorption, it is desired to include a commercially available glyphosate formulation in the study.
Borggaard (2011) reported that the interaction of phosphate and glyphosate sorption remains unclear, even though this interaction has been studied for four decades. de Jonge et al. (2001) is the only study in these four decades to have examined the effect of “aged” phosphate on glyphosate sorption. Utilizing soil from field plots that had received various amounts of phosphate and lime fertilizers for more than 60 years, batch equilibrium studies indicated that less glyphosate was sorbed in soils with greater Olsen-P concentrations. To the best of our knowledge, the combined effect of fresh and aged phosphate on glyphosate sorption in soil has not been investigated.

The objective of this study was to examine under a range of pH conditions, the impact of field-aged phosphate and cadmium concentrations on glyphosate sorption by soil, with and without phosphate co-applications in the laboratory. The pH conditions were chosen to include the range of possible glyphosate speciations, as described by Wang et al. (2004). In general, studies have reported that glyphosate sorption generally decreases with increasing soil pH (McConnel and Hossner, 1985; de Jonge and de Jonge, 1999; Gimsing et al., 2004).

2. Materials and Methods

2.1. Experimental Design and Soil Characteristics

Soil samples (0-15cm) with a sandy clay loam texture were collected in the spring 2013 from research plots situated under a durum wheat and flax rotation near Carman (49° 29.7’ N, 98° 2.4’ W), Manitoba, Canada. The soil is classified as an Orthic Black Chernozem based on the Canadian System of Soil Classification, which is approximately equivalent to the Udic Boroll subgroup in the U.S. Soil Taxonomy (CSSC, 1998). The experimental plot was a randomized complete block
design with 10 treatments and 4 replicates per treatment. In each of the forty plots, the composite soil sample consisted of ten samples collected in the plot using a Dutch augur. Treatments were a control (neither phosphate nor Cd applications), and plots receiving from 2002 to 2009 annual applications of mono ammonium phosphate (MAP) fertilizers that originated from three different phosphate rock sources containing 0.4, 70 or 210 mg Cd kg$^{-1}$, or low, medium and high Cd, respectively (Grant et al., 2013). MAP from these three sources was applied to plots at 20, 40 and 80 kg P ha$^{-1}$, or 20P, 40P and 80P, respectively. For all plots that received MAP, 20 kg P ha$^{-1}$ was placed near the seed to enhance fertilizer use efficiency, a common practice in Canadian Prairie agriculture. For the 40 and 80 kg P ha$^{-1}$ treatments, to avoid seedling toxicity, the additional MAP was broadcasted and then incorporated in soil. From 2010 to 2013, the rotation was continued but no phosphate or Cd was applied. Nitrogen fertilizer varied by year to optimize yields. The typical rate of N applied was 90 kg N ha$^{-1}$ in durum wheat and 50 kg N ha$^{-1}$ in flax.

Soil samples were air-dried and sieved (<2mm) prior to soil properties analysis and sorption studies. Soil was digested with nitric acid and total Cd was determined by *inductively coupled plasma (ICP)* (U.S. EPA, 1996). Extractable Cd was extracted with *diethylene triamine pentaacetic acid (DTPA)* following procedures as recommended by Lindsay and Norvell (1978), and extracts were analyzed by *ICP* (Whitney, 2011). Various factors have been shown to influence the efficiency of micronutrient extraction by DTPA, including extraction temperature and shaking time (Lindsay and Norvell, 1978).

Available phosphate was extracted using Olsen (NaHCO$_3$) phosphorus test (Frank et al., 2011).

Soil physical and chemical properties that are known to influence glyphosate and phosphate sorption by soil, but did not significant vary across the plots by treatment, were also determined. Soil organic carbon content was determined using combustion technique with a high temperature.
induction furnace (Nelson and Sommers, 1996). Extractable Fe$_2$O$_3$ and Al$_2$O$_3$ were extracted with DTPA (Whitney, 2011) and 0.01M CaCl$_2$ (Barnhisel and Bertsch, 1982), respectively, and extracts were analyzed by ICP. Extractable Ca was also measured by ICP using ammonium acetate as an extractant (Warncke and Brown, 2011). Results were soil organic carbon content: 2.80% (mean) ± 0.04 (standard error) (n=16, number of plots analyzed); extractable Fe$_2$O$_3$: 246 ± 5 mg kg$^{-1}$ (n=40), extractable Al$_2$O$_3$: 6.4 ± 0.65 mg kg$^{-1}$ (n=16); and extractable Ca: 2,252 ± 40.57 mg kg$^{-1}$ (n=16). Given that the study focused on Cd and P applications as treatments, the concentrations of extractable and total Cd, as well as Olsen-P in all plots were determined. We did not expect to see treatment differences for the other parameters that were measured (i.e., extractable Fe$_2$O$_3$, Al$_2$O$_3$, and Ca). Fe$_2$O$_3$ was also measured in all plots as previous studies have demonstrated that there is a strong positive association between Fe$_2$O$_3$ concentrations and phosphate or glyphosate sorption in soils. Since our results indicated no treatment differences induced by Cd and P applications on Fe$_2$O$_3$ concentrations extractable Al$_2$O$_3$, and Ca were quantified for 16 plots only (i.e., Control, 20P, 40P and 80P plots).

2.2. Sorption Studies

Chemicals used in the sorption studies were: analytical grade ammonium phosphate monobasic (98% chemical purity) and glyphosate (99.9% purity) from Sigma-Aldrich Co., St. Louis, MO, $^{14}$C-labelled glyphosate [phosphonomethyl-$^{14}$C] (99% radiochemical purity; specific activity 50 µCi), from American Radiolabeled Chemicals Inc. and Roundup Ultra 2 (49% active ingredient and 51% other ingredients, CAS No. 70901-12-1) from Monsanto Chemical Company. Active ingredient was potassium salt of N-(phosphonomethyl) glycine.
Glyphosate sorption was determined by batch equilibrium with the initial solution containing 1 mg L\(^{-1}\) glyphosate and 6.67 X 10\(^{4}\) Bq L\(^{-1}\) \(^{14}\)C-labelled glyphosate. Batch equilibrium procedures followed the OECD guideline 106 using a soil/solution ratio of 1:5 and an equilibrium time of 24 h (OECD, 2000). Initial solution (10 mL) was added to soil (2 g) in 50-mL centrifuge Teflon tubes (duplicates) and slurries were rotated in the dark at 5\(^\circ\)C for 24 h. A constant 5\(^\circ\)C temperature was utilized to minimize risks for biodegradation. To our knowledge, there have been no studies on the effect of temperature on glyphosate sorption. Studies using other herbicides have found that 2,4-D sorption decreases (Aksu and Kabasakal, 2005; Gupta et al. 2006) or increases (Gaultier et al., 2009) with increasing temperature. Regardless, the effect of temperature on sorption is small because, between 5 and 25 \(^\circ\)C, the 2,4-D sorption distribution coefficient only varied by 1 to 3\% (Gaultier et al., 2009).

Equilibrium solution was centrifuged (10,000 revmin\(^{-1}\) for 10 minutes) and subsamples (1 mL) of supernatant were added in duplicated 7-mL scintillation vials containing 5 mL 30% Scintisafe scintillation cocktail (Fisher Scientific, Fairlawn, NJ). Vials were lightly shaken and stored in the dark for 24 h to disperse the chemiluminescence before the radioactivity was measured. Radioactivity was quantified by Liquid Scintillation Counting (LSC) with automated quench correction (#H method) (LS 6500 Beckman Instruments, Fullerton, CA). The glyphosate sorption distribution constant, \(K_d\) (L kg\(^{-1}\)) was calculated by \(C_s/C_e\), whereby \(C_s = \) glyphosate sorption by soil at equilibrium (mg kg\(^{-1}\)), and \(C_e = \) glyphosate concentration of equilibrium solution (mg L\(^{-1}\)). The effects of field-aged phosphate and Cd concentrations on glyphosate sorption were examined at pH conditions ranging from 3.6 to 7.3. This first experiment utilized soils from all forty plots and the range in pH was induced using different types of ions in the initial solution (0.01M HCl, 0.01M CaCl\(_2\), 0.01M KCl, 0.01M KOH or dH\(_2\)O). For the control and high Cd 80P plots, the
experiments were repeated but then using the Tier 2 parallel method (OECD, 2000) with tubes being sampled at 1, 2, 4, 6, 8 and 24 h. The two subsequent experiments utilized soils from the plots labeled as low Cd and with 20P, 40P or 80P levels. In one experiment, for slurry pH conditions ranging from 3.6 to 7.3, batch equilibriums procedures were repeated but using Roundup Ultra 2 in 0.01M HCl, 0.01M CaCl$_2$, 0.01M KCl, 0.01M KOH or dH$_2$O to verify experimental results for a formulated product. In the other experiment, for slurry pH conditions range from 4.7 to 5.4, the effect of fresh phosphate additions on glyphosate sorption by soil was examined by adding analytical grade MAP to analytical glyphosate in 0.01M CaCl$_2$, 0.01M KCl and dH$_2$O solutions. The amounts of MAP added was equivalent to 11, 22 and 44 mg P kg$^{-1}$, or an estimated 20, 40 and 80 P kg ha$^{-1}$, respectively, assuming the fertilizer being present in the top 15 cm layer of a soil with a bulk density of 1,200 kg m$^{-3}$.

Statistical analyses were completed using SAS software version 9.3 for Windows (SAS Institute Inc. 2002-2010). Two-way analysis of variance (ANOVA) and multiple means comparison (Tukey’s) tests were conducted to determine the effect of phosphate fertilizer (20P, 40P, 80P) and Cd (low, medium, high) treatment on Olsen-P concentrations, extractable Cd concentrations and total Cd concentrations in soil. For each pH (ionic solution), multiple linear regression analyses were carried out to predict glyphosate Kd values by using Olsen-P and extractable Cd concentrations as independent variables. Repeated measure analysis was used to determine the effect of shaking time (0.5, 1, 2, 4, 6, 8 and 24 h) by using phosphate levels and time as independent variables. Two-way ANOVA and multiple means comparison (Tukey’s) tests were utilized to quantify the effects of field-aged (20P, 40P, 80P) and fresh phosphate additions (11, 22 and 44 mg P kg$^{-1}$) on glyphosate Kd values. One-way ANOVA and multiple means comparison (Tukey’s)
tests were applied to quantify the impact of using Roundup Ultra 2 versus analytical-grade glyphosate on Kd values in soils.

3. Results and Discussion

Glyphosate Kd values ranged from 43 to 1,173 L kg\(^{-1}\) which is in agreement with glyphosate Kd values reported in agricultural soils (Sørensen et al., 2006). There were no significant differences in glyphosate sorption by soil when using either Roundup Ultra 2 or analytical-grade glyphosate, suggesting that other ingredients in the commercial formulation had no impact on the sorption behaviour of the active ingredient glyphosate in soil.

The additions of MAP fertilizers from 2002 to 2009 had a significant effect on phosphate concentrations in 2013 (Figure 1). Olsen-P concentrations ranged from 13 to 99 mg kg\(^{-1}\) across plots and significantly decreased from 80P > 40P > 20P plots. North American agricultural soils have a median phosphate concentration of 25 mg kg\(^{-1}\) (Fixen et al., 2010). Total Cd concentrations in soil ranged from 0.42 to 0.98 mg kg\(^{-1}\) across plots but there were no significant treatment effects. Thus, the amount of Cd in the MAP fertilizers applied had no significant effect on the total Cd concentrations in 2013. \(DTPA\)-extractable Cd concentration ranged from 0.19 to 0.41 mg kg\(^{-1}\), within the typical range of 0.1 to 0.5 mg kg\(^{-1}\) reported for soils (International Cadmium Association, 2015). There was a significant interaction, between the rate of phosphate fertilizer applied and the amount of Cd that the phosphate fertilizer contained, on extractable Cd concentrations in soil (Figure 2). For the 80P plots, extractable Cd concentrations significantly decreased in the order of high Cd > med Cd > low Cd. For the 40P plots, extractable Cd concentrations significantly decreased in the order of high Cd > (med Cd = low Cd). In 20P plots,
only the high and low Cd treatments had significantly different extractable Cd concentrations. Despite these significant differences, extractable Cd concentrations in soil had no significant influence on glyphosate Kd values (Table S1- Supplementary information). The Cd concentrations in our field plots are those typically encountered in agricultural soils, but we recognize that in a batch equilibrium experiment, Zhou, et al. (2004) demonstrated that the co-application of exceptionally large quantities of Cd to glyphosate solutions (i.e., 562 mg Cd kg\(^{-1}\) soil) can increase glyphosate sorption by approximately 1.6 times fold, relative to control soil (Zhou et al., 2004).

Increased Olsen-P concentrations in soil was a significant factor (P<0.0001) in the regression analysis to explain reduced glyphosate Kd values in soil (Table S1- Supplementary information). Regardless of the ionic solution used in the batch equilibrium experiments, increased Olsen P concentrations significantly decreased glyphosate sorption by soil (Figure 3). A maximum reduction in glyphosate sorption occurred at a pH of 5 (0.01M KCl solution) when the Olsen-P concentrations was on average 89 mg kg\(^{-1}\) Olsen P and the glyphosate Kd value was reduced by 57\%, relative to the control plots that contained on average 18.75 mg kg\(^{-1}\) Olsen-P (Figure 3). Our results are in agreement with the findings of de Jonge et al. (2001) who also reported that field-aged phosphate in soil reduces glyphosate sorption by soil. Iron oxides are important sorption sites for glyphosate and phosphate in soil (Gimsing and Borggaard, 2002b; Gimsing et al., 2004; Ololade, et al., 2014). The iron oxides content of the Orthic Black Chernozem used is within the range of that observed in other Prairie soils in Canada (Obikoya, 2016) suggesting the competitive effect of phosphate on glyphosate sorption could be applicable to a wider range of soils in the Prairie region of Canada particularly with low pH and high Fe content.

At pH 5.4, in both 80P and control, time had no significant effect on glyphosate Kd values and sorption was always significantly smaller in 80P than control plots. For all other pH conditions,
glyphosate sorption approached equilibrium at approximately 8 h because there were no significant
differences in glyphosate Kd values between 8 and 24 h (Figure 4). For these pH conditions,
glyphosate sorption was almost always significantly smaller in 80P than control plots, regardless
of the time, except for 0.5, 1 and 2 h under pH 3.6 and 0.5 h under pH 5.0 (Figure 4). In general,
longer shaking hours resulted in greater numerically differences in glyphosate Kd values between
control and 80P plots.

Regardless of the ionic solution used (Figure 5), there was a significant interaction (P < 0.01)
between field-aged and fresh phosphate on glyphosate sorption (Table S2- Supplementary
information). In general, regardless of the amount of aged phosphate in soil, the addition of fresh
MAP to the ionic solutions numerically reduced glyphosate Kd values, suggesting that phosphate
and glyphosate compete for the same sorption sites in soil and that phosphate is preferentially
sorbed when added with glyphosate to soil. Additions of 11 mg P kg\(^{-1}\) to the 0.01M CaCl\(_2\) solutions
had no significant effect on glyphosate Kd values, except in the 20 P plots containing relatively
small Olsen-P concentrations (Figure 5). The addition of 22 or 44 mg P kg\(^{-1}\) to the 0.01M CaCl\(_2\)
solutions always significantly reduced glyphosate Kd values, except the addition of 22 mg P kg\(^{-1}\)
to 80 P plots (Figure 5). For the largest co-application (44 mg P kg\(^{-1}\)), glyphosate Kd values were
reduced on average by 52% in 20P plots, but by only 37% in the 80P plots. Additions of 11, 22 or
44 mg P kg\(^{-1}\) to 0.01M KCl solutions always significantly reduced glyphosate Kd values except
for 80 P plots for which only the addition of 44 mg P kg\(^{-1}\) resulted in a significant reduction in
glyphosate Kd values (Figure 5). For the 44 mg P kg\(^{-1}\) co-application, glyphosate Kd values were
reduced on average by 54% in 20P plots, but by 42% in the 80P plots. Thus, the largest impact of
fresh MAP applications on reducing sorption sites for glyphosate occurred in soils with smaller
field-aged phosphate concentrations because more sorption sites were available for competition in the plots that had low field-aged phosphate concentrations.

In general, glyphosate Kd values were largest at pH 4.7 (0.01M CaCl₂) when glyphosate molecules mainly exist as $\text{H}_2\text{G}^-\text{G}^-$ (~85%) and $\text{HG}^2-$ (~15%), and at pH 5.0 (0.01M KCl) when glyphosate molecules mainly exist as $\text{H}_2\text{G}^-\text{H}^-$ (~75%) and $\text{HG}^2-$ (~25%) (Wang et al., 2004). The use of 0.01M CaCl₂ would have enhanced the amount of exchangeable $\text{Ca}^{2+}$ associated with organic-clay complexes in soil, and glyphosate has been shown to form stable complexes with divalent cations such as Ca, Mg and Fe (Cakmak et al., 2009). The soil used in this study had already a relatively large $\text{Ca}^{2+}$ content (2,252 ± 40.57 mg kg$^{-1}$), and using 0.01M KCl, would allow $\text{K}^+$ to replace $\text{Ca}^{2+}$ on the exchange site of organic-clay complexes (Brady and Weil, 2008) which may interact with glyphosate forming stable complexes. Glyphosate Kd values were greater at pH 3.6 (0.01M HCl), than pH 5.4 (dH₂O) (Figure 3). At pH 3.6, a greater amount of soil colloids is net positively-charged, promoting the sorption of glyphosate molecules that mainly exist as $\text{H}_2\text{G}^-\text{G}^-$ (~95%) and $\text{H}_2\text{G}^-\text{H}^-$ (~5%) (McConnell and Hossner, 1985; Wang et al., 2004). Sorption was less at pH 5.4 than at pH 3.6 because the amount of negatively-charged soil colloids increases with soil pH, and glyphosate molecules mainly exist as $\text{H}_2\text{G}^-\text{H}^-$ (~60%) and $\text{HG}^2-$ (~40%) at pH 5.4 (McConnell and Hossner, 1985; Wang et al., 2004). The lowest sorption was observed at pH 7.3 (0.01M KOH), as the negatively-charged soil colloids increased and glyphosate molecules existed as $\text{HG}^2-$ (~100%) (McConnell and Hossner, 1985; Wang et al., 2004).

4. Conclusion
Analytical-grade glyphosate showed similar results as a commercially-available glyphosate formulation. Long-term additions of phosphate fertilizers to soils will reduce the capacity of the soil to bind glyphosate under a wide range of pH conditions, but the impurities of Cd in these fertilizers have no impact on glyphosate sorption. Fresh applications of phosphate fertilizers to most soils will significantly reduce the availability of sorption sites for glyphosate. However, this reduction in sorption site availability will be small in soils that have exceptionally high phosphate levels and do not have many sorption sites available for phosphate or glyphosate. Cd concentrations typically found in agricultural fields are not high enough to influence the binding capacity of glyphosate in soil.

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Figure 1: Effect of phosphate fertilizers with different Cd levels on Olsen P concentrations in soil. The solid line indicates the concentration of Olsen P in control plots.

Figure 2: Effect of phosphate fertilizers with different Cd levels on DTPA-extractable Cd in soil. The solid line indicates the concentration of extractable Cd in control plots.
Figure 3: Relation between Olsen-P concentrations in soil and the glyphosate sorption distribution constant, Kd, with soil slurries being under different pH conditions. All regression equations are significant at P<0.0001, and the equations are given in Table S1- Supplementary information.
Figure 4. Time dependent sorption study of glyphosate Kd values in control and 80P plots.

Figure 5: Effect of co-applying mono ammonium phosphate with glyphosate in solution, for batch equilibrium experiments using 0.01M CaCl$_2$ (pH 4.7) and 0.01M KCl (pH 5.0).
Supplementary Information:

Table S1. Multiple regression results using Olsen-P and extractable Cd as independent variables to predict glyphosate Kd.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Parameter Estimate</th>
<th>Standard Error</th>
<th>F Value</th>
<th>P value</th>
</tr>
</thead>
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<td><strong>0.01M HCl (pH 3.6)</strong></td>
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<tr>
<td>Intercept</td>
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<td>29.07</td>
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<td>Olsen-P</td>
<td>-1.74</td>
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<td>Cd</td>
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<td><strong>0.01M CaCl₂ (pH 4.7)</strong></td>
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<tr>
<td>Intercept</td>
<td>937.68</td>
<td>81.25</td>
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<td>390.49</td>
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<tr>
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Table S2. Effect of field P-level (20P, 40, 80P kg ha\(^{-1}\)) and fresh-P applications (0P 20P 40P 80P kg ha\(^{-1}\)) on glyphosate Kd values in batch equilibrium experiments utilizing either 0.01M CaCl\(_2\) or 0.01M KCl as a background liquid.

<table>
<thead>
<tr>
<th>Effect</th>
<th>Num DF</th>
<th>Den DF</th>
<th>F Value</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>0.01M CaCl(_2) (pH 4.7)</strong></td>
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