1   **Phosphate fertilizer impacts on glyphosate sorption by soil**

2

3   **Sirajum Munira[*1], Annemieke Farenhorst[1], Don Flaten[1] and Cynthia Grant[2]**

4

5   [1] Department of Soil Science, Faculty of Agricultural & Food Sciences, University of Manitoba,

6   362 Ellis Building, Winnipeg, MB, Canada, R3T 2N2

7   [2]Agriculture and Agri-Food Canada, Brandon, MB, Canada R7A 5Y3

8

9   * Corresponding author:

10   Sirajum Munira

11   E-mail address: muniras@myumanitoba.ca

12   +1 204 558 0504

13

14   **Abstract**

15   This research examined the impact of field-aged phosphate and cadmium (Cd) concentrations, and

16   fresh phosphate co-applications, on glyphosate sorption by soil. Soil samples were collected in

17   2013 from research plots that had received, from 2002 to 2009, annual applications of mono

18   ammonium phosphate (MAP) at 20, 40 and 80 kg P ha^{-1} and from products containing 0.4, 70 or

19   210 mg Cd kg^{-1} as an impurity. A series of batch equilibrium experiments were carried out to

20   quantify the glyphosate sorption distribution constant, Kd. Extractable Cd concentrations in soil

21   had no significant effect on glyphosate sorption. Glyphosate Kd values significantly decreased

22   with increasing Olsen-P concentrations in soil, regardless of the pH conditions studied.

23   Experiments repeated with a commercially available glyphosate formulation showed statistically

1

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,

47 1999) and hence phosphorus applications may influence the bioavailability and transport potential

48 of glyphosate in soil (Gimsing and Borggaard, 2002a). Phosphate fertilizers contain cadmium (Cd)

49 as an impurity and repeated application of phosphate fertilizer results in Cd accumulation in soil

50 (Lambert et al., 2007). The addition of Cd to glyphosate solutions in the laboratory has been shown

51 to enhance glyphosate sorption by soil because Cd and glyphosate form complexes that are

52 retained on the surface of negatively-charged soil colloids (Zhou et al., 2004). The effect of

53 impurities of Cd in phosphate fertilizers on glyphosate sorption in soil is not known.

54 The batch equilibrium procedure is a common method to examine the effect of phosphate additions

55 on glyphosate sorption. All researchers, except de Jonge et al. (2001), added phosphate to soil in

56 the laboratory prior to or during the batch equilibrium experiment, and hence exclusively studied

57 the effect of "*fresh*" phosphate on glyphosate sorption. Phosphate did compete with glyphosate

58 sorption in a wide range of soils (Sprankle et al., 1975; de Jonge and de Jonge 1999; de Jonge et

59 al., 2001; Kanissery et al., 2015), but not in other soils (Gimsing et al., 2004). Some research

60 demonstrates that phosphate is preferentially sorbed by clay minerals (Gimsing and Borggaard,

61 2001), for example because the phosphate molecule (0.25 nm) is smaller than the glyphosate

62 molecule (0.43 nm) (Cáceres-jensen et al., 2009). The impact of phosphate on reducing glyphosate

63 sorption was stronger for synthesized Fe and Al- oxides than for pure clay-minerals (Gimsing and

64 Borggaard, 2002b). All batch equilibrium studies utilized analytical-grade glyphosate, while

65 herbicide products applied on agricultural land contain other ingredients that could impact the

66 sorption of the active ingredient by soil (Farenhorst and Bowman 1998). Hence, in evaluations of

67 the impact of phosphate additions on glyphosate sorption, it is desired to include a commercially

68 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

92   design with 10 treatments and 4 replicates per treatment. In each of the forty plots, the composite

93   soil sample consisted of ten samples collected in the plot using a Dutch augur. Treatments were a

94   control (neither phosphate nor Cd applications), and plots receiving from 2002 to 2009 annual

95   applications of mono ammonium phosphate (MAP) fertilizers that originated from three different

96   phosphate rock sources containing 0.4, 70 or 210 mg Cd kg^{-1}, or low, medium and high Cd,

97   respectively (Grant et al., 2013). MAP from these three sources was applied to plots at 20, 40 and

98   80 kg P ha^{-1}, or 20P, 40P and 80P, respectively. For all plots that received MAP, 20 kg P ha^{-1} was

99   placed near the seed to enhance fertilizer use efficiency, a common practice in Canadian Prairie

100   agriculture. For the 40 and 80 kg P ha^{-1} treatments, to avoid seedling toxicity, the additional MAP

101   was broadcasted and then incorporated in soil. From 2010 to 2013, the rotation was continued but

102   no phosphate or Cd was applied. Nitrogen fertilizer varied by year to optimize yields. The typical

103   rate of N applied was 90 kg N ha^{-1} in durum wheat and 50 kg N ha^{-1} in flax.

104   Soil samples were air-dried and sieved (<2mm) prior to soil properties analysis and sorption

105   studies. Soil was digested with nitric acid and total Cd was determined by *inductively coupled*

106   *plasma (ICP)* (U.S. EPA, 1996). Extractable Cd was extracted with *diethylene triamine*

107   *pentaacetic acid* (*DTPA*) following procedures as recommended by Lindsay and Norvell (1978),

108   and extracts were analyzed by *ICP* (Whitney, 2011). Various factors have been shown to influence

109   the efficiency of micronutrient extraction by DTPA, including extraction temperature and shaking

110   time (Lindsay and Norvell, 1978).

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

112   Soil physical and chemical properties that are known to influence glyphosate and phosphate

113   sorption by soil, but did not significant vary across the plots by treatment, were also determined.

114   Soil organic carbon content was determined using combustion technique with a high temperature

115   induction furnace (Nelson and Sommers, 1996). Extractable Fe_2O_3 and Al_2O_3 were extracted with

116   *DTPA* (Whitney, 2011) *and* 0.01M $CaCl_2$ (Barnhisel and Bertsch, 1982), respectively, and extracts

117   were analyzed by *ICP*. Extractable Ca was also measured by ICP using ammonium acetate as an

118   extractant (Warncke and Brown, 2011). Results were soil organic carbon content: 2.80% (mean)

119   ± 0.04 (standard error) (n=16, number of plots analyzed); extractable Fe_2O_3: 246 ± 5 mg kg^{-1}

120   (n=40), extractable Al_2O_3: 6.4 ± 0.65 mg kg^{-1} (n=16); and extractable Ca: 2,252 ± 40.57 mg kg^{-1}

121   (n=16). Given that the study focused on Cd and P applications as treatments, the concentrations of

122   extractable and total Cd, as well as Olsen-P in all plots were determined. We did not expect to see

123   treatment differences for the other parameters that were measured (i.e., extractable Fe_2O_3, Al_2O_3,

124   and Ca). Fe_2O_3 was also measured in all plots as previous studies have demonstrated that there is

125   a strong positive association between Fe_2O_3 concentrations and phosphate or glyphosate sorption

126   in soils. Since our results indicated no treatment differences induced by Cd and P applications on

127   Fe_2O_3 concentrations extractable Al_2O_3, and Ca were quantified for 16 plots only (i.e., Control,

128   20P, 40P and 80P plots).

129

130   *2.2. Sorption Studies*

131

132   Chemicals used in the sorption studies were: analytical grade ammonium phosphate monobasic

133   (98% chemical purity) and glyphosate (99.9% purity) from Sigma-Aldrich Co., St. Louis, MO,

134   ^{14}C-labelled glyphosate [phosphonomethyl-^{14}C] (99% radiochemical purity; specific activity 50

135   µCi), from American Radiolabeled Chemicals Inc. and Roundup Ultra 2 (49% active ingredient

136   and 51% other ingredients, CAS No. 70901-12-1) from Monsanto Chemical Company. Active

137   ingredient was potassium salt of N-(phosphonomehyl) glycine.

138   Glyphosate sorption was determined by batch equilibrium with the initial solution containing 1 mg

139   L^{-1} glyphosate and 6.67 X 10^4 Bq L^{-1} ^{14}C-labelled glyphosate. Batch equilibrium procedures

140   followed the OECD guideline 106 using a soil/solution ratio of 1:5 and an equilibrium time of 24

141   h (OECD, 2000). Initial solution (10 mL) was added to soil (2 g) in 50-mL centrifuge Teflon tubes

142   (duplicates) and slurries were rotated in the dark at 5°C for 24 h. A constant 5°C temperature was

143   utilized to minimize risks for biodegradation. To our knowledge, there have been no studies on the

144   effect of temperature on glyphosate sorption. Studies using other herbicides have found that 2,4-

145   D sorption decreases (Aksu and Kabasakal, 2005; Gupta et al. 2006) or increases (Gaultier et al.,

146   2009) with increasing temperature. Regardless, the effect of temperature on sorption is small

147   because, between 5 and 25 ^{o}C, the 2,4-D sorption distribution coefficient only varied by 1 to 3%

148   (Gaultier et al., 2009).

149   Equilibrium solution was centrifuged (10,000 revmin^{-1} for 10 minutes) and subsamples (1 mL) of

150   supernatant were added in duplicated 7-mL scintillation vials containing 5 mL 30% Scintisafe

151   scintillation cocktail (Fisher Scientific, Fairlawn, NJ). Vials were lightly shaken and stored in the

152   dark for 24 h to disperse the chemiluminescence before the radioactivity was measured.

153   Radioactivity was quantified by Liquid Scintillation Counting (LSC) with automated quench

154   correction (#H method) (LS 6500 Beckman Instruments, Fullerton, CA). The glyphosate sorption

155   distribution constant, Kd (L kg^{-1}) was calculated by Cs/Ce, whereby Cs = glyphosate sorption by

156   soil at equilibrium (mg kg^{-1}), and Ce = glyphosate concentration of equilibrium solution (mg L^{-1}).

157   The effects of field-aged phosphate and Cd concentrations on glyphosate sorption were examined

158   at pH conditions ranging from 3.6 to 7.3. This first experiment utilized soils from all forty plots

159   and the range in pH was induced using different types of ions in the initial solution (0.01M HCl,

160   0.01M CaCl$_2$, 0.01M KCl, 0.01M KOH or dH$_2$O). For the control and high Cd 80P plots, the

161 experiments were repeated but then using the Tier 2 parallel method (OECD, 2000) with tubes

162 being sampled at 1, 2, 4, 6, 8 and 24 h. The two subsequent experiments utilized soils from the

163 plots labeled as low Cd and with 20P, 40P or 80P levels. In one experiment, for slurry pH

164 conditions ranging from 3.6 to 7.3, batch equilibriums procedures were repeated but using

165 Roundup Ultra 2 in 0.01M HCl, 0.01M CaCl$_2$, 0.01M KCl, 0.01M KOH or dH$_2$O to verify

166 experimental results for a formulated product**.** In the other experiment, for slurry pH conditions

167 range from 4.7 to 5.4, the effect of fresh phosphate additions on glyphosate sorption by soil was

168 examined by adding analytical grade MAP to analytical glyphosate in 0.01M CaCl$_2$, 0.01M KCl

169 and dH$_2$O solutions. The amounts of MAP added was equivalent to 11, 22 and 44 mg P kg^{-1}, or an

170 estimated 20, 40 and 80 P kg ha^{-1}, respectively, assuming the fertilizer being present in the top 15

171 cm layer of a soil with a bulk density of 1,200 kg m^{-3}.

172 Statistical analyses were completed using SAS software version 9.3 for Windows (SAS Institute

173 Inc. 2002-2010). Two-way analysis of variance (ANOVA) and multiple means comparison

174 (Tukey's) tests were conducted to determine the effect of phosphate fertilizer (20P, 40P, 80P) and

175 Cd (low, medium, high) treatment on Olsen-P concentrations, extractable Cd concentrations and

176 total Cd concentrations in soil. For each pH (ionic solution), multiple linear regression analyses

177 were carried out to predict glyphosate Kd values by using Olsen-P and extractable Cd

178 concentrations as independent variables. Repeated measure analysis was used to determine the

179 effect of shaking time (0.5, 1, 2, 4, 6, 8 and 24 h) by using phosphate levels and time as independent

180 variables. Two-way ANOVA and multiple means comparison (Tukey's) tests were utilized to

181 quantify the effects of field-aged (20P, 40P, 80P) and fresh phosphate additions (11, 22 and 44 mg

182 P kg^{-1}) on glyphosate Kd values. One-way ANOVA and multiple means comparison (Tukey's)

183 tests were applied to quantify the impact of using Roundup Ultra 2 versus analytical-grade

184 glyphosate on Kd values in soils.

185

186 **3. Results and Discussion**

187

188 Glyphosate Kd values ranged from 43 to 1,173 L kg^{-1} which is in agreement with glyphosate Kd

189 values reported in agricultural soils (Sørensen et al., 2006). There were no significant differences

190 in glyphosate sorption by soil when using either Roundup Ultra 2 or analytical-grade glyphosate,

191 suggesting that other ingredients in the commercial formulation had no impact on the sorption

192 behaviour of the active ingredient glyphosate in soil.

193 The additions of MAP fertilizers from 2002 to 2009 had a significant effect on phosphate

194 concentrations in 2013 (Figure 1). Olsen-P concentrations ranged from 13 to 99 mg kg^{-1} across

195 plots and significantly decreased from 80P > 40 P > 20P plots. North American agricultural soils

196 have a median phosphate concentration of 25 mg kg^{-1} (Fixen et al., 2010). Total Cd concentrations

197 in soil ranged from 0.42 to 0.98 mg kg^{-1} across plots but there were no significant treatment effects.

198 Thus, the amount of Cd in the MAP fertilizers applied had no significant effect on the total Cd

199 concentrations in 2013. *DTPA*-extractable Cd concentration ranged from 0.19 to 0.41 mg kg^{-1},

200 within the typical range of 0.1 to 0.5 mg kg^{-1} reported for soils (International Cadmium

201 Association, 2015). There was a significant interaction, between the rate of phosphate fertilizer

202 applied and the amount of Cd that the phosphate fertilizer contained, on extractable Cd

203 concentrations in soil (Figure 2). For the 80P plots, extractable Cd concentrations significantly

204 decreased in the order of high Cd > med Cd > low Cd. For the 40P plots, extractable Cd

205 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,

229 glyphosate sorption approached equilibrium at approximately 8 h because there were no significant

230 differences in glyphosate Kd values between 8 and 24 h (Figure 4). For these pH conditions,

231 glyphosate sorption was almost always significantly smaller in 80P than control plots, regardless

232 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,

233 longer shaking hours resulted in greater numerically differences in glyphosate Kd values between

234 control and 80P plots.

235 Regardless of the ionic solution used (Figure 5), there was a significant interaction (P < 0.01)

236 between field-aged and fresh phosphate on glyphosate sorption (Table S2- Supplementary

237 information). In general, regardless of the amount of aged phosphate in soil, the addition of fresh

238 MAP to the ionic solutions numerically reduced glyphosate Kd values, suggesting that phosphate

239 and glyphosate compete for the same sorption sites in soil and that phosphate is preferentially

240 sorbed when added with glyphosate to soil. Additions of 11 mg P kg^{-1} to the 0.01M CaCl$_2$ solutions

241 had no significant effect on glyphosate Kd values, except in the 20 P plots containing relatively

242 small Olsen-P concentrations (Figure 5). The addition of 22 or 44 mg P kg^{-1} to the 0.01M CaCl$_2$

243 solutions always significantly reduced glyphosate Kd values, except the addition of 22 mg P kg^{-1}

244 to 80 P plots (Figure 5). For the largest co-application (44 mg P kg^{-1}), glyphosate Kd values were

245 reduced on average by 52% in 20P plots, but by only 37% in the 80P plots. Additions of 11, 22 or

246 44 mg P kg^{-1} to 0.01M KCl solutions always significantly reduced glyphosate Kd values except

247 for 80 P plots for which only the addition of 44 mg P kg^{-1} resulted in a significant reduction in

248 glyphosate Kd values (Figure 5). For the 44 mg P kg^{-1} co-application, glyphosate Kd values were

249 reduced on average by 54% in 20P plots, but by 42% in the 80P plots. Thus, the largest impact of

250 fresh MAP applications on reducing sorption sites for glyphosate occurred in soils with smaller

251   field-aged phosphate concentrations because more sorption sites were available for competition in

252   the plots that had low field-aged phosphate concentrations.

253   In general, glyphosate Kd values were largest at pH 4.7 (0.01M $CaCl_2$) when glyphosate molecules

254   mainly exist as H_2G^- (~ 85%) and HG^{2-} (~ 15%), and at pH 5.0 (0.01M KCl) when glyphosate

255   molecules mainly exist as H_2G^- (~ 75%) and HG^{2-} (~ 25%) (Wang et al., 2004). The use of 0.01M

256     $CaCl_2$ would have enhanced the amount of exchangeable Ca^{2+} associated with organic-clay

257   complexes in soil, and glyphosate has been shown to form stable complexes with divalent cations

258   such as Ca, Mg and Fe (Cakmak et al., 2009). The soil used in this study had already a relatively

259   large Ca^{2+} content (2,252 ± 40.57 mg kg^{-1}), and using 0.01M KCl, would allow K^+ to replace Ca^{2+}

260   on the exchange site of organic-clay complexes (Brady and Weil, 2008) which may interact with

261   glyphosate forming stable complexes. Glyphosate Kd values were greater at pH 3.6 (0.01M HCl),

262   than pH 5.4 (dH_2O) (Figure 3). At pH 3.6, a greater amount of soil colloids is net positively-

263   charged, promoting the sorption of glyphosate molecules that mainly exist as H_2G^- (~ 95%) and

264   H_3G (~ 5%) (McConnell and Hossner, 1985; Wang et al., 2004). Sorption was less at pH 5.4 than

265   at pH 3.6 because the amount of negatively-charged soil colloids increases with soil pH, and

266   glyphosate molecules mainly exist as H_2G^- (~ 60%) and HG^{2-} (~ 40%) at pH 5.4 (McConnell and

267   Hossner, 1985; Wang et al., 2004). The lowest sorption was observed at pH 7.3 (0.01M KOH), as

268   the negatively-charged soil colloids increased and glyphosate molecules existed as HG^{2-} (~100%)

269   (McConnell and Hossner, 1985; Wang et al., 2004).

270

271   **4. Conclusion**

272

273 Analytical-grade glyphosate showed similar results as a commercially-available glyphosate

274 formulation. Long-term additions of phosphate fertilizers to soils will reduce the capacity of the

275 soil to bind glyphosate under a wide range of pH conditions, but the impurities of Cd in these

276 fertilizers have no impact on glyphosate sorption. Fresh applications of phosphate fertilizers to

277 most soils will significantly reduce the availability of sorption sites for glyphosate. However, this

278 reduction in sorption site availability will be small in soils that have exceptionally high phosphate

279 levels and do not have many sorption sites available for phosphate or glyphosate. Cd

280 concentrations typically found in agricultural fields are not high enough to influence the binding

281 capacity of glyphosate in soil.

282

283 **Acknowledgements**

290

291 **References**

292

293 Aksu, Z., Kabasakal, E., 2005. Adsorption characteristics of 2, 4-dichlorophenoxyacetic acid (2,4-

294 D) from aqueous solution on powdered activated carbon. J. Environ. Sci. Health Part B 40, 545 –

295 70. (DOI: 10.1081/PFC-200061533).

296   Barnhisel, R., Bertsch, P.M., 1982. Total Aluminum. In Methods of Soil Analysis. Part 2.

297   Chemical and Microbiological Properties, Agronomy 9, 2nd ed. 275–300. American Society of

298   Agronomy, Soil Science Society America, Madison, WI. USA.

299   Benbrook, C.M., 2012. Impacts of genetically engineered crops on pesticide use in the U.S. -- the

300   first sixteen years. Environ. Sci. Euro. 24, 24. (DOI: 10.1186/2190-4715-24-24).

301   Borggaard, O.K., 2011. Does phosphate affect soil sorption and degradation of glyphosate? – A

302   review. Trends Soil Sci. Plant. Nutr. J. 2, 16-27

303   (http://www.academyjournal.net/asj/index.php/TSPN/article/view/193/pdf_46).

304   Brady, N.C., Weil, R.R., 2008. Soil Phosphorous and Potassium. In The Nature and Properties of

305   Soils. 14th ed. 594-638. Pearson Education, Inc. Upper Saddle River, NJ. USA.

306   Cáceres-jensen, L., Gan, J., Báez, M., Fuentes, R., Escudey, M., 2009. Adsorption of glyphosate

307   on variable-charge volcanic ash-derived soils. J. Environ. Qual. 38, 1449-1457. (DOI:

308   10.2134/jeq2008.0146).

309   Cakmak I., Yazici, A., Tutus, Y., Ozturk, L., 2009. Glyphosate reduced seed and leaf

310   concentrations of calcium, manganese, magnesium, and iron in non-glyphosate resistant soybean.

311   Eur. J. Agronomy 31, 114–119. (DOI:10.1016/j.eja.2009.07.001).

312   CSSC, 1998. Correlation of Canadian taxonomy with their systems, chapter 6. In the Canadian

313   system of soil classification. 155. Agriculture and Agri-food Canada.

314   (http://sis.agr.gc.ca/cansis/publications/manuals/1998-cssc-ed3/cssc3_manual.pdf).

315   de Jonge, H., de Jonge, L.W., 1999. Influence of pH and solution composition of glyphosate and

316   prochloraz to a sandy loam soil. Chemosphere 39, 753-763. (DOI: 10.1016/S0045-

317   6535(99)00011-9).

318 de Jonge, H., de Jonge, L.W., Jacobsen, O.H., Yamaguchi, T., Moldrup, P., 2001. Glyphosate

319 sorption in soils of different pH and phosphorus content. Soil Sci. 166, 230–238.

320 (DOI: 10.1097/00010694-200104000-00002).

321 FAO, 2012. The world fertilizer outlook, Supply, Phosphate. In current world fertilizer trends and

322 outlook to 2016, 18. Food and agriculture organization of the United Nations, Rome.

323 (ftp://ftp.fao.org/ag/agp/docs/cwfto16.pdf).

324 Farenhorst, A., Bowman, B.T., 1998. Pesticides, food contaminants, and agricultural wastes. J.

325 Environ. Sci. Health, Part B 33, 671-682. (DOI: 10.1080/03601239809373171).

326 Fixen, P.E., Bruulsema, T.W., Jensen, T.L., Mikkelsen, R., Murrell, T.S., Phillips, S.B., Rund, Q.,

327 Stewart, W.M., 2010. The fertility of North American soils. Adapted from better crops with plant

328 food. 94. (Reference #10134/Nov. 2010)

329 (http://extension.umd.edu/sites/default/files/_docs/programs/anmp/IPNI_Fertility%20NA%20Soi

330 ls%202010.pdf).

331 Frank, K., Beegle, D., Denning, J., 2011. Phosphorus. In recommended chemical soil test

332 procedures for the North Central region. North Central regional research publication no. 221

333 (Revised). 21-26. Missouri Agricultural Experiment Station, SB 1001, USA.

334 (http://msue.anr.msu.edu/uploads/234/68557/Rec_Chem_Soil_Test_Proce55c.pdf).

335 Gaultier, J., Farenhorst, A., Kim, S.M., Saiyed, I., Messing, P., Cessna, A.J., Glozier, N.E., 2009.

336 Sorption-desorption of 2,4-dichlorophenoxyacetic acid by wetland sediments. Wetlands 29, 837–

337 844. (DOI: 10.1672/08-42.1).

338 Gimsing, A.L., Borggaard O.K., 2001. Effect of KCl and $CaCl_2$ as background electrolytes on the

339 competitive adsorption of glyphosate and phosphate on goethite. Clays Clay Miner. 49, 270-275.

340 (http://ccm.geoscienceworld.org/content/49/3/270).

341 Gimsing, A.L., Borggaard O.K., 2002a. Effect of phosphate on the adsorption of glyphosate on

342 soils, clay minerals and oxides. Int. J. Environ. Anal. Chem. 82, 545-552. (DOI:

343 10.1080/0306731021000062964).

344 Gimsing, A.L., Borggaard O.K., 2002b. Competitive adsorption and desorption of glyphosate and

345 phosphate on clay silicates and oxides. Clay Miner. 37, 509-515.

346 (DOI:10.1180/0009855023730049).

347 Gimsing, A.L., Borggaard. O.K., Bang, M., 2004. Influence of soil composition on adsorption of

348 glyphosate and phosphate by contrasting Danish surface soils. Eur. J. Soil Sci. 55, 183-191.

349 (DOI: 10.1046/j.1365-2389.2003.00585.x).

350 Gimsing, A.L., Borggaard, O.K., 2007. Phosphate and glyphosate adsorption by hematite and

351 ferrihydrite and composition with other variable-charge minerals. Clays Clay Miner. 55, 108-114.

352 (DOI: http://dx.doi.org.uml.idm.oclc.org/10.1346/ccmn.2007.0550109).

353 Gimsing, A.L., Szilas, C., Borggaard, O.K., 2007. Sorption of glyphosate and phosphate by

354 variable-charge tropical soils from Tanzania. Geoderma 138, 127-132. (DOI:

355 10.1016/j.geoderma.2006.11.001).

356 Global strategic business report (GSBR), 2011. Glyphosate: A global strategic business report.

357 Global Industry Analysis, Inc., USA.

358 (http://www.strategyr.com/gia_new/strategyr_new/pressMCP-6447.asp).

359 Grant, C., Flaten, D., Tenuta, M., Malhi, S., Akinremi, W., 2013. The effect of rate and cd

360 concentration of repeated phosphate fertilizer applications on seed Cd concentration varies with

361 crop type and environment. Plant Soil. 372, 221–233 (DOI: 10.1007/s11104-013-1691-3).

362 Gupta, V.K., Imran, A., Suhas, Saini, V.K., 2006. Adsorption of 2,4-D and carbofuran pesticides

363 using fertilizer and steel industry wastes. J. Colloid Interface Sci. 299, 556-63. (DOI:

364   10.1016/j.jcis.2006.02.017).

365   International Cadmium Association, 2015. Environment. Levels of cadmium in the environment.

366   (www.cadmium.org accessed 20th September, 2015).

367   Kanissery, R.G., Welshb, A., Sims, G. K., 2015. Effect of soil aeration and phosphate addition on

368   the microbial bioavailability of carbon-14-glyphosate. J. Environ. Qual. 44, 137-144. (DOI:

369   10.2134/jeq2014.08.0331).

370   Lambert, R., Grant, C. Sauvé. S., 2007. Cadmium and zinc in soil solution extracts following the

371   application   of   phosphate   fertilizers.   Sci.   Total   Environ.   378,   293-305

372   (DOI:10.1016/j.scitotenv.2007.02.008).

373   Lindsay, W.L., Norvell. W.A., 1978. Development of a DTPA soil test for zinc, iron, manganese,

374   and   copper.   Soil   Sci.   Soc.   Am.   J.   42,   421-428.

375   (DOI:10.2136/sssaj1978.03615995004200030009x).

376   McConnell, J.S., Hossner, L.R., 1985. pH - dependent adsorption isotherms of glyphosate. J.

377   Agric. Food Chem. 33, 1075-1078. (DOI: 10.1021/jf00066a014).

378   Nelson, D.W., Sommers, L.E., 1996. Total carbon, organic carbon, and organic matter. In methods

379   of soil analysis. Part 3. Chemical methods. 961-1010. American Society of Agronomy, Soil

380   Science Society America, Madison, WI. USA.

381   (https://dl.sciencesocieties.org/publications/books/abstracts/sssabookseries/methodsofsoilan3/96

382   1/preview).

383   Obikoya, O.A., 2016. Changes in Soil Test Phosphorus and Phosphorus Forms with Continuous

384   Phosphorus Fertilizer Addition to Contrasting Prairie soils. Master's thesis, department of soil

385   science, university of Manitoba, Canada.

386 OECD, 2000. Guideline for the testing of chemicals 106. Adsorption–desorption using a batch

387 equilibrium method. Adopted 21st January, 2000. (DOI: 10.1787/20745753).

388 Ololade, I.A., Oladoja, N.A., Oloye, F.F., Alomaja, F., Akerele, D.D., Iwaye, J., Aikpokpodion,

389 P., 2014. Sorption of glyphosate on soil components: The roles of metal oxides and organic

390 materials. Soil sed. contam. 23, 571–585. (DOI: 10.1080/15320383.2014.846900).

391 Sørensen, S.R., Schultz, A., Jacobsen, O.S., Aamand, J., 2006. Sorption, desorption and

392 mineralisation of the herbicides glyphosate and MCPA in samples from two Danish soil and

393 subsurface profiles. Environ. Pollut. 141, 84–194. (DOI:10.1016/j.envpol.2005.07.023).

394 Sprankle, P., Meggitt, W.F., Penner, D., 1975. Adsorption, mobility and microbial degradation of

395 glyphosate in the soil. Weed Sci. 23, 229-234. (http://www.jstor.org/stable/4042279).

396 U.S. Environmental Protection Agency (EPA), 1996. Method 3050B: Acid digestion of sediments

397 sludges and soils. In Hazardous Waste Test Methods / SW-846-Test methods for evaluating solid

398 waste, physical/chemical methods, 3000 series-inorganic samples preparation. 4-12.

399 (https://www.epa.gov/sites/production/files/2015-06/documents/epa-3050b.pdf).

400 Wang, Y., Zhou, D., Luo, X., Sun, R., Chen, H., 2004. Cadmium adsorption in montmorillonite as

401 affected by glyphosate. J. Environ. Sci. 16, 881-884.

402 (http://www.ncbi.nlm.nih.gov/pubmed/15900712).

403 Warncke, D., Brown, J.R., 2011. Potassium and Other Basic Cations. In recommended chemical

404 soil test procedures for the North Central region. North Central regional research publication no.

405 221 (Revised). 31-33. Missouri Agricultural Experiment Station SB 1001.

406 (http://www.naptprogram.org/files/napt/north-central-states-methods-manual-2012.pdf).

407 Whitney, D.A., 2011. Micronutrients: Zinc, Iron, Manganese and Copper. In recommended

408 chemical soil test procedures for the North Central region. North Central regional research

409   publication no. 221 (Revised). 41-44. Missouri Agricultural Experiment Station, SB 1001, USA.

410   (http://msue.anr.msu.edu/uploads/234/68557/Rec_Chem_Soil_Test_Proce55c.pdf).

411   Zhou, D.M., Wang, Y.J., Cang, L., Hao, X.Z., Luo, X.S., 2004. Adsorption and cosorption of

412   cadmium and glyphosate on two soils with different characteristics. Chemosphere 57, 1237-1244.

413   (DOI:10.1016/j.chemosphere.2004.08.043).
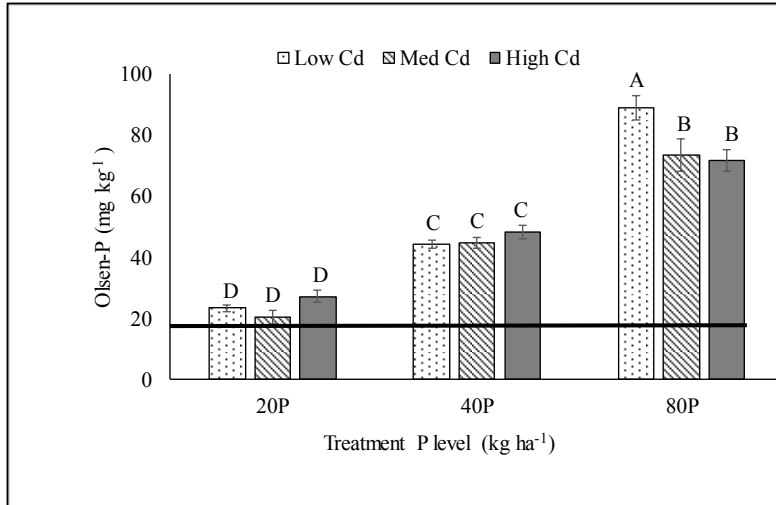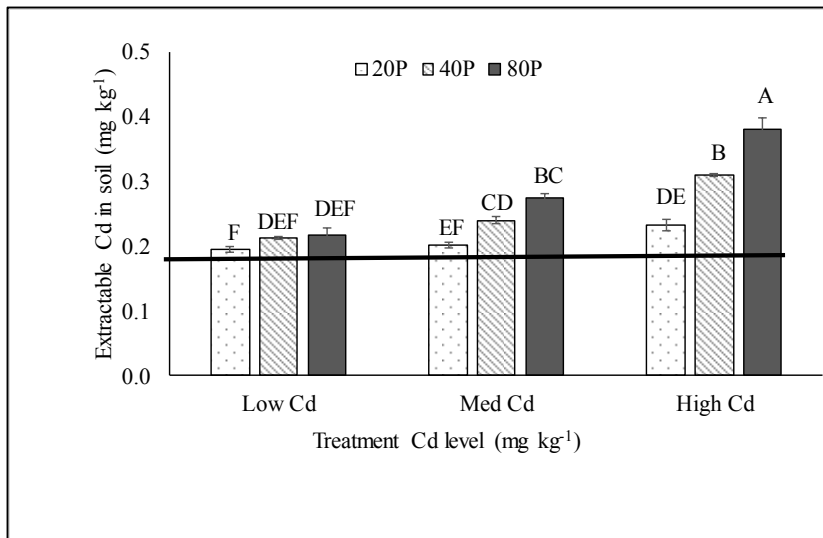
414

415

416

417

418

419

420

421

422

423

424

425

426

427

428

429

Figure 1: Effect of phosphate fertilizers with different Cd levels on Olsen P concentrations in soil.

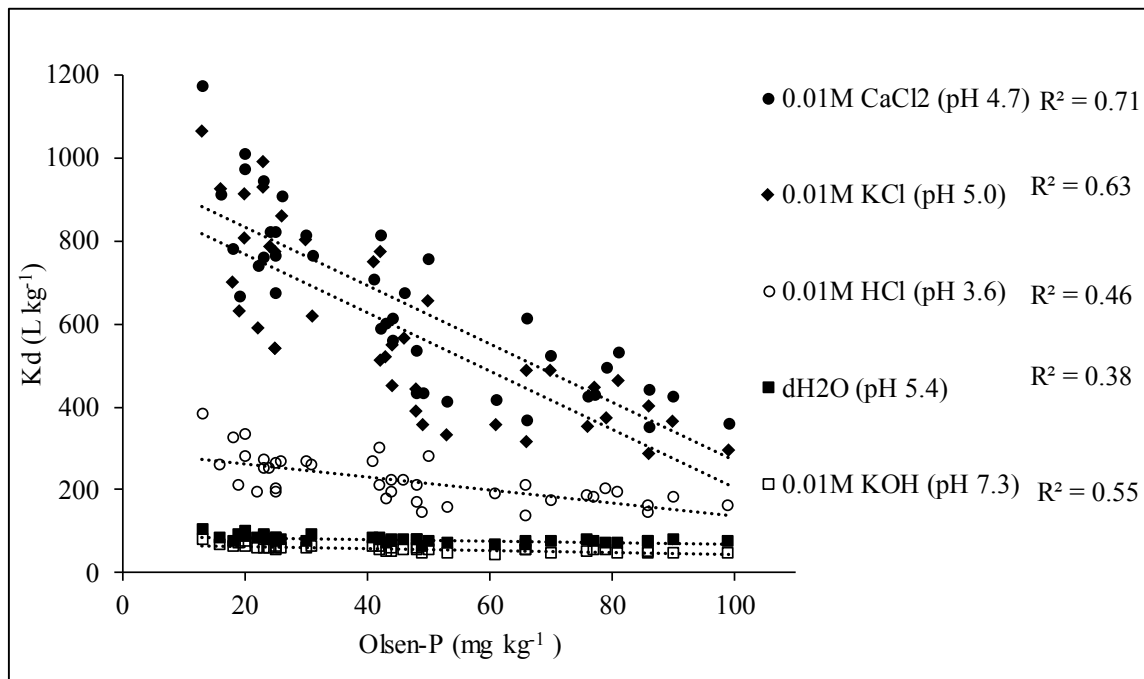The solid line indicates the concentration of Olsen P in control plots.

432

433

434

435

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.

438

439

440

441

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

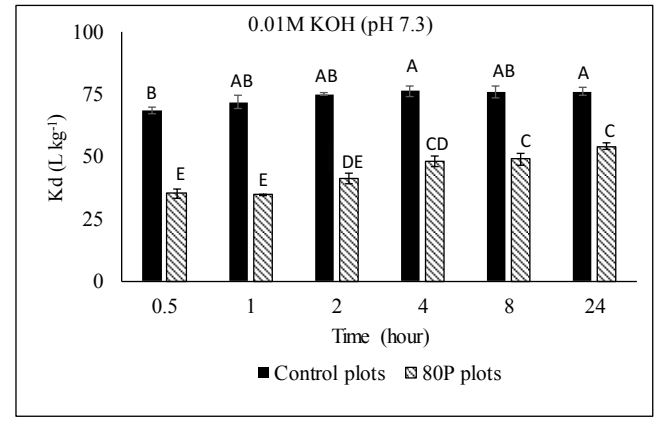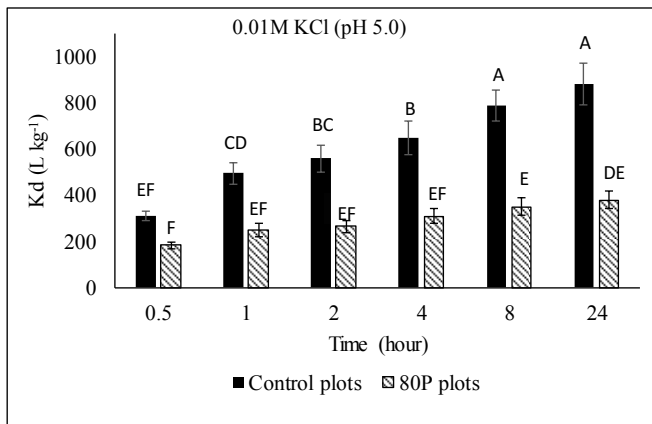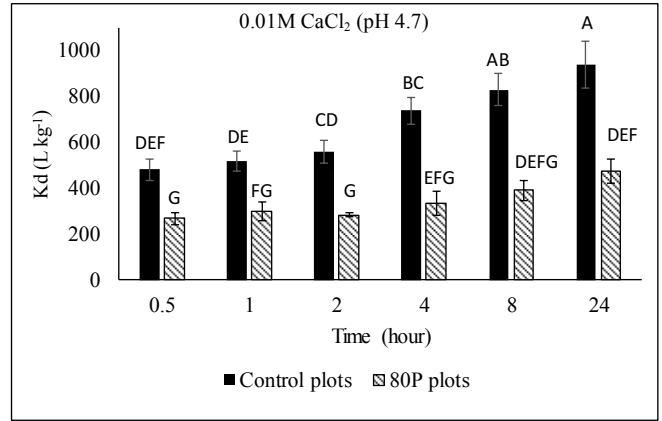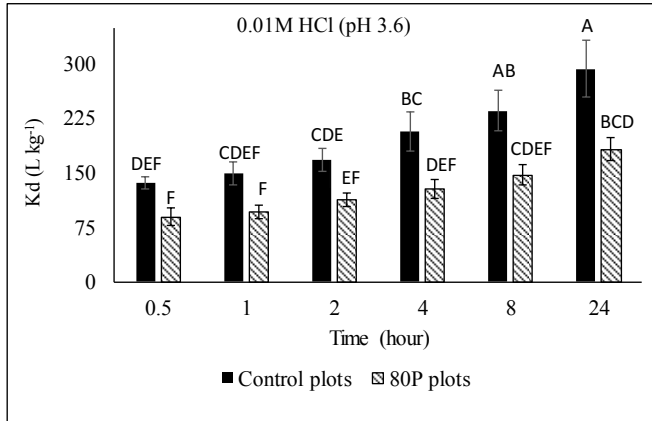significant at P<0.0001, and the equations are given in Table S1- Supplementary information.
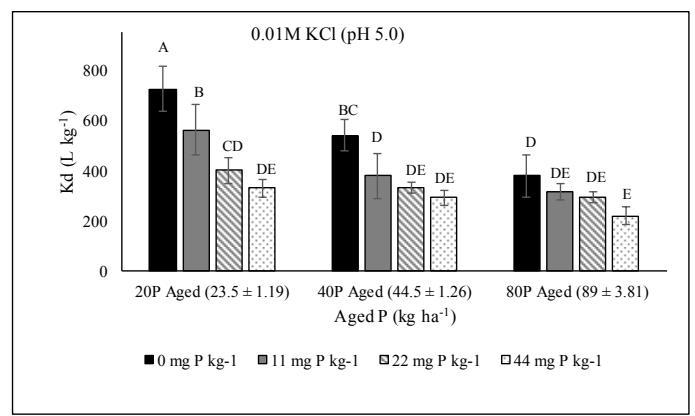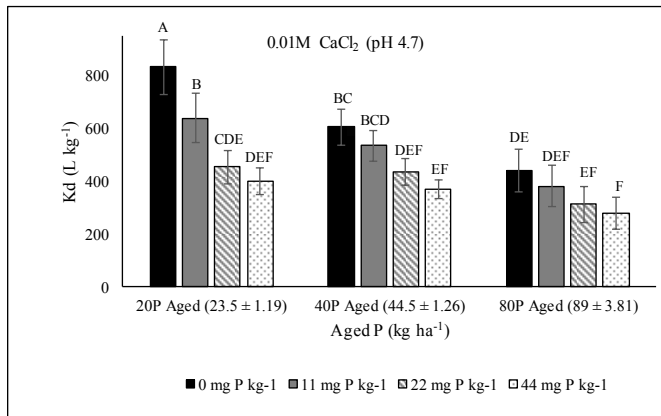
445

446

447

448

449

450

451

452

Figure 4. Time dependent sorption study of glyphosate Kd values in control and 80P plots.

453

454

455

Figure 5: Effect of co-applying mono ammonium phosphate with glyphosate in solution, for batch

456

equilibrium experiments using 0.01M CaCl$_2$ (pH 4.7) and 0.01M KCl (pH 5.0).

457

458
459   Supplementary Information:

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

Variable	Parameter Estimate	Standard Error	F Value	P value
0.01M HCl (pH 3.6)				
Intercept	272.07	29.07	87.59	<.0001
Olsen-P	-1.74	0.34	27.04	<.0001
Cd	125.99	139.72	0.81	0.3730
0.01M CaCl$_2$ (pH 4.7)				
Intercept	937.68	81.25	133.18	<.0001
Olsen-P	-7.32	0.94	61.01	<.0001
Cd	209.13	390.49	0.29	0.5955
0.01M KCl (pH 5.0)				
Intercept	921.07	93.34	97.38	<.0001
Olsen-P	-6.90	1.08	41.01	<.0001
Cd	-78.13	448.57	0.03	0.8627
d.H$_2$O (pH 5.4)				
Intercept	88.82	4.66	362.72	<.0001
Olsen-P	-0.21	0.05	15.85	0.0003
Cd	3.65	22.41	0.03	0.8715
0.01M KOH (pH 7.3)				
Intercept	66.37	4.11	259.89	<.0001
Olsen-P	-0.29	0.05	36.07	<.0001
Cd	15.73	19.78	0.63	0.4314

460

461

462

463

464

465

466

467

468

469

470

471

472

473

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.

Effect	Num DF	Den DF	F Value	Pr > F
0.01M CaCl$_2$ (pH 4.7)				
Field-P	2	33	52.00	<.0001
Fresh-P	3	33	45.56	<.0001
Fresh-P X Field-P	6	33	4.31	<.0026
0.01M KCl (pH 5.0)				
Field-P	3	33	40.56	<.0001
Fresh-P	3	33	39.18	<.0001
Fresh-P X Field-P	6	33	3.54	0.0081

474
475
476

477