COMPETITIVE SORPTION INTERACTIONS OF ORGANIC AND INORGANIC CHEMICALS IN SOIL

by

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ABSTRACT

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Agricultural soils in the Prairies encompasses 80% of Canada's farmland that may contain a range of chemical compounds, including phosphate and cadmium from fertilizer application, glyphosate and MCPA from herbicide application and/or the antibiotic tetracycline following livestock manure application. The aim of this PhD research was to examine the competitive sorption effects of these inorganic and organic chemicals in soil. Soil samples were collected in 2013 from two research sites that had received repeated annual application (2002-2009) of mono ammonium phosphate at 20, 40 and 80 kg P ha^{-1} with cadmium added at low, medium or high rates. A series of batch equilibrium experiments were conducted to quantify sorption of phosphate, glyphosate, MCPA and tetracycline. Results showed that cadmium concentrations in soil had no significant effect on sorption of glyphosate. Sorption of glyphosate significantly decreased with increasing phosphate concentrations in soils regardless of whether phosphate levels arose from repeated applications in the field, or from fresh applications in the laboratory. Similarly, sorption of phosphate significantly decreased with increasing phosphate concentrations in soils, suggesting that the capacity of soils to retain phosphate or glyphosate was reduced resulting from previous (repeated) phosphate fertilizer applications. The effect of phosphate on reducing phosphate and glyphosate sorption was greater in an acidic soil that

had high Fe/Al-oxides than in slightly alkaline calcareous soil. Analytical-grade glyphosate and commercially-available glyphosate formulation, Roundup Ultra2, behaved similarly for their sorption pattern. Phosphate sorption was not significantly impacted when Roundup Ultra2 was added to soil slurries in the laboratory. Repeated application of phosphate in the field had no significant impact on MCPA and tetracycline sorption, but fresh addition of phosphate in the laboratory significantly reduced MCPA and tetracycline sorption. Phosphate addition significantly increased desorption of glyphosate, MCPA and tetracycline but the impact was numerically very small. Thus, phosphate had a greater impact on sorption than desorption of the three organic chemicals. The presence of MCPA significantly reduced glyphosate sorption and increased desorption only when MCPA was added at high amounts and no phosphate was added. Overall, the competitive effect of phosphate on glyphosate sorption was strongest among the chemicals studied.

FOREWARD

This thesis was written in manuscript style in accordance with the guideline of the Department of Soil Science and Faculty of Graduate Studies, University of Manitoba. This consists of five chapters including introduction (Chapter 1), three stand-alone specific research chapters (2 to 4) and the overall synthesis (Chapter 5) that includes the summary, implications and provides recommendation for future studies.

Chapter 2 and 4 have been published:

Munira, S., Farenhorst, A., Flaten, D, Grant, C. 2016. Phosphate fertilizer impacts on glyphosate sorption by soil. Chemosphere **153**: 471-477.

Munira, S., Farenhorst, A. 2017. Sorption and desorption of glyphosate, MCPA and tetracycline and their mixtures in soil as influenced by phosphate. Journal of Environmental Science and Health, Part B 1-9. (Published online September 29, 2017).

Chapter 3 has been submitted:

Munira, S., Farenhorst, A., Akinremi, W. 2017. Phosphate and glyphosate sorption in soils following repeated application of phosphate fertilizer. Geoderma (Accepted with revisions reference number GEODER_2017_228_R2)

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1. INTRODUCTION

1.1. Global Use of Pesticides

Pesticides are the chemical substances used to control harmful organisms by preventing, destroying or mitigating pests (Costa and Aschner, 2014). As an example, herbicides are used as plant growth regulator, desiccator or defoliant. Commercialization of synthetic organic chemicals as pesticides began in the 1940s and included the introduction of the herbicide 2,4-D (1942), the fungicide zineb (1943) and the insecticide DDT (Matthews, 2006). Following the second World War, synthetic organic chemicals become an increasingly important component of modern agricultural production thus decreasing the share of the earlier inorganic chemicals used in pest control practices. Global food production has increased by 145% since 1960 because of the improvements of pesticide and fertilizer product, plant varieties and other technologies (Matthews, 2006; Pretty, 2008). Currently, approximately 1,814 million kg of pesticides are used in the world each year, of which 47.5% are herbicides, and the US market occupies 25% of the total world pesticide market (De et al., 2014). In US, pesticide use expenditures in agriculture sector was an estimated $9.2 billion per year between 2002-2009 (Popp et al., 2013) and $12.1 billion in 2016 (Freedonia, 2016). In Canada, over 101 million kg of pesticide active ingredients were sold in 2014, of these amount 74% were agricultural pesticide use (Health Canada, 2014). Herbicides are applied on 70% of the crop lands in Canada and on 74% of the crop land in the Prairie Provinces (Statistics Canada, 2013).

1.2. Herbicide Tolerant (HT) Crops

Recent progress in gene technology has resulted in the development of crop genotypes that are resistant to a specific herbicide and, since the mid-1990s, herbicide tolerant (HT) crops have been cultivated on a large scale (Schwember, 2008). It has been reported that 85% of all genetically modified crops grown are HT tolerant crop (CBAN, 2015). Globally, about million hectares of land were seeded to HT crops in 2015 including s 96oybean, corn, canola, cotton, sugarbeet, wheat, rice, potato, linseed, chicory, carnation, alfalfa and bentgrass (ISAAA, 2016). HT crops such as cotton, corn and soybean are widely cultivated in the United States. For example, in 2011, HT varieties occupied 73%, 72% and 94% of total acreage of cotton, corn and soybean, respectively, grown in the US (Osteen et al., 2012).

Canada is the fifth largest genetically-modified-food producing country in the world, with canola (95%), corn (80%), soybean (60%) and sugar beet (100%) being the four major HT crops grown (CBAN, 2015). HT canola was first grown commercially in 1995 and within ten years, 95% of the canola grown in the Prairie Region accounted for glyphosate, glufosinate ammonium and imidazoline resistant varieties (Beckie et al., 2006; Duke, 2005). Manitoba is one of three Prairie Provinces where canola (93% of all canola grown), soybeans (89%) and corn (48%) are the dominant HT crops grown (Wilson, 2012) and the most common HT varieties of these crops are glyphosate tolerant (ISAAA, 2016).

Studies have examined the impact of the adoption of HT crops on the amount of pesticides used in agriculture. It is estimated that pesticide use in the United States increased from

1996 to 2011 by 239 million kg due to the adoption of HT crop. Glyphosate is the herbicide that has seen the greatest increases in use as a result of the adaptation of HT crops in agriculture (Benbrook, 2012; ISAAA, 2016). For example, since 1995 to 2014, globally glyphosate use has increased by 14.6 fold (Benbrook, 2016).

1.3. Benefits and Adverse Impacts of Glyphosate Use

Glyphosate [N-(phosphonomethyl) glycine] is a broad spectrum, non-selective, systemic, post-emergent, foliar-applied herbicide. It controls annual broadleaf weeds, grass and perennial weeds by inhibiting the ability of plants to synthesize amino acids. More specifically, glyphosate inhibits the enzyme, 5-enolpyruvoyl-shikimate-3-phosphate synthetase (EPSPS), essential for the formation of the aromatic amino acid such as phenylalanine, tyrosine and tryptofan (Székács and Darvas, 2012). Glyphosate was first synthesized in 1950 by Swiss chemist Dr. Henri Martin. In 1970, Dr. John E Franz from the crop protection company Monsanto identified glyphosate to be an effective herbicide and the company launched the glyphosate containing product Roundup© in 1974 (Dill et al., 2010; Duke and Powles, 2008; Myers et al., 2016). Glyphosate is now registered for use in 130 countries (Dill et al., 2010). Globally, an estimated 826 million kg glyphosate was applied in 2014, with the United States accounting for the largest share (19%) of the global glyphosate use (Benbrook, 2016). In 2014, according to a report of global industry analysts, glyphosate accounted for one fourth of global herbicide sales and will reach a global use of 1,225 million kg in 2017 (Global Industry Analysts, 2017). It has also been estimated that global glyphosate sales will exceed US$10 billion by 2021 (Abnewswire, 2016).

Glyphosate plays an important role in global food security system. There is a range of benefits reported for glyphosate use. For example, some viewpoints are that glyphosate is among the least toxic and most environmentally-friendly pesticides on the market (Benbrook, 2016). It simplifies weed control by minimizing the need for mechanical tillage thereby also reducing the risk of soil erosion associated with intensively-tilled soils and CO_2 emissions associated with tillage operations (Held et al., 2016). However, there are concerns about the wide spread use of glyphosate (Kremer and Means, 2009; Zobiole et al., 2012; Gomes et al., 2016). For example, the over use and sole reliance on glyphosate for weed control evolved glyphosate resistant weeds (Green, 2016). In 2017, 37 weed species have been identified with glyphosate resistant biotypes (Heap, 2017). To combat this problem, farmers apply multiple herbicides that increases agricultural production costs and may harm the environment (Myers et al., 2016). In US, herbicide use has been increased by 70% for soybean and 50% for cotton (Benbrook, 2012).

There are contrasting studies to whether glyphosate application in agricultural production has the potential to reduce crop health. Some studies report reduced chlorophyll content in crops and reduced uptake of essential nutrients such as Ca, Mg, Fe and Mn by soybean (Cakmak et al., 2009; Petter et al., 2016), but other studies report that glyphosate applied at recommended rate has no adverse impact on the uptake of nutrients by crops (Duke et al., 2012; Loecker et al., 2010). Other studies reported that the continuous use of glyphosate can weaken plant defence mechanisms and increase virulence due to immobilization of essential elements, thus increases the risk of disease development in crops (Johal and

Huber, 2009; Kremer and Means, 2009). Application of glyphosate has also been shown to increase *Pythium* and *Fusarium* fungi in soil (Kremer and Means, 2009; Meriles et al., 2006). Other studies report that the presence of glyphosate in soil reduced Arbuscular mycorrhizal fungi (AMF) spores viability and root colonization ability (Druille et al., 2013; Zaller et al., 2014) and glyphosate has no effect on increasing sudden death syndrome caused by *Fusarium* in soybean (Kandel et al., 2015). A range of other studies have reported the potential negative effects of glyphosate on soil microbial communities, including reducing Mn and N_2 fixing bacteria such as *Rhizobium* (Zobiole et al., 2011), leading to reduced root nodulation in glyphosate-resistant soybean crops (Kremer and Means, 2009; Zobiole et al., 2012). However, other studies reported that glyphosate application has no adverse effect on AMF, Mn, and N_2 fixing bacteria (Duke et al., 2012; Hart et al., 2009; Powell et al., 2009).

1.3.1. Glyphosate Detections in the Environment

Glyphosate has been detected in rainfall (Farenhorst et al., 2015), wetlands (Messing et al., 2011), rivers (Battaglin et al., 2014), lakes (Crowe et al., 2011) and ground water (Sanchís et al., 2012). It has been reported that glyphosate at concentrations ranging from 4 to 350 mg L^{-1} in the laboratory experiment can have negative impacts on aquatic organisms such as *Daphnia*, algae, and amphibians (Baier et al., 2016; Le et al., 2010). Glyphosate has frequently been detected in surface waters in Canada (Byer et al., 2008; Environment Canada, 2011; Struger et al., 2015). However, the detection in surface water are typically below the Canadian Drinking Water Quality Guideline which is set at 0.28 mg L^{-1} (Health Canada, 2017; Struger et al., 2015).

Half-life of glyphosate in soil range from 30 to 197 days (Smith and Aubin, 1993; Giesy et al., 2000). The major metabolite of glyphosate in soil is aminomethylphosphonic acid (AMPA) (Dill et al., 2010). Generally, it is considered that glyphosate and AMPA have low potential to move downward from soil to groundwater (Borggaard and Gimsing, 2008; Giesy et al., 2000). However, studies showed that glyphosate (or AMPA) has been detected in groundwater in agricultural areas (Battaglin et al., 2014; Crowe et al., 2011; Kjaer et al., 2011). A review by Borggaard and Gimsing (2008) reported that glyphosate can be transported by preferential flow through macropores such as root channels or earthworm burrows (Kjaer et al., 2011), specially when glyphosate application coincides with a heavy rainfall events in clay soils. Glyphosate movement to groundwater in sandy soil is considered relatively lower than clay soils in the absence of macropores. However, repeated application of glyphosate can increase the risk of groundwater pollution in shallow water table areas in coarse-textured soils such as sandy poor oxidic soil (Borggaard and Gimsing, 2008). In addition, glyphosate downward movement is influenced by the changes in hydrology (wet vs dry period), land management practices, irrigation intensity, and timing of glyphosate application (Van Stempvoort et al., 2016). It has been reported that the detection of glyphosate in surface and groundwater occurred more frequently in the summer (May-June) than in the fall (September) because glyphosate was applied during spring and early summer (Coupe et al., 2012; Van Stempvoort et al., 2016).

1.3.2. Glyphosate Sorption in Soil

Sorption is an important process that determines the transport and availability of glyphosate in soil (Duke et al., 2012). Sorption refers to absorption, adsorption and precipitation of

organic compounds (Koskinen and Harper, 1990). The sorption distribution coefficient (Kd) is an important sensitive parameter in pesticide fate models needed to predict the risk of pesticide transport to surface water and groundwater (Farenhorst et al., 2008). Kd is the degree of sorption of an organic chemical per unit of soil. The batch equilibrium method is commonly used to measure Kd in the laboratory. Soil and solution is mixed and kept rotated for 24 hours which is considered the standard equilibrium time. Soil is separated from the liquid phase by centrifugation and the equilibrium solution is analysed to determine equilibrium pesticide concentration, Ce (mg L^{-1}). Amount pesticide sorbed, Cs (mg kg^{-1}) is calculated by subtracting the equilibrium pesticide concentration from initial pesticide concentration in soil slurry. Kd (L kg^{-1}) is calculated by Cs/Ce (Wauchope et al., 2002). Generally, Kd value in the order of 100 is considered strongly sorbed and immobile in soil (Wauchope et al., 2002). Desorption is also an important process influencing the transport of pesticides as it releases a pesticide from the solid to the water phase in soil (Huang et al., 1998). The percentage of desorption is calculated by dividing the total mass (mg) of pesticide present in the supernatant after the desorption step by the mass of pesticide (mg) that was in soil following the sorption step and then multiplying by 100 (OECD, 2000). Glyphosate Kd values have ranged from 108 to 1,140 L kg^{-1} (Sørensen et al., 2006; Farenhorst et al., 2008; Kumari et al., 2016) and its percentage desorption from 0.1 to 23.6% (Cheah et al., 1998; Sørensen et al., 2006).

Glyphosate is a zwitterion with three functional groups: phosphonic, amino and carboxylic acid groups (Tévez and Afonso, 2015). It has four acid dissociation constant (pK_a) values (2, 2.6, 5.6 and 10.6) (Sprankle et al., 1975) (Table 1.1). Sorption of glyphosate by soil constituents occurs mainly through the phosphonic acid moiety (Sprankle et al., 1975),

specifically by surface complexation with mineral fractions such as clays and amorphous soil Fe/Al-oxides (Borggaard and Gimsing, 2008). Glyphosate sorption is pH dependent because soil pH influences the surface charges of soil constituents as well as the net charge of the glyphosate molecule (McConnell and Hossner, 1985). Many previous studies report that glyphosate sorption decreases with increasing soil pH (de Jonge et al., 2001; McConnell and Hossner, 1985), but the influence of soil organic carbon content on glyphosate sorption is not clear (Farenhorst et al., 2009). For example, some studies reported that organic carbon content decreased glyphosate sorption (Arroyave et al., 2016; Day et al., 1997), while others reported that organic carbon content increased glyphosate sorption (Piccolo et al., 1996; Albers et al., 2009). Soil oxides such as Fe/Al-oxides are the principal sorption sites for glyphosate (Gimsing et al., 2007; Gimsing and Borggaard, 2007; Sprankle et al., 1975). Glyphosate forms inner sphere complexes with the surface hydroxyl groups of Fe/Al-oxides by ligand exchange reaction (Prata et al., 2003). Negatively charged phosphonate group of glyphosate (Table 1.1) interacts with positively charged surface hydroxyl groups on the Fe/Al-oxides surfaces (Barja and Afonso, 2005; Prata et al., 2003). Also, glyphosate has a tendency to form complexes with divalent/trivalent cations such as Ca^{2+}, Mg^{2+}, Fe^{3+} (Glass, 1987; McConnell and Hossner, 1985). Glyphosate can be sorbed in the interlayer spaces of clay minerals by forming glyphosate-cation complexes (Glass, 1987). The sorption of glyphosate in soil depends on several factors, including the presence of inorganic or organic molecules in soil (Boivin et al., 2005; de Jonge and de Jonge, 1999).

1.4. Phosphate and its Interaction with Glyphosate in Soil

Phosphorus is an essential element for plant growth (Gomes et al., 2015). Phosphate fertilizer demand is anticipated to reach 4.45 billion kg in North America by 2018 (FAO, 2015). Only a small portion of the phosphate fertilizer is utilized by the plants (Holford, 1997) and repeated application of phosphate fertilizer leads to the build-up of phosphate in soils (de Jonge et al., 2001; Pizzeghello et al., 2011). Residual phosphate in soil can be transported to surface water by runoff and erosion, and to groundwater by leaching (Gomes et al., 2015; Sheppard, 2011; Smith et al., 2016). Phosphate contamination has been shown to reduce surface water quality and lead to surface water eutrophication (Schindler et al., 2012; Scott et al., 2011; Withers et al., 2014). Phosphate residuals in soil occupy sorption sites and hence can influence the capacity of soil to retain newly-added phosphate when fertilizers are applied (de Jonge et al., 2001; Pizzeghello et al., 2011). For example, in a recent batch equilibrium experiment, more phosphate remained in soil solution when the soil was pre-treated with a phosphate fertilizer at the rate of 150 mg P kg^{-1} (Shafqat and Pierzynski, 2014). In fact, the equilibrium phosphate concentration in the experiment exceeded 0.025 mg L^{-1} which is the threshold level for phosphate in runoff to lead to fresh water eutrophication (US EPA, 1988).

Sorption of phosphate in soil includes both adsorption (a surface phenomenon) and precipitation (a new chemical phase) (Siebsen 1981; Akinremi, 1990) and these two processes limit the availability and solubility of phosphate over time (Brady and Well, 2008). Phosphate sorption in soil is greatly influenced by pH, carbonate content, organic carbon content and clay content of soils (Gustafsson et al., 2012; Ige et al., 2007), as well

as the concentrations of amorphous Fe/Al-oxides in soil (Gimsing et al., 2004). Studies indicate that phosphate sorption in acidic soils is positively correlated with the amount of amorphous Fe/Al-oxides in soil (Börling et al., 2004). Phosphate is adsorbed by Fe/Al-oxides or hydroxides through ligand exchange reactions (Börling et al., 2001; Brady and Well, 2008). In calcareous soils, phosphate sorption occurs by both adsorption and precipitation (Freeman and Rowell, 1981; von Wandruszka, 2006). Phosphate particularly forms complexes and precipitated with divalent cations such as calcium and magnesium (Tunesi et al., 1999).

Phosphate content in soil has been shown to influence glyphosate sorption because of the resemblance of the functional groups of glyphosate and phosphate as both molecules are sorbed through phosphonic acid moiety (Sprankle et al., 1975) (Table 1.1). Glyphosate and phosphate can compete for the same sorption sites in soil, such as Fe/Al-oxides, and this has been observed in some (Cruz et al., 2007; Kanissery et al., 2015; Piccolo et al., 1994; Sprankle et al., 1975), but not in other soils (Gimsing et al., 2004). In fact, the competition of glyphosate and phosphate for sorption sites remains unclear due to the heterogeneous soil systems (Borggaard, 2011; Paradelo et al., 2015).

Phosphate fertilizers contain cadmium (Cd) as an impurity and repeated application of phosphate fertilizer causes accumulation of Cd in soil (Lambert et al., 2007). Thus, it is possible that both Cd and glyphosate are present in agricultural soils. The presence of Cd has been shown to enhance the sorption of glyphosate in soil because Cd and glyphosate form complexes that are retained on the surface of negatively-charged soil colloids (Zhou

et al., 2004). The combined effect of phosphate and Cd on glyphosate sorption in soil is unknown.

1.5. MCPA and its Interaction with Phosphate and Glyphosate in Soil

The phenoxyacetic acid herbicide MCPA (2-methyl-4-chlorophenoxyacetic acid) is used extensively to control broadleaf weeds in crops grown in the Prairie Provinces in Canada. MCPA has two functional groups, polar carboxylic acid and lipophilic phenyl moiety (Haberhauer et al., 2001), and its acid dissociation constant (pK_a) is 3.73 (IUPAC, 2017) (Table 1.1). The soil pH range of most agricultural soils is North America is between 5 to 8 and hence MCPA is anionic form (Hiller et al., 2006). MCPA is weakly retained in soil with Kd ranging from 0.01 to 9.3 L kg^{-1} (Sørensen et al., 2006; Jacobsen et al., 2008; Alister et al., 2011) and desorption from 13 to 100% (Hiller et al., 2008, 2006; Sørensen et al., 2006). Soil half-lives for MCPA vary from 15 to 50 days (Sattar and Paasivirta, 1980; Soderquist and Crosby, 1975). As a consequence of extensive use and weak retention, MCPA has frequently been detected in surface and groundwater, and MCPA transports to the broader environment can be a result of spray drift, surface runoff and leaching (Health Canada, 2017). MCPA has been detected in Manitoba river water in concentrations up to 0.07 mg L^{-1} (Dr. Annemieke Farenhorst Unpublished Data) which is below the maximum allowable concentration set by Canadian drinking water quality guideline (0.1 mg L^{-1}) (Health Canada, 2017).

MCPA sorption in soil is positively correlated with organic carbon content and negatively correlated with soil pH because of the electrostatic repulsion between negatively charged

soil colloids and anionic MCPA in alkaline soils (Fredslund et al., 2008; Hiller et al., 2006; Jacobsen et al., 2008). MCPA is sorbed onto organic matter through H-bonding and lipophilic interaction (Haberhauer et al., 2001; Kah and Brown, 2006; Paszko, 2011). MCPA can also interact with positively charged soil Fe/Al-oxides complexes in soil (Waldner et al. 2012; Palma et al. 2016).

In one study it has been shown that addition of inorganic phosphate significantly reduced MCPA sorption in soil (Hiller et al., 2012). Perhaps, it is also possible that glyphosate and MCPA compete for the sorption sites in soil, particularly because glyphosate and MCPA molecules both have a carboxylic acid moiety (Gimsing and Borggaard, 2001; Haberhauer et al., 2001). However, to date no study has examined the effect of MCPA on glyphosate sorption.

1.6. Tetracycline and its Interaction with Phosphate and Glyphosate in Soil
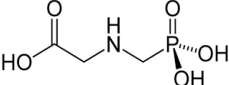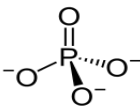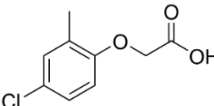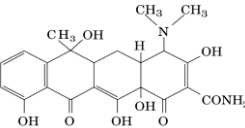
Tetracycline is a board-spectrum antibiotic and among the most widely used antibiotics in the world (De Briyne et al., 2014; Gu and Karthikeyan, 2005; Wan et al., 2010). Tetracycline is used to treat a number of infectious bacterial diseases affecting humans and other animals, including urinary tract and respiratory diseases (De Briyne et al., 2014; Lamb et al., 2015). It has been estimated that about 70 to 90% of an ingested antibiotic is excreted by animals, and the antibiotic is mainly excreted as the original active ingredient (parent compound) or in the form of the first transformation product (Massé et al., 2014). In agricultural fields that received application of animal manures, tetracycline has been detected in soil at concentrations of up to 500 ug kg^{-1} (Karcı and Balcıoğlu, 2009). Soil

half-lives for tetracycline range from 23 to 87 days (Aga et al., 2005; Pan and Chu, 2016). The persistence of antibiotics in soil or the broader environment could contribute to the development and spread of antibiotic resistance genes associated with some bacteria and antibiotic resistance is a serious threat to human and animal health (Munir and Xagoraraki, 2011). Tetracycline has been detected in surface and groundwater (Javid et al., 2016; Karthikeyan and Meyer, 2005; Lindsey et al., 2001).

Tetracycline has three acid dissociation constant (pK_a) values (3.3, 7.7 and 9.7) (Tolls, 2001) (Table 1.1) and, depending on soil pH, tetracycline exists as a cation (acidic soils), zwitterion (moderately acidic to neutral soils) or anion (alkaline soils) (Bao et al., 2009; Zhang et al., 2011). For soil pH conditions, it has been shown that tetracycline decreases with increasing soil pH (Zhang et al., 2011). A wide range of Kd values have been reported for tetracycline sorption in soil, ranging from 74 to 1,093 L kg^{-1} (Bao et al., 2009; Pan and Chu, 2016) but tetracycline desorption has been shown to only range from 1 to 9% (Fernández-Calviño et al., 2015; Pils and Laird, 2007). Soil organic matter and Fe/Al-oxides provide for sorption sites for tetracycline because the tricarbonyl amide and carbonyl functional groups of the molecule bind to these soil constituents (Gu and Karthikeyan, 2005). Presence of cations (Cu^{2+}, Ca^{2+}, and Mg^{2+}) increases tetracycline sorption because of the formation of metal-tetracycline complexes particularly involving clays and humic substances in soil (Pils and Laird, 2007; Zhang et al., 2010; Zhao et al., 2012, 2011).

Only one study has examined the competitive sorption of antibiotics and herbicides and this study showed that the herbicide simazine and metsulfuron-methyl reduced tetracycline sorption by biochar (Zhang et al., 2013). The presence of phosphate also reduced tetracycline sorption in soil (Wang et al., 2010). However, the competitive effect of tetracycline on glyphosate sorption and desorption in soils is unknown.

Table 1.1. Physicochemical properties of glyphosate, phosphate, MCPA and tetracycline

Properties	Glyphosate	Phosphate	MCPA	Tetracycline
Structure				
Molecular Mass (g mol^{-1})	169.1 [a]	94.97 [c]	200.62 [a]	444.43 [c]
Solubility in water (mg L^{-1})	10500 [a]	1000 [c]	29390 [a]	1700 [e]
Octanol-water partition coefficient (log K_{ow})	-3.2 [a]	-	0.81 [a]	-1.73 [e]
pKa	2, 2.6, 5.6, 10.6 [b]	2.2, 7.21, 12.67 [d]	3.73 [a]	3.3, 7.68, 9.69 [c]

Adatpted from: [a] (IUPAC, 2017), [b] (Sprankle et al., 1975), [c] (PubChem Compound Database, 2017), [d] (Weast, 1983), [e] (Tolls, 2001)

1.7. Objectives

Phosphate, cadmium, glyphosate, MCPA and/or tetracycline can be present in agricultural soils at the same time. The overall objective of this research was to examine the competitive sorption effects of various combinations of these inorganic and organic chemicals in soil. Specific objectives were to: 1) examine under a range of pH conditions, the impact of field-aged phosphate and cadmium concentrations on glyphosate sorption in an acidic sandy clay loam soil, with and without phosphate co-applications in the laboratory (Study 1), 2) using $0.01M$ KCl and $0.01M$ $CaCl_2$ as background electrolyte solutions, to examine the impact of a) field-aged phosphate concentrations and a commercially available glyphosate formulation on the sorption of phosphate in soil rich in iron oxides or calcium carbonates and b) field-aged phosphate concentrations and fresh phosphate on sorption of glyphosate in these two contrasting soils (Study 2) and 3) the impacts of phosphate concentrations on glyphosate, MCPA and tetracycline sorption and desorption in soil, and of MCPA and tetracycline and their mixtures, in the presence and absence of phosphate, on glyphosate sorption and desorption (Study 3).

1.8. Thesis outline

This thesis was written as manuscript style in accordance with the guideline of the Department of Soil Science and Faculty of Graduate Studies, University of Manitoba. It has five chapters including introduction (Chapter 1), three stand-alone specific research chapters (2 to 4) and overall synthesis (Chapter 5).

Chapter 2: Phosphate fertilizer impacts on glyphosate sorption by soil

Munira, S., Farenhorst, A., Flaten, D, Grant, C. 2016. Phosphate fertilizer impacts on glyphosate sorption by soil. Chemosphere **153**: 471-477.

Chapter 3: Phosphate and glyphosate sorption in soils following repeated application of phosphate fertilizer

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Chapter 4: Sorption and desorption of glyphosate, MCPA and tetracycline and their mixtures in soil as influenced by phosphate

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My contributions to chapters 2 to 4 includes the design of the experiments, sample collections, laboratory experiments and analyses, data management, statistical analysis, writing the manuscripts including preparation of figures and tables, submission to co-authors for their review, addressing their comments, and manuscript submission to journals and addressing reviewers comments as suggested.

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2. PHOSPHATE FERTILIZER IMPACTS ON GLYPHOSATE SORPTION BY SOIL

2.1. 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 mono ammonium 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 decreased significantly 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.

2.2. 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 been 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 demonstrate 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/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).

2.3. Materials and Methods

2.3.1. Experimental Design and Soil Characteristics

Soil samples (0-15 cm) with a sandy clay loam texture were collected near Carman (49° 29.7' N, 98° 2.4' W), Manitoba, Canada in the spring of 2013 from research plots situated from 2002-2012 under an annual crop rotation of durum wheat and flax. 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 four replicates per treatment. In each of the forty plots, the composite soil sample consisted of ten samples collected in the plot using a Dutch augar. 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 broadcast 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 urea applied was 90 kg N ha^{-1} in durum wheat and 50 kg N ha^{-1} in flax as in soil banding prior to or at the time of seeding.

Soil samples were air-dried and sieved (<2 mm) prior to soil properties analysis and sorption studies. Soil was digested with nitric acid and total Cd was determined by *inductively coupled plasma (ICP) analysis* (U.S. EPA, 1996). Extractable Cd was extracted with *diethylene triamine pentaacetic acid* (*DTPA*) following procedures 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 vary significantly 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_2O_3: 246 ± 5 mg kg^{-1} (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} (n=16).

2.3.2. 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 Ultra2 (49% active ingredient and 51% other ingredients, CAS No. 70901-12-1) from Monsanto Chemical Company. Active ingredient was potassium salt of N-(phosphonomehyl) glycine.

Glyphosate sorption was determined by batch equilibrium with the initial solution containing 1 mg L^{-1} glyphosate and 6.67×10^4 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°C for 24 h. 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, Kd (L kg^{-1}) was calculated by Cs/Ce, whereby Cs = glyphosate sorption by soil at equilibrium (mg kg^{-1}), and Ce = 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 0.5, 1, 2, 4, 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 Ultra2 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 grade 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}.

2.3.3. Statistical Analysis

Statistical analyses were completed using SAS software version 9.3 for Windows (SAS Institute Inc. 2002-2010). Prior to each analysis, data were examined for outliers, conformation of the residuals to the Gaussian distribution (Shapiro-Wilk test) and homogeneity of variances (quantile-quantile plot). Data were analysed using the normal error distribution. 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, two-way analysis of variance (ANOVA) ($p<0.05$) (3 X 3 factorial layout) and multiple means comparison (Tukey's) tests ($p<0.05$) in PROC GLIMMIX were conducted. Phosphate fertilizer, Cd, and their interaction were considered as fixed effect while block as random effect. For each pH (ionic solution), multiple linear regression analyses ($p<0.05$) were carried out to predict glyphosate Kd values by using Olsen P and extractable Cd concentrations as independent variables. Repeated measure analysis ($p<0.05$) in PROC GLIMMIX was used to determine the effect of shaking time (0.5, 1, 2, 4, 8 and 24 h) by using phosphate levels and time as independent variables and considering time as the repeated measures effect. In this repeated measure analysis, the spatial power [SP(POW)] covariance structure was used. Shaking time, phosphate levels and their interaction were considered as fixed effect while block as random effect. Two-way ANOVA ($p<0.05$) (3 × 4 factorial layout) and multiple means comparison (Tukey's) tests ($p<0.05$) in PROC GLIMMIX were utilized to quantify the effects of field-aged (20P, 40P, 80P) and fresh phosphate additions (0, 11, 22 and 44 mg P kg^{-1}) on glyphosate Kd values. Field-aged phosphate, fresh phosphate and their interaction were considered as fixed effect while block as random effect. To quantify the impact of using Roundup Ultra2

versus analytical-grade glyphosate on Kd values in soils, one-way ANOVA (P<0.05) and multiple means comparison (Tukey's) tests (P<0.05) in PROC GLIMMIX were applied. Formulation (commercial and analytical grade) was considered as fixed effect and block as random effect.

2.4. 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 (Table 2S1) in glyphosate sorption by soil when using either Roundup Ultra2 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 2.1). Olsen P concentrations ranged from 13 to 99 mg kg^{-1} among individual field plots and decreased significantly from 80P > 40 P > 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 (Table 2S2). Thus, the amount of Cd in the MAP fertilizers applied from 2002-2009 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} (Figure 2.2) which is 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 2.2). For the 80P plots, extractable Cd concentrations decreased significantly in the order of high Cd > med Cd > low Cd. For the 40P plots, extractable Cd concentrations decreased significantly 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 2S3- Supplementary information). The Cd concentrations in our field plots are similar 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) increased glyphosate sorption by approximately 1.6-fold, relative to control soil.
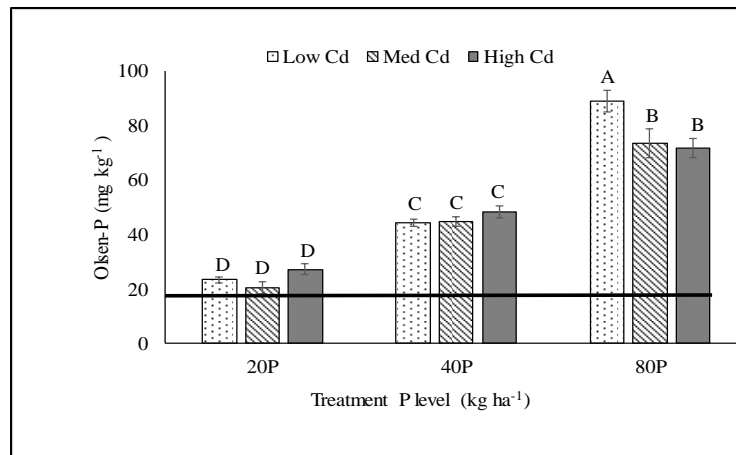
Figure 2.1. Effect of phosphate fertilizers with different Cd levels on Olsen P concentrations in soil. The solid line indicates the concentration of Olsen P in control plots.
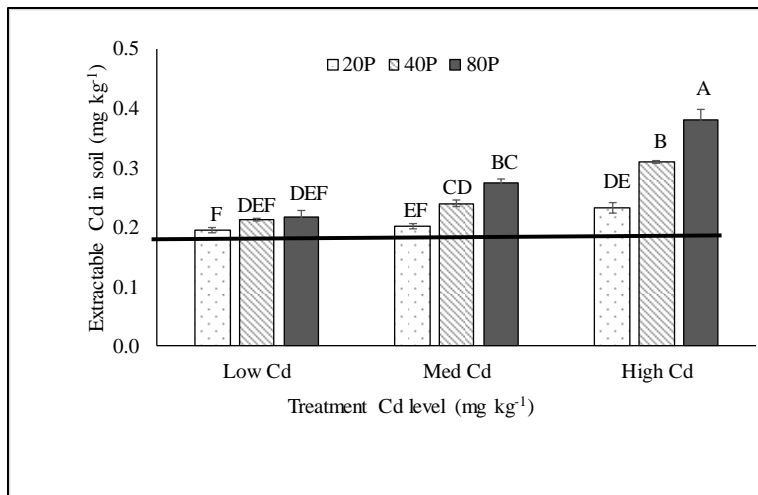
Figure 2.2. Effect of phosphate fertilizers with different Cd levels on DTPA-extractable Cd in soil. The solid line indicates the concentration of extractable Cd in control plots.

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 2S3-Supplementary information). Regardless of the ionic solution used in the batch equilibrium experiments, increased Olsen P concentrations significantly decreased glyphosate sorption by soil (Figure 2.3). 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 17 mg kg^{-1} Olsen P (Figure 2.3). 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 treatments, time had no significant effect on glyphosate Kd values and sorption was always significantly lower in 80P than in 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 2.4). For these pH conditions, glyphosate sorption was almost always significantly lower 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 2.4). In general, longer shaking hours resulted in greater numerically differences in glyphosate Kd values between control and 80P plots.
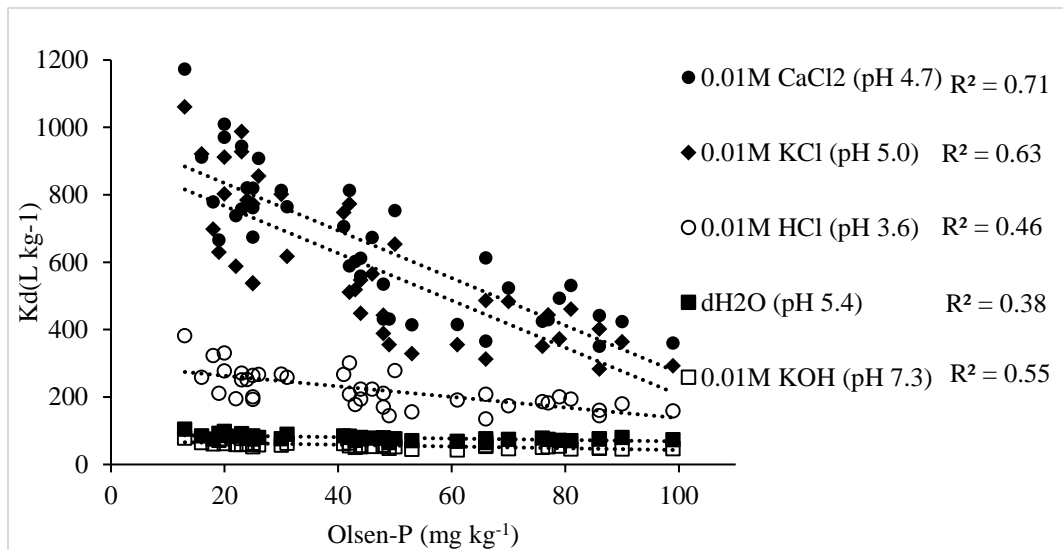
Figure 2.3. Relation between Olsen-P concentrations in soil and the glyphosate sorption distribution constant, Kd, with soil slurries being under different pH conditions. All regression equations are significant at P<0.05, and the equations are given in Table 2S3-Supplementary information.

At pH 5.4 (dH$_2$O), addition of fresh phosphate had no significant effect (P = 0.1920) on glyphosate sorption. For two other pH conditions (pH 4.7 and 5.0), regardless of the ionic solution used (Figure 2.5), there was a significant interaction (P<0.01) between field-aged and fresh phosphate on glyphosate sorption (Table 2.2S4- 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 sorbed preferentially when added with glyphosate to soil.
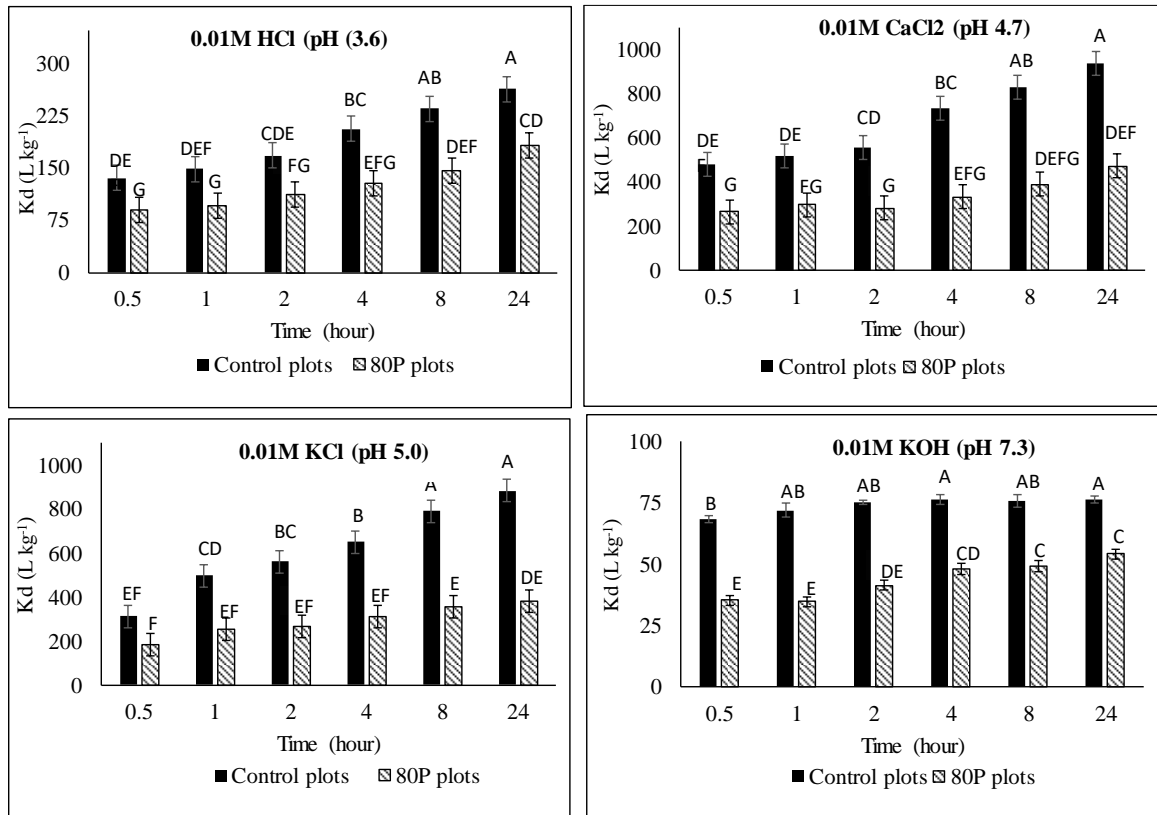
Figure 2.4. Time dependent sorption study of glyphosate Kd values in control and 80P plots.
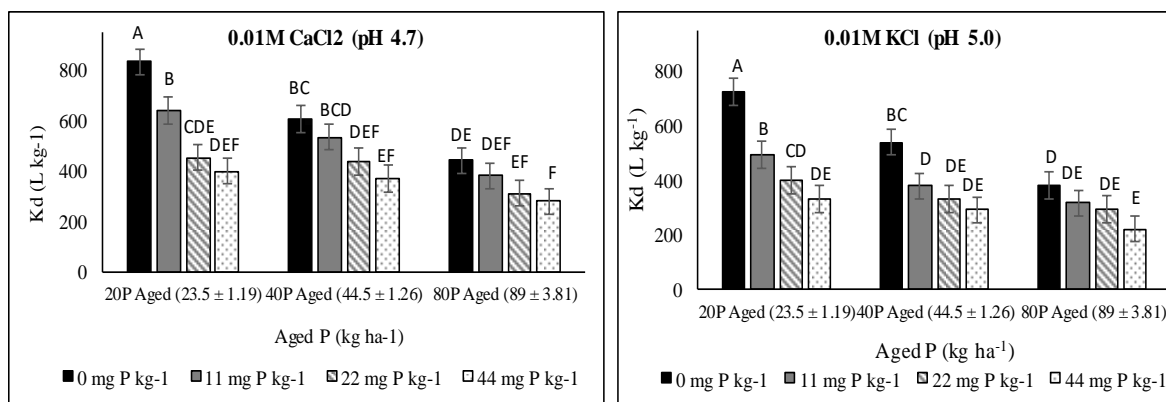
Figure 2.5. Effect of co-applying mono ammonium phosphate with glyphosate in solution, for batch equilibrium experiments using 0.01M $CaCl_2$ (pH 4.7) and 0.01M KCl (pH 5.0).

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 20P plots containing relatively small Olsen P concentrations (Figure 2.5). 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 80P plots (Figure 2.5). 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 80P plots for which only the addition of 44 mg P kg^{-1} resulted in a significant reduction in glyphosate Kd values (Figure 2.5). 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 H_2G^- (~ 85%) and HG^{2-} (~ 15%), and at pH 5.0 (0.01M KCl) when glyphosate molecules mainly exist as H_2G^- (~ 75%) and HG^{2-} (~ 25%) (Wang et al., 2004). The use of 0.01M CaCl₂ would have enhanced the amount of exchangeable 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 a relatively large Ca^{2+} content ($2,252 \pm 40.57$ mg kg^{-1}), and using 0.01M KCl, would allow K^+ to replace Ca^{2+} on the exchange sites 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 2.3). At pH 3.6, a greater amount of soil colloids is net positively-charged, promoting the sorption of glyphosate molecules that mainly exist as H_2G^- (~ 95%) and H_3G (~ 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 H_2G^- (~ 60%) and 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 HG^{2-} (~100%) (McConnell and Hossner, 1985; Wang et al., 2004).

2.5. Conclusion

Analytical-grade glyphosate showed similar results as a commercially-available glyphosate formulation. Repeated application 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.

2.6. Acknowledgements

2.7. References

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3. PHOSPHATE AND GLYPHOSATE SORPTION IN SOILS FOLLOWING REPEATED APPLICATION OF PHOSPHATE FERTILIZER

3.1. Abstract

Phosphate and glyphosate molecules compete for sorption sites in soil. The objective of this study was to quantify the impact of Olsen P concentrations in two contrasting soils on phosphate and glyphosate sorption. Soils were a sandy clay loam soil rich in iron oxides (SCL-Fe_2O_3) and a clay loam soil rich in calcium carbonates (CL-$CaCO_3$). The phosphate Freundlich sorption coefficient (Kf) ranged from 3 to 68 $L^{1/n} mg^{1-1/n} kg^{-1}$ in the SCL-Fe_2O_3 and from 21 to 76 $L^{1/n} mg^{1-1/n} kg^{-1}$ in the CL-$CaCO_3$. Glyphosate sorption coefficient (Kd) ranged from 293 to 1173 $L kg^{-1}$ in the SCL-Fe_2O_3 but only 99 to 141 $L kg^{-1}$ in the CL-$CaCO_3$. Glyphosate Kd and phosphate Kf values decreased significantly with increasing Olsen P concentrations in both soils. Glyphosate Kd values were further significantly reduced when phosphate was added to the slurry solutions, but phosphate Kf values were not impacted by the presence of glyphosate in solutions. We conclude that annual phosphate fertilizer applications leave phosphate concentrations in Prairie soils to the extent that soils have a lesser capacity to retain glyphosate and phosphate that are subsequently applied, but glyphosate residues will not influence phosphate sorption.

3.2. Introduction

Sorption is an important process that influences the transport and availability of nutrients and pesticides in soil (Duke et al., 2012; Hiller et al., 2012; Ige et al., 2005). Phosphate is an essential nutrient for crop growth and annually applied to agricultural soils (Gomes et al., 2015). Glyphosate [*N*-(phosphonomethyl) glycine] is a broad spectrum, non-selective

systemic herbicide and among the most widely used pesticides in the world (Duke and Powles, 2009; FOEE, 2013).

Amorphous Fe and Al oxides in soil are the preferred sorption sites for phosphate and glyphosate molecules (Barja and Afonso, 2005; Gimsing et al., 2004; Piccolo et al., 1994). Phosphate is preferentially sorbed by Fe-oxides because the phosphate molecule (0.25 nm) is smaller than the glyphosate molecule (0.43 nm) (Gimsing et al., 2007; Gimsing and Borggaard, 2001), but glyphosate is retained by Fe-oxides through both phosphonic acid and carboxylic acid moieties (Gimsing and Borggaard, 2001; Sprankle et al., 1975; Tévez and Afonso, 2015). Phosphate readily forms complexes with Ca^{2+} in calcareous soils (Busman et al., 2009) and glyphosate can form stable complexes with Ca^{2+} in solution and sorb (Glass, 1987) or form weak bonds with exchangeable Ca^{2+} associated with clays (de Jonge et al., 2001). Glyphosate can also be sorbed onto clay minerals (de Jonge et al., 2001), divalent cations of inter layer clay minerals (Piccolo et al., 1994) and organic matter (Morillo et al., 2000).

Sorption of phosphate and glyphosate by soil is typically determined using soil slurries in batch-equilibrium experiments (de Jonge et al., 2001; Gimsing et al., 2004; Gimsing and Borggaard, 2002; Ige et al., 2005). Phosphate and glyphosate molecules compete for sorption sites in soil and adding phosphate and glyphosate to soil slurries at the same time can influence sorption of the individual chemicals (de Jonge and de Jonge, 1999). For example, in a range of batch equilibrium studies, glyphosate sorption by soil was less when phosphate was added with glyphosate (Gimsing et al., 2007; Gimsing and Borggaard, 2007; Kanissery et al., 2015). A recent study generated rainfall runoff at one day after glyphosate

applications on a silty clay loam soil and showed that glyphosate concentrations in runoff were greater for plots that had also received phosphate applications (Sasal et al., 2015).

Recently, concerns have been raised about the possibility of glyphosate increasing the transport of phosphate to surface water thereby increasing the risk for lake eutrophication (Barrera, 2016). In batch equilibrium studies, glyphosate additions to soils were able to release some phosphate in acidic soil particularly when high in Al and Fe-oxides (Gimsing et al., 2004). Additions of glyphosate also released phosphate molecules bound to synthesized Al-oxides and but this release was not observed with synthesized Fe-oxides (Gimsing et al., 2004; Gimsing and Borggaard, 2002, 2001). It is unknown whether glyphosate influences phosphate retention in calcarious soils.

Batch equilibrium studies of glyphosate or phosphate sorption typically use either 0.01M $CaCl_2$ or 0.01M KCl as background electrolyte solutions (Bhattacharyya et al., 2015; de Jonge et al., 2001; Gimsing and Borggaard, 2001; 2007; Okada et al., 2016). The batch equilibrium process does not distinguish between sorption and precipitation (Siebsen 1981; Akinremi, 1990; Muhammad, 1992). At pH 7, the background electrolyte solution strongly influenced the sorption of phosphate by Fe-oxides because with 0.01M $CaCl_2$, phosphate formed complexes with Ca^{2+} and precipitated, while this did not occur with 0.01M or 0.1M KCl (Gimsing and Borggaard, 2001). However, under the same conditions, the background electrolyte solution had little influence on glyphosate sorption by Fe-oxides (Gimsing and Borggaard, 2001).

Repeated phosphate fertilizer applications result in the build-up of phosphate in agricultural soils because crops only utilize a small portion of the phosphate applied (de Jonge et al., 2001; Holford, 1997). Repeated phosphate-fertilizer applications to sandy clay loam and loamy sand soils in India reduced the ability of the soils to retain phosphate molecules, as determined by batch equilibrium experiments (Bhattacharyya et al., 2015; Varinderpal-Singh et al., 2006). Repeated phosphate fertilizer applications to a sandy clay loam soil in Canada and coarse sand and sandy loam soils in Denmark resulted in fertilized soils showing significantly lesser glyphosate sorption than untreated soils, also as determined by batch equilibrium experiments (de Jonge et al., 2001; Munira et al., 2016). The combination of fresh phosphate applications to soils containing field-aged phosphate and the impact of these phosphate concentrations on glyphosate sorption has received no attention except for Munira et al. (2016).

It is unknown whether increasing field-aged-phosphate concentrations have the same impact on reducing phosphate sorption as they have on reducing glyphosate sorption. In this study, we used a soil high in Fe-oxides (pH 4.7 to 5) versus a soil high in calcium carbonates (pH 7.3 to 7.5) that were both subjected to repeated phosphate fertilizer applications at 20, 40 and 80 kg P ha^{-1} resulting in a range of Olsen P concentrations due to the build-up of phosphate in soil during eight years of annual applications. Using 0.01M KCl and 0.01M CaCl$_2$ as background electrolyte solutions, the objective of this study was to examine the impact of 1) field-aged phosphate concentrations and a commercially available glyphosate formulation on phosphate sorption in soils rich in iron oxides or

calcium carbonates and 2) field-aged phosphate concentrations in combination with fresh phosphate on glyphosate sorption in these two contrasting soils.

3.3. Materials and Methods

3.3.1. Chemicals

Chemicals used were analytical grade glyphosate (99.9% purity) from Sigma-Aldrich Co., St. Louis, MO; [phosphonomethyl-^{14}C]glyphosate (99% radiochemical purity; specific activity 50 mCi/mmol) from American Radiolabeled Chemicals Inc., St. Louis, MO; Roundup Ultra2 (49% active ingredient and 51% other ingredients, CAS No. 70901-12-1) from Monsanto Chemical Company; and analytical grade potassium dihydrogen phosphate (KH_2PO_4) (99% chemical purity), potassium chloride (100% chemical purity) and calcium chloride, dihydrate (>95% chemical purity) from Fisher Scientific, Fair Lawn, NJ.

3.3.2. Soil Characteristics and Experimental Design

This study utilized soil samples (0-15 cm) obtained from long-term experimental plots under a durum wheat and flax rotation near Carman (49° 29.7' N, 98° 2.4' W) and near Forrest (50° 1.2' N, 99° 53.3' W) Manitoba, Canada. Soil profiles at both sites were classified based on the Canadian System of Soil Classification as Orthic Black Chernozems, which is equivalent to the Udic Boroll subgroup in the U.S. Soil Taxonomy (Soil Classification Working Group, 1998). The experimental design at each site was a randomized complete block design with four mono ammonium phosphate fertilizer treatments and four replicates plots. Treatments were a control (no phosphate applications), and plots receiving annual applications of mono ammonium phosphate fertilizers at 20, 40,

and 80 kg P ha^{-1}, (Grant et al., 2013) or 20P, 40P, and 80P, respectively, from 2002 to 2009. For all plots that received mono ammonium phosphate, 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 mono ammonium phosphate was broadcast and then incorporated. From 2010 to 2013, the rotation was continued but no phosphate was applied. Application of urea fertilizer differed by year. Generally, durum wheat received 90 kg N ha^{-1} and flax 50 kg N ha^{-1}.

From each plot, composite samples were collected in spring, 2013 using a Dutch auger with ten (Carman) to eight (Forrest) samples per plot and cleaning the auger between plots. Soil samples were air-dried and sieved (<2 mm) prior to soil property analyses and sorption experiments. The Carman soil has a sandy clay loam texture and is relatively high in iron oxides (SCL-Fe$_2$O$_3$), whereas the Forrest soil has a clay loam texture and is relatively high in calcium carbonates (CL-CaCO$_3$) (Table 3.1). Available phosphate was extracted using the Olsen (0.5 M NaHCO$_3$, pH 8.5) phosphorus test. 2 g of air dried soil and 40 ml of 0.5N NaHCO$_3$ solution was mixed in a 50 mL Erlenmeyer flask. Flasks (duplicates) were shaken horizontally (200 excursions min^{-1}). Equilibrium solutions were filtered through Whatman No. 2 filter paper and phosphate concentrations were determined colorimetrically (Frank et al., 2011).

Table 3.1. Selected soil physical and chemical properties as mean with standard error

Soil	Organic Carbon [a] (%)	pH[b]	Fe_2O_3[c] (mg kg^{-1})	Al_2O_3[c] (mg kg^{-1})	Ca[d] (mg kg^{-1})	Clay[e] %	Silt[e] %	Sand[e] %
SCL-Fe_2O_3	2.81 ± 0.04	4.7± 0.02	237±7.93	6.41±0.64	2252±35	20	20	60
CL-$CaCO_3$	3.2 ± 0.07	7.3± 0.02	12.52±0.22	1.07±0.47	4791±158	30	39	31

[a] Soil organic carbon content was determined using combustion technique with a high temperature induction furnace (Nelson and Somemers, 1996). [b] Soil pH was determined using a 10 ml 0.01M $CaCl_2$ solution and 2 g soil solution ratio (Jones Jr, 2001). [c] Extractable Fe and Al were extracted with diethylenetriaminepentaacetic acid *(DTPA)* (Whitney, 2011) and 0.01M $CaCl_2$, (Barnhisel and Bertsch, 1982) respectively, and extracts were analyzed by *ICP*. [d] Extractable Ca was also measured by *ICP* using ammonium acetate as an extractant (Warncke and Brown, 2011) [e] data adapted from Grant et al. (2013).

3.3.3. Phosphate Sorption

Phosphate sorption was determined by batch equilibrium using either 0.01M $CaCl_2$ or 0.01M KCl as the background electrolyte. Batch equilibrium procedures followed standard protocols using a soil/solution ratio of 1:10 and an equilibrium time of 24h (Ige et al., 2005). Two experiments were conducted utilizing soil samples: (1) from all plots at each site to quantify the effect of Olsen P concentrations on phosphate sorption in soil and (2) from control and 80P plots at each site to quantify the effect of Roundup Ultra2 additions to soil slurries on phosphate sorption in soil.

3.3.3.1. Effect of Field-Aged Phosphate Concentrations on Sorption of Phosphate

In the first experiment, potassium dihydrogen phosphate solutions (20 mL) at concentrations of 5, 10, 25, 50, 100, 150, 250 or 500 mg P L^{-1} were added to air-dried soil (2 g) in 50-mL centrifuge tubes (duplicates) and shaken horizontally (120 excursions min^{-}

[1]) at room temperature ($23\pm2^{\circ}$C) for 24h. Equilibrium solution was centrifuged (10,000 rev min^{-1} for 10 min) and filtered (0.45 µm). Phosphate concentration was determined colorimetrically by the molybdate blue method (Murphy and Riley, 1962). Linearized Freundlich isotherm has been specified as: The phosphate sorption coefficient, Kf ($L^{1/n}$ mg$^{1-1/n}$ kg[-1]), was calculated using the linearized form of Freundlich equation:

log q = log Kf + 1/n log C

Where q represents phosphate sorption in soil at equilibrium (mg kg^{-1}), C represents phosphate concentration of equilibrium solution (mg L^{-1}), and 1/n represents the Freundlich slope. In addition, the Freundlich P sorption isotherm was used to determine the equilibrium P concentration (EPCo) at log q = 0, which is the concentration at which neither sorption nor desorption occurs and hence can be used to define whether a soil is likely to act as a sink (sorption) or source (desorption) of P (Indiati and Sharpley, 1998; Sharpley et al., 1994). EPCo levels above 0.025 mg L^{-1} (US EPA, 1988) suggest an increased risk of eutrophication because of P transport in soluble form.

3.3.3.2. Effect of Glyphosate Formulation on Sorption of Phosphate

In the second experiment, stock solutions of 150 mg P L^{-1} were prepared with and without 100 mg L^{-1} Roundup Ultra2 in the solution. The 150 mg P L^{-1} solution was used because previous studies have proposed that this parameter (P150) is the most optimum single point in the isotherm reflective of the phosphate sorption capacity in soils (Ige et al., 2005). Batch equilibrium procedures were carried out as described above. The phosphate sorption coefficient, Kd (L kg^{-1}), was calculated by q/C, where q represents phosphate sorption by

soil at equilibrium (mg kg^{-1}) and C represents phosphate concentration of equilibrium solution (mg L^{-1}).

3.3.4. Glyphosate Sorption

Glyphosate sorption was determined by batch equilibrium with the initial glyphosate solution containing 1 mg L^{-1} analytical-grade glyphosate and 6.67×10^4 Bq L^{-1} ^{14}C-labelled glyphosate. Two experiments were conducted utilizing soil samples: (1) from all plots to quantify at each site the effect of Olsen P concentrations on glyphosate sorption, and (2) from control and 80P plots to quantify at each site the effect of fresh phosphate additions to soil slurries on glyphosate sorption in soil.

3.3.4.1. Impact of Field-Aged Phosphate Concentrations on Sorption of Glyphosate

Batch equilibrium procedures followed the OECD guideline 106 using a soil/solution ratio of 1:5, an equilibrium time of 24h and 0.01M CaCl$_2$ or 0.01M KCl as background electrolyte (OECD, 2000). Glyphosate solutions (10 mL) were added to air-dried soil (2 g) in 50-mL centrifuge Teflon tubes (duplicates) and slurries were rotated in the dark at 5°C for 24h. Equilibrium solution was centrifuged (10,000 rev min^{-1} for 10 min) and subsamples (1 mL) of supernatant were added in duplicated 7-mL scintillation vials containing 5 mL of 30% Scintisafe scintillation cocktail (Fisher Scientific, Fair Lawn, NJ). 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, Kd (L kg^{-1}), was calculated by Cs/Ce, where Cs represents glyphosate sorption by soil at equilibrium (mg kg^{-1}) and Ce represents

glyphosate concentration of equilibrium solution (mg L^{-1}). The difference between the added radioactivity and radioactivity in the supernatant was assumed to be the proportion of glyphosate having been sorbed.

3.3.4.2. Impact of Fresh Phosphate Addition on Sorption of Glyphosate

Experiments followed similar batch equilibrium sorption protocols as described above. In this experiment, potassium dihydrogen phosphate was added to the initial glyphosate solution at rates equivalent to 11, 22 and 44 mg P kg^{-1} soil, or an estimated 20, 40 and 80 P kg ha^{-1}, respectively, when assuming the fertilizer being present in the top 15-cm layer of a soil with a bulk density of 1,200 kg m^{-3}.

3.3.5. Statistical analysis

Statistical analyses were carried out using SAS software version 9.3 for Windows (SAS Institute Inc. 2002-2010). Prior to each analysis, data were examined for outliers, conformation of the residuals to the Gaussian distribution (Shapiro-Wilk test) and homogeneity of variances (quantile-quantile plot). Data were analysed using the normal error distribution. The paired t-test ($P<0.05$) was used to test for the effect of background electrolyte solution (0.01M CaCl$_2$ versus 0.01M KCl) on glyphosate Kd or phosphate Kf and EPCo. For both background electrolyte solutions and at each site, simple linear regression analyses ($P<0.05$) were carried out to estimate glyphosate Kd and phosphate Kf values using Olsen P concentration as the independent variable. In each of the glyphosate Kd and phosphate Kf figures, the slopes of regression lines developed for SCL-Fe$_2$O$_3$ and CL-CaCO$_3$ were compared by including dummy variables in PROC REG to test whether

the responses of sorption to increasing Olsen P concentrations was influenced by soil type. Simple linear regression analysis was also carried out to estimate glyphosate Kd values using the added fresh phosphate concentration as the independent variable. The slopes of the regression lines developed for the 0P (control) and 80P plots in both soils were compared by including dummy variables in PROC REG to test whether the responses of sorption to increasing potassium dihydrogen phosphate concentration was influenced by Olsen P concentrations (0P, 80P). Simple linear regression analyses were carried out to determine the relationship between glyphosate Kd and phosphate Kf values by using Kf as an independent variable. Simple linear regression analyses ($P<0.05$) were also carried out to estimate EPCo values by using Olsen P as an independent variable for CL-CaCO$_3$ soil. Graphical plot fitting of EPCo as a function of Olsen P showed that data did not fit well (lack-of fit test) with simple linear regression for the SCL-Fe$_2$O$_3$ soil. Therefore, data was fitted to an exponential non-linear model ($y = ae^{bx}$ or EPCo $= ae^{bOlsenP}$) using PROC NLIN. One-way ANOVA ($P<0.05$) in Proc GLIMMIX was used to estimate the effect of Roundup Ultra2 on phosphate sorption in soils. Roundup Ultra2 formulation was considered as fixed effect and block as random effect.

3.4. Results and Discussion

3.4.1. Effect of Background Electrolyte Solutions on Sorption of Phosphate and Glyphosate

The types of ions in solution had a significant effect on phosphate and glyphosate sorption, except for glyphosate sorption in the CL-CaCO$_3$ soil (Table 3.2). Phosphate Kf values in

both soils were significantly greater in experiments with 0.01M $CaCl_2$ than experiments with 0.01M KCl (Table 3.2).

Table 3.2. Statistical parameters (Paired t-tests) on the effect of background electrolyte solution (0.01M $CaCl_2$ versus 0.01M KCl) on glyphosate (L kg^{-1}), phosphate sorption parameters ($L^{1/n}$ mg $^{1-1/n}$ kg^{-1}) and phosphate equilibrium concentrations (mg L^{-1}) in soils.

Sorption Parameter	Soil	Mean		DF	t Value	Pr > |t|
		0.01M $CaCl_2$	0.01M KCl			
Phosphate sorption	SCL-Fe_2O_3	38.47	23.11	15	11.14	<.0001
coefficient, Kf	CL-$CaCO_3$	54.08	36.04	15	10.94	<.0001
Glyphosate sorption	SCL-Fe_2O_3	703	632	15	5.89	<0.0001
coefficient, Kd	CL-$CaCO_3$	116	117	15	-1.55	0.1430
Phosphate Equilibrium	SCL-Fe_2O_3	0.007	0.04	15	-2.29	<.0366
Concentration, EPCo	CL-$CaCO_3$	0.006	0.015	15	-4.72	<.0003

Phosphate Kf values were on average 54 $L^{1/n}$ mg $^{1-1/n}$ kg^{-1} in CL-$CaCO_3$ and 38 $L^{1/n}$ mg $^{1-1/n}$ kg^{-1} SCL-Fe_2O_3 with $CaCl_2$ but on average 36 $L^{1/n}$ mg $^{1-1/n}$ kg^{-1} in CL-$CaCO_3$ and 23 $L^{1/n}$ mg $^{1-1/n}$ kg^{-1} in SCL-Fe_2O_3 with KCl. Thus, when 0.01M $CaCl_2$ was used with the SCL-Fe_2O_3 and CL-$CaCO_3$ soils but also when KCl was used with the CL-$CaCO_3$ soil, phosphate likely formed stable complexes with a portion of Ca^{2+} in soil solution and precipitated (Carreira et al., 2006; Devau et al., 2011; Gimsing and Borggaard, 2001). In batch equilibrium experiments with 0.01M $CaCl_2$, precipitation with Ca^{2+} occurs more readily for phosphate than glyphosate (Gimsing and Borggaard, 2001). For glyphosate sorption, Kd values were on average 116 L kg^{-1} in CL-$CaCO_3$ and 703 L kg^{-1} SCL-Fe_2O_3 with $CaCl_2$, and on average 117 L kg^{-1} in CL-$CaCO_3$ and 632 L kg^{-1} SCL-Fe_2O_3 with KCl. In calcareous soils, Ca^{2+} in forms a bridge between negatively charged soil colloids and glyphosate molecules in soil (Zhao et al., 2015) and, because of the already high free calcium content

in the CL-CaCO$_3$ soil, the addition of Ca with 0.01M CaCl$_2$ solution had no impact on glyphosate sorption. For the SCL-Fe$_2$O$_3$ soil, glyphosate sorption was greater with 0.01M CaCl$_2$ than 0.01M KCl, suggesting that glyphosate was able to form complexes with Ca in solution for enhanced sorption (Glass, 1987).

3.4.2. Effect of Field-Aged Phosphate Concentrations on Sorption of Phosphate

Despite being exposed to similar repeated application of phosphate fertilizer treatments, Olsen P ranged from 13 to 99 mg kg^{-1} in the acidic SCL-Fe$_2$O$_3$ soil but only from 8 to 48 mg kg^{-1} in the calcareous CL-CaCO$_3$ soil. Olsen P concentrations by treatment were on average 17 (control), 24 (20P), 44 (40P) and 89 (80P) mg kg^{-1} in the SCL-Fe$_2$O$_3$ soil and 13 (control), 18 (20P), 24 (40P) and 41 (80P) mg kg^{-1} in the CL-CaCO$_3$ soil. The Olsen P test was originally developed for calcareous soils and can overestimate plant available P in acidic soils (Kane et al., 2013), such as the SCL-Fe$_2$O$_3$. Olsen P measures the NaHCO$_3$ extractable phosphate in soil, but calcareous soil may also contain slow release inorganic phosphate (apatite minerals) extracted by 1M HCl (Cross and Schlesinger, 1995; Hedley et al., 1982; Yu et al., 2014). Olsen P concentrations ranged from 8 to 99 mg kg^{-1} in this research which is within the typical range of 8 to 114 mg kg^{-1} that has been reported for soils in North America (McDowell et al., 2001). Hence, the findings from this research on the sorption pattern of phosphate and glyphosate in soil would be applicable to a wider range of soils in North America.

Phosphate Kf values significantly decreased with the increasing concentrations of Olsen P in soil (Figure 3.1). The SCL-Fe$_2$O$_3$ and CL-CaCO$_3$ soils showed relatively similar

phosphate sorption (Figure 3.2). Phosphate Kf values ranged from 3.2 to 68 $L^{1/n}$ mg $^{1-1/n}$ kg^{-1} in the SCL-Fe_2O_3 soil with 1/n values between 0.37 to 0.92, and from 21 to 76 $L^{1/n}$ mg $^{1-1/n}$ kg^{-1} in the CL-$CaCO_3$ soil with 1/n values between 0.68 to 0.92. These values are within the range of other studies (Bertrand et al., 2003; Jalali, 2007; Shafqat and Pierzynski, 2014). A maximum reduction of phosphate Kf value was observed in SCL-Fe_2O_3 soil. The phosphate Kf value in SCL-Fe_2O_3 was reduced by 95% in soil containing 99 mg kg^{-1} Olsen P relative to soil containing 13 mg kg^{-1} Olsen P. Thus, P accumulation in soil reduced the capacity of soil to hold P. Wang et al. (2015) also reported that sorption of P decreased with the increasing concentrations of Olsen P because repeated application of P fertilizer leads to the accumulation of P in soil. In their study, they showed that repeated (5 to 15 years) application of phosphate significantly reduced phosphate sorption by 56% in soil containing 53 mg kg^{-1} Olsen P relative to soil containing 15 mg kg^{-1} Olsen P.

Olsen P concentrations significantly predicted phosphate Kf (Figure 3.1) in both SCL-Fe_2O_3 and CL-$CaCO_3$. The effect of Olsen P concentrations on reducing phosphate sorption was more pronounced for SCL-Fe_2O_3 than CL-$CaCO_3$ (Table 3S6). For the phosphate Kf, the regression slopes were significantly different between the soils in case of 0.01M KCl but not with 0.01M $CaCl_2$ because the presence of Ca in solution led to the possibility of precipitation of phosphate-Ca^{2+} complexes in both soils (Busman et al., 2009; Carreira et al., 2006; Devau et al., 2011). Generally, in calcareous soil, Ca forms precipitation with the added phosphate in soil solution (Carreira et al., 2006). For 0.01M KCl, the CL-$CaCO_3$ showed a significantly steeper slope than SCL-Fe_2O_3 (Figure 3.1) because, with increasing Olsen P concentrations, more sorption sites remained available in SCL-Fe_2O_3. CL-$CaCO_3$

soil has less sorption sites available for the added phosphate than SCL-Fe_2O_3 soil because calcareous soils contain slow-release phosphate (e.g, octacalcium phosphate and apatite) which occupy sorption sites that otherwise would be available for the added phosphate (Cross and Schlesinger, 1995; Hedley et al., 1982; Shariatmadari et al., 2007).

EPCo significantly increased with increasing concentrations of Olsen P in both SCL-Fe_2O_3 and CL-$CaCO_3$ (Figure 3.2). EPCo values ranged from 0 to 0.281 mg L^{-1}, depending on the background electrolyte solution and soil (Figure 3.2). EPCo values in both soils were significantly greater in the experiments with 0.01M KCl than experiments with 0.01M $CaCl_2$ (Table 3.2) because of the formation of Ca^{2+}-phosphate complexes in both soils with 0.01M $CaCl_2$. All EPCo levels were below the threshold value of 0.025 mg L^{-1} (US EPA, 1988) except in the 80P plots. The average calculated EPCo values for the four replicated 80P plots was 0.031 mg L^{-1} for CL-$CaCO_3$ and 0.190 mg L^{-1} for SCL-Fe_2O_3 with 0.01M KCl, and 0.025 mg L^{-1} for SCL-Fe_2O_3 with 0.01M $CaCl_2$. Although this suggest that prairie soils have a low risk for soluble P transport, a recent review reported that a significant portion of phosphate in Prairie soils can be transported as dissolved P during snow melt runoff (Flaten, 2016). Phosphate can be transported from the agricultural soil when phosphate fertilizer is applied in excess of crop requirements (Kleinman et al., 2011) and also from plant residues during snow melt (Flaten, 2016).
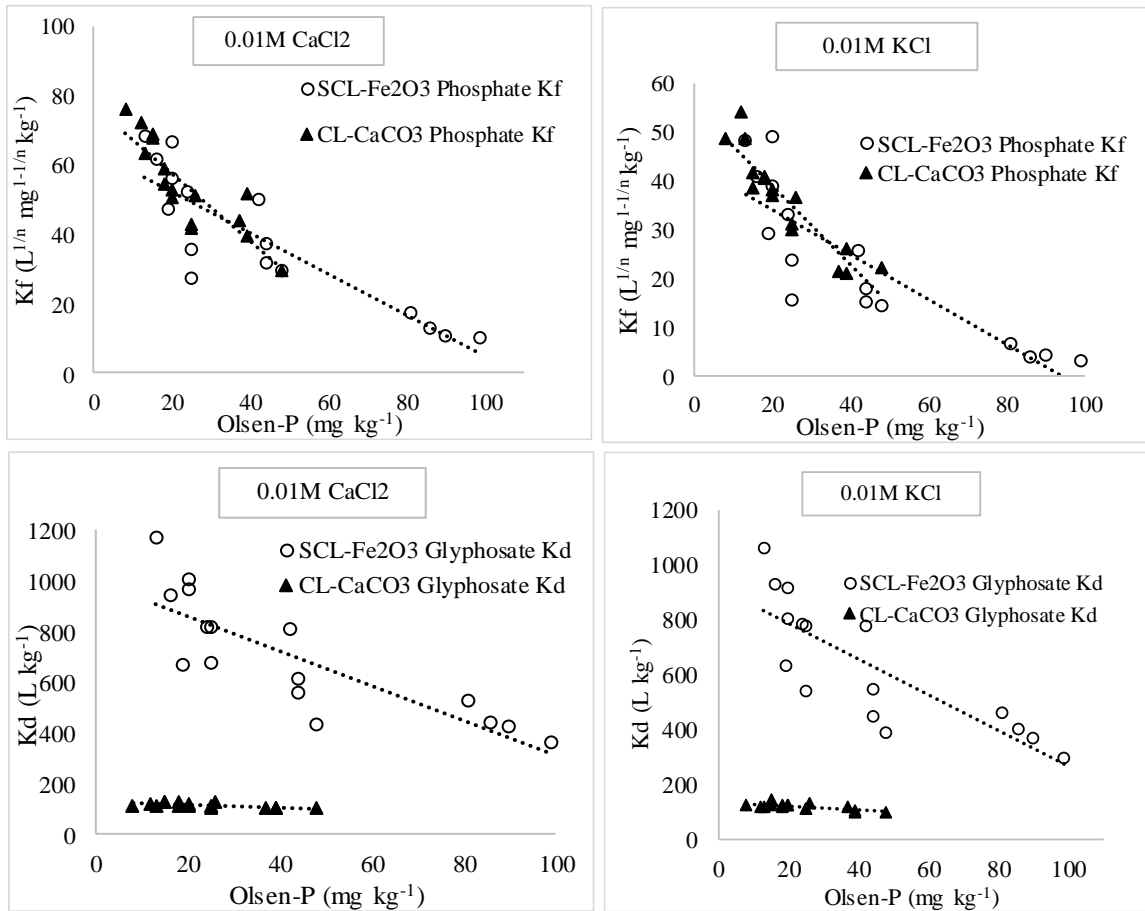
Figure 3.1. Effect of Olsen P concentrations in soil on glyphosate and phosphate sorption in SCL- Fe_2O_3 and CL-$CaCO_3$ soils, as determined by batch equilibrium experiments using 0.01M $CaCl_2$ or 0.01M KCl as background electrolyte solutions. All regression equations are significant at $P<0.05$ and the equations are given in Table 3S1-Supplementary information.
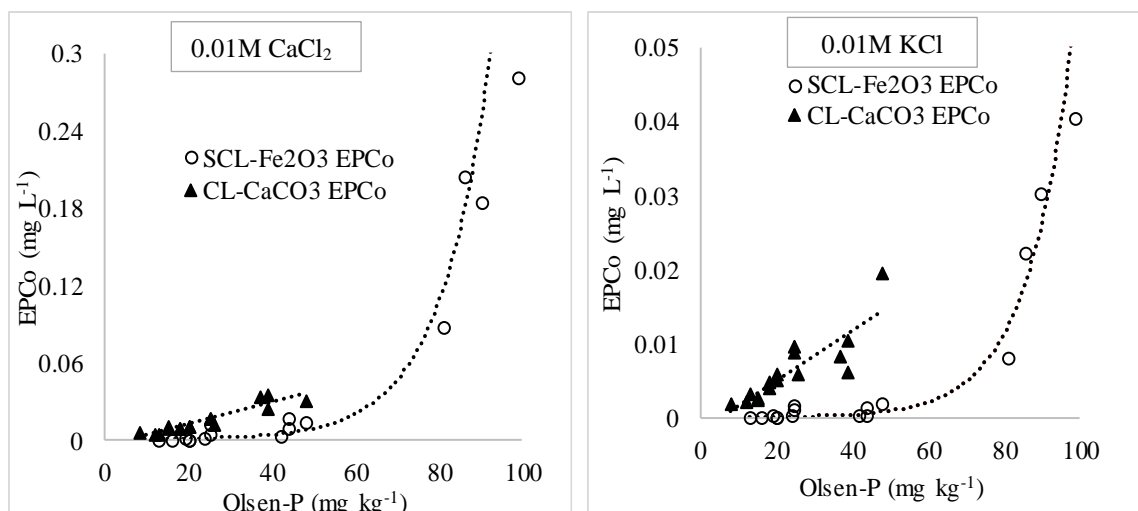
Figure 3.2. Effect of Olsen P concentrations in soil on the phosphate equilibrium experiments using 0.01M $CaCl_2$ or 0.01M KCl as background electrolyte solutions. Olsen P All regression equations are significant at $P<0.05$ and the equations are given in Table 3S1, 3S2-Supplementary information.

3.4.3. Effect of Glyphosate Formulation on Sorption of Phosphate

Commercially available glyphosate formulation had no impact on phosphate sorption in soil because there were no significant differences in phosphate sorption between treatments with and without Roundup Ultra2 additions to soil slurries (Table 3S3). Gimsing and Borggaard (2001) also found that, when glyphosate was added following phosphate additions to goethite, glyphosate did not displace the sorbed phosphate. Therefore, this finding indicates that recent concerns regarding transport of phosphate to surface water due to glyphosate (Barrera, 2016) are not fully justified. It is unlikely that phosphate transport to surface water is increased due to glyphosate application in agricultural fields.

3.4.4. Effect of Field-Aged Phosphate on Sorption of Glyphosate

Glyphosate Kd values significantly decreased with the increasing concentrations of Olsen P in both SCL-Fe$_2$O$_3$ and CL-CaCO$_3$ (Figure 3.1). Glyphosate Kd values ranged from 293 to 1173 L kg^{-1} in the acidic SCL-Fe$_2$O$_3$ soil and from only 99 to 141 L kg^{-1} in the calcareous CL-CaCO$_3$ soil (Figure 3.1), and these values are within the range of other studies (Farenhorst et al., 2008; Kumari et al., 2016; Sørensen et al., 2006). Repeated application of phosphate fertilizer in soil reduced glyphosate sorption because pre-sorbed phosphate occupied the sorption sites that would otherwise be available to glyphosate (Gimsing et al., 2007). A maximum reduction in glyphosate Kd value was observed in SCL-Fe$_2$O$_3$ soil. The Kd value in SCL-Fe$_2$O$_3$ was reduced by 75% in soil containing 99 mg kg^{-1} Olsen P relative to soil containing 13 mg kg^{-1} Olsen P. Thus, suggesting that glyphosate and phosphate compete for the same sorption sites in soil. Similar observations have been made by de Jonge et al. (2001) who reported that long-term (60 to 100 years) application of phosphate significantly reduced glyphosate sorption by 50% in soil containing 59 mg kg^{-1} Olsen P relative to soil containing 6 mg kg^{-1} Olsen P.

Olsen P concentrations significantly predicted glyphosate Kd (Figure 3.1) in both SCL-Fe$_2$O$_3$ and CL-CaCO$_3$. With both 0.01M CaCl$_2$ and 0.01M KCl, the slopes of the regressions predicting glyphosate Kd were significantly different between soils with the SCL-Fe$_2$O$_3$ showing steeper slopes than CL-CaCO$_3$ (Figure 3.1). Regardless of the solution used, the sorption of glyphosate was greater in SCL-Fe$_2$O$_3$ than CL-CaCO$_3$ because of the importance of Fe$_2$O$_3$ in providing sorption sites for the negatively charged glyphosate in acidic soils. Research findings indicate that the presence of iron-oxide and soil pH had a

stronger influence on glyphosate than phosphate sorption (Gimsing et al., 2004). The SCL-Fe_2O_3 soil contained 94% more Fe-oxides and 83% more Al-oxides than the CL-$CaCO_3$ soil (Table 3.1), and glyphosate sorption was greater in SCL-Fe_2O_3 soil because glyphosate sorption has been shown to be positively correlated with Fe/Al-oxides (Gimsing et al., 2004; Ololade et al., 2014). In addition, glyphosate sorption was greater in SCL-Fe_2O_3 (pH 4.7 to 5) than CL-$CaCO_3$ (pH 7.3 to 7.5) soil because glyphosate sorption is negatively correlated with soil pH (Gimsing et al., 2004). This is because with increasing soil pH, an increasing portion of the glyphosate molecules become negatively charged with glyphosate molecules existing as HG^{2-} (~100%) at pH 7.3-7.5 (Yu-jun et al., 2004), and soil colloid deprotonation increases (McConnell and Hossner, 1985) with soil colloids having a net negative charge in Prairie soils when soil pH > 6 (Lavkulich and Arocena, 2011). Hence, regardless of the background electrolyte solutions, the sorption of glyphosate was always relatively low in the CL-$CaCO_3$ soil (Figure 3.1). Thus, the effect of Olsen P concentrations on reducing glyphosate sorption was more pronounced for SCL-Fe_2O_3 than CL-$CaCO_3$ (Table 3S6). For example, with 0.01M KCl, glyphosate Kd was reduced by 39% when the phosphate concentration increased from 17 mg kg^{-1} (control) to 44 mg kg^{-1} (40P plots) in SCL-Fe_2O_3 but by only 11% when the phosphate concentration increased from 13 mg kg^{-1} (control) to 41 mg kg^{-1} (80P plots) in CL-$CaCO_3$ (Table 3S6).
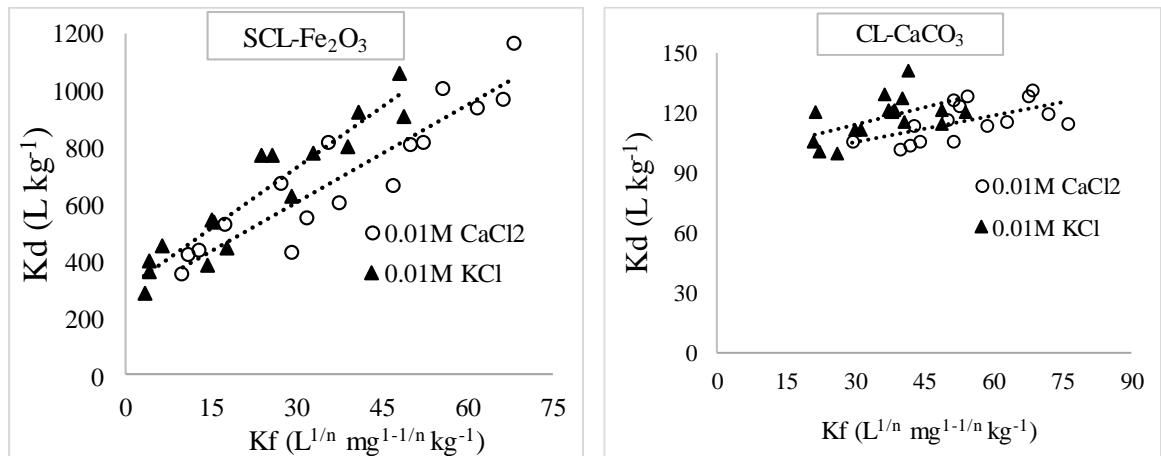
Figure 3.3. Association between glyphosate K_d and Phosphate K_f in SCL-Fe$_2$O$_3$ and CL-CaCO$_3$ soils with sorption being determined by batch equilibrium experiments using 0.01M CaCl$_2$ or 0.01M KCl as background electrolyte solutions. All regression equations are significant at $P<0.05$ and the equations are given in Table 3S4-Supplementary information.

3.4.5. Association Between Glyphosate Kd and Phosphate Kf in Relation to Field-Aged Phosphate

Phosphate Kf and glyphosate Kd values were positively correlated (Figure 3.3). Thus, agreeing with previous studies suggesting phosphate and glyphosate have similar sorption pattern in soil (Barja and Afonso, 2005; Gimsing et al., 2007). However, regardless of the background electrolyte solution, phosphate Kf and glyphosate Kd were more strongly correlated in SCL-Fe$_2$O$_3$ than CL-CaCO$_3$ (Table 3S4). Hence, glyphosate and phosphate may compete more strongly for sorption sites in acidic soils with high Fe/Al-oxides content than in calcareous soils. In both soils and under both electrolyte background solutions, phosphate sorption was more strongly reduced by Olsen P concentrations than glyphosate sorption was reduced by Olsen P concentrations (Table 3S6). Thus, repeated application of phosphate fertilizer has an overall greater impact on reducing phosphate sorption than glyphosate sorption.

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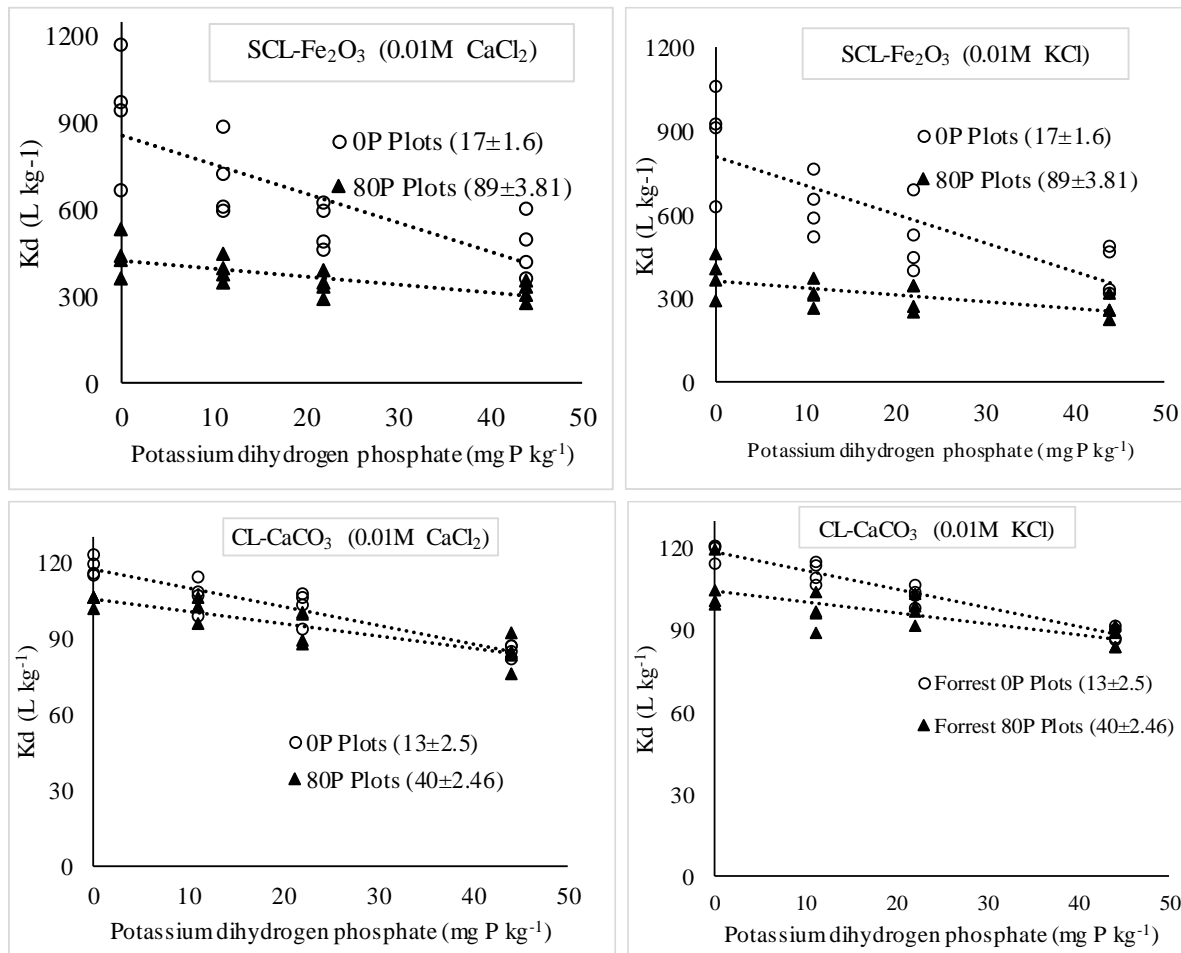
Figure 3.4. Effect of potassium dihydrogen phosphate concentrations on glyphosate sorption in SCL-Fe$_2$O$_3$ and CL-CaCO$_3$ soils with low (0P) or high (80P) Olsen P concentrations. Potassium dihydrogen phosphate was added to glyphosate in soil sluries during batch equilibrium experiments using 0.01M CaCl$_2$ and 0.01M KCl. All regression equations are significant at $P<0.05$ and the equations are given in Table 3S5-Supplementary information. The values in parentheses in each legend represent mean values of Olsen P and standard error.

3.4.6. Effect of Fresh Phosphate Addition on the Sorption of glyphosate

Regardless of the background electrolyte solution and soil, the potassium dihydrogen phosphate additions to soil slurries significantly decreased glyphosate Kd values (Figure 3.4). Addition of fresh phosphate significantly reduced glyphosate sorption because the

chemicals competed for the same sorption sites as they have similar phosphonate functional groups (Gimsing and Borggaard, 2001; Sprankle et al., 1975). Gimsing and Borggaard, (2002) studied the competitive sorption effect of fresh phosphate on glyphosate in soil and concluded that phosphate is preferentially sorbed over glyphosate. In addition to this, sorption of phosphate lowers the zero point charge of sorption sites such as Fe/Al-oxides, potentially increases the net negative charge on the oxide surfaces and thereby increasing the electrostatic repulsion between glyphosate and soil oxides (Gimsing et al., 2007).

Fresh phosphate significantly predicted glyphosate Kd (Figure 3.4) in both SCL-Fe_2O_3 and CL-$CaCO_3$. The regression slope was significantly steeper for 0P plots (control) than 80P plots in both soils and regardless of the background electrolyte solution (Figure 3.4). Thus, the effect of potassium dihydrogen phosphate addition in reducing glyphosate Kd values was less in soils that had greater Olsen P concentrations because less sorption sites were available for the added phosphate to compete with glyphosate molecules. This impact of phosphate already in soil was larger in SCL-Fe_2O_3 than CL-$CaCO_3$ (Table 3S7) because in CL-$CaCO_3$ soil at pH 7.3-7.5, glyphosate molecule existed as HG^{2-} (~100%) (Yu-jun et al., 2004) leading to less sorption, both in the presence and absence of fresh phosphate (Table 3S7). Thus, the competitive effect of phosphate on glyphosate is stronger in soils that are acidic and contain substantial amount of Fe-oxides than in calcareous soils.

3.5. Conclusion

The sorption of phosphate and glyphosate was reduced due to the repeated application of phosphate fertilizer in two Prairie soils. The impact of Olsen P on reducing glyphosate

sorption was more pronounced in the acidic (iron-oxide rich) sandy clay loam than the calcareous (calcium carbonate rich) clay loam soil, both with or without the addition of potassium dihydrogen phosphate. Regardless of the background electrolyte and soil type, phosphate sorption was more strongly reduced by the Olsen P concentrations than glyphosate sorption. The reduction of glyphosate sorption due to the application of potassium dihydrogen phosphate was greater in soils containing low Olsen P concentrations. The equilibrium phosphate concentration was above the threshold level for eutrophication only in soils that had exceptionally high phosphate concentrations i.e., the soils had received annual applications of mono ammonium phosphate at rates of 80 kg ha^{-1} for eight years. Commercially formulated glyphosate had no influence on phosphate sorption suggesting that glyphosate residues in soils have no impact on phosphate sorption or mobility.

3.6. Acknowledgements

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4. SORPTION AND DESORPTION OF GLYPHOSATE, MCPA AND TETRACYCLINE AND THEIR MIXTURES IN SOIL AS INFLUENCED BY PHOSPHATE

4.1. Abstract

Phosphate fertilizers and herbicides such as glyphosate and MCPA are commonly applied to agricultural land, and antibiotics such as tetracycline have been detected in soils following the application of livestock manures and biosolids to agricultural land. Utilizing a range of batch equilibrium experiments, this research examined the competitive sorption interactions of these chemicals in soil. Soil samples (0-15 cm) collected from long-term experimental plots contained Olsen P concentrations in the typical (13 to 20 mg kg^{-1}) and elevated (81 to 99 mg kg^{-1}) range of build-up phosphate in agricultural soils. The elevated Olsen P concentrations in field soils significantly reduced glyphosate sorption up to 50%, but had no significant impact on MCPA and tetracycline sorption. Fresh phosphate additions in the laboratory, introduced to soil prior to, or at the same time with the other chemical applications, had a greater impact on reducing glyphosate sorption (up to 45%) than on reducing tetracycline (up to 13%) and MCPA (up to 8%) sorption. The impact of fresh phosphate additions on the desorption of these three chemicals was also statistically significant, but numerically very small namely < 1% for glyphosate and tetracycline and 3% for MCPA. The presence of MCPA significantly reduced sorption and increased desorption of glyphosate, but only when MCPA was present at concentrations much greater than environmentally relevant and there with no phosphate added to the MCPA solution. Tetracycline addition had no significant effect on glyphosate sorption and desorption in soil. For the four chemicals studied, we conclude that when mixtures of phosphate,

herbicides and antibiotics are present in soil, the greatest influence of their competitive interactions is phosphate decreasing glyphosate sorption and the presence of phosphate in solution lessens the potential impact of MCPA on glyphosate sorption. For the organic chemicals studied in this experiment, the presence of chemical mixtures in soil solution have an overall greater impact on the sorption than desorption of individual organic chemicals in soil.

4.2. Introduction

The herbicides glyphosate and MCPA are among the top 5 most widely applied pesticides in Canada. Glyphosate half-lives in soil range from 30 to 197 days (Giesy et al., 2000; Smith and Aubin, 1993) and glyphosate is typically strongly retained by soil with the sorption distribution constant, Kd, ranging from 108 to 1,140 L kg^{-1} (Giesy et al., 2000; IUPAC, 2017). Soil half-lives for MCPA range from 15 to 50 days (Sattar and Paasivirta, 1980; Soderquist and Crosby, 1975) and the herbicide is weakly sorbed in soil with Kd ranging from 0.01 to 9.3 L kg^{-1} (Sørensen et al., 2006; Jacobsen et al., 2008; Alister et al., 2011). MCPA (26-100%) is more readily desorbed than glyphosate (0.6-23.6%) in soil (Sørensen et al., 2006). The antibiotic, tetracycline, accounts for more than three-quarters of the total sales of antibiotics in USA livestock production (FDA, 2015). Tetracycline is also registered for human use, for example, for the treatment of urinary tract and respiratory diseases (De Briyne et al., 2014; Lamb et al., 2015). Tetracycline is detected in soils following the application of livestock manure and biosolids on agricultural land (Hamscher et al., 2002; Pan and Chu, 2016; Wang et al., 2010). Soil half-lives for tetracycline range from 23 to 87 days (Aga et al., 2005; Pan and Chu, 2016) and its Kd ranges from 74 to

1,093 L kg^{-1} (Bao et al., 2009; Pan and Chu, 2016). Tetracycline desorption has been shown to only range from 1 to 9% (Fernández-Calviño et al., 2015; Pils and Laird, 2007). Sorption and desorption are important processes that determine the mobility of glyphosate, MCPA and tetracycline in soils (Duke et al., 2012; Hiller et al., 2012; Zhang et al., 2010). The sorption and desorption of herbicides and antibiotics may be influenced by phosphate concentrations in soil (Hiller et al., 2012; Kanissery et al., 2015; Wang et al., 2010).

Batch equilibrium studies have demonstrated that repeated applications of phosphate fertilizer significantly reduced glyphosate sorption in soil (de Jonge et al., 2001; Munira et al., 2016). Using similar procedures, glyphosate sorption was also reduced when phosphate was added in the laboratory at the same time as glyphosate was added in solution to soils (Cruz et al., 2007; Kanissery et al., 2015). For a clay loam soil, Gimsing et al. (2007) demonstrated that the competition was even stronger when phosphate was added prior to glyphosate additions because the pre-sorbed phosphate increased the net negative charge of the soil colloids and repelled the negatively charged glyphosate molecules. In contrast, in another study (Gimsing et al., 2004), the differential timing of phosphate applications, relative to glyphosate additions, produced the same reducing impact on glyphosate sorption in sandy to sandy clay loam soils.

Fewer studies have examined the impact of phosphate on the sorption of other pesticides or antibiotics in soils (Hiller et al., 2012; Vasudevan and Cooper, 2004; Wang et al., 2010). The impact of phosphate on MCPA (Hiller et al., 2012) and tetracycline (Wang et al., 2010) sorption was recently examined but both studies utilized phosphate and organic chemical

concentrations far exceeding their potential concentrations in agricultural soils. Hiller et al. (2012) found that added phosphate reduced MCPA sorption but only in two of the three soils examined. Wang et al. (2010) reported that phosphate significantly reduced tetracycline sorption in two soils. The effect of phosphate on MCPA and tetracycline desorption in soil is unknown. However, two studies have examined the impact of phosphate on glyphosate desorption with phosphate being added to soil either two (Laitinen et al., 2008) or four (Prata et al., 2003) weeks prior to glyphosate addition. In both cases, phosphate additions increased glyphosate desorption, by ~2-10% in Prata et al. (2003) and by 6-13% in Laitinen et al. (2008).

Herbicides and antibiotics can be present in agricultural soils as mixtures. Studies have shown that herbicide mixtures in soil can influence the sorption of an individual herbicide (De Wilde et al., 2009; Farenhorst and Bowman, 1998; Hernández-Soriano et al., 2007; Leistra and Matser, 2004). No studies have examined the impact of antibiotics on herbicide sorption in soil, but it has been reported that the presence of some antibiotics increase pesticide persistence (Nordenholt et al., 2016).

Utilizing a range of batch equilibrium experiments, this research examined the competitive sorption of chemicals as mixtures, particularly focusing on the impacts of phosphate concentrations on glyphosate, MCPA and tetracycline sorption and desorption in soil, and on the impact of MCPA and tetracycline and their mixtures, in the presence and absence of phosphate, on glyphosate sorption and desorption.

4.3. Materials and Methods

4.3.1. Chemicals

Analytical grade glyphosate (99.9% purity), MCPA (99%), tetracycline (98%) were obtained from Sigma-Aldrich Co., St. Louis, MO; and potassium dihydrogen phosphate (KH_2PO_4) (99% chemical purity) and potassium chloride (100% chemical purity) from Fisher Scientific, Fair Lawn, NJ. Radioactive phosphonomethyl-[14]C Glyphosate (99% radiochemical purity; specific activity 50 mCi/mmol), 2-methyl -4-chlorophenoxyacetic acid ^{14}C MCPA (98% radiochemical purity; specific activity 55 mCi/mmol) and 7-[3]H (N) tetracycline (98% radiochemical purity; specific activity 20 Ci/mmol) were obtained from American Radiolabeled Chemicals Inc., St. Louis, MO.

4.3.2. Soil Characteristics and Experimental Design

Soil samples (0-15 cm) were collected in spring 2013 from experimental plots that were arranged in a randomized complete block design with four replications and were located at the University of Manitoba Carman Field Research Station (49° 29.7' N, 98° 2.4' W), Manitoba, Canada. All plots were under a flax and durum wheat rotation and received urea fertilizers at an annual rate of 50 and 90 kg N ha^{-1}, respectively. For this study, samples were collected from the replicated plots that had also received eight years (2002-2009) of annual mono ammonium phosphate (MAP) applications at rates of 80 kg P ha^{-1}, as well as from control plots that did not receive MAP application during these years (Grant et al., 2013). The rotation was continued from 2010 to 2013 but after 2010 no phosphate was applied. In each plot, composite soil samples were collected using a Dutch auger with ten samples per plot and the auger was cleaned in between plots. Samples were air-dried and

sieved (<2 mm) prior to use in batch equilibrium experiments. Available phosphate was determined by the Olsen (0.5N NaHCO$_3$, pH 8.5) phosphorus test (Olsen P) (Frank et al., 2011). Olsen P concentrations ranged from 81 to 99 mg kg^{-1} in soil from the 80P plots that had received MAP applications and from 13 to 20 mg kg^{-1} in soil from the 0P plots that had received no phosphate fertilizers. 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).

Soil organic carbon content was determined using combustion technique with a high temperature induction furnace (Nelson and Somemers, 1996). Soil pH was determined using a 10 mL 0.01M CaCl$_2$ solution and 2 g soil solution ratio (Jones Jr, 2001). Extractable Fe and Al were extracted with diethylenetriaminepentaacetic acid (*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). Soil texture data was adapted from Grant et al. (2013). Results were (in all cases n=16, number of plots analyzed): soil organic carbon content: 2.81 (mean) ± 0.04 (standard error) %; soil pH: 4.7± 0.02; extractable Fe-oxides: 237±7.93 mg kg^{-1}; Al-oxides: 6.41±0.64 mg kg^{-1}; extractable Ca: 2,252±35 mg kg^{-1}; clay: 20%; silt: 20% and sand: 60%.

4.3.3. Impact of Phosphate in Solution on Herbicides and Antibiotic Sorption and Desorption

Batch equilibrium procedures using 50-mL centrifuge Teflon tubes (duplicates) followed the OECD guideline 106 (OECD, 2000) with air-dried soil (2 g) and a soil/solution ratio of 1:5 with 0.01M KCl as the background electrolyte. Soil slurries were rotated in the dark at 5°C from 0 to 24h (pre-incubation), from 24 to 48h (sorption) and from 48 to 72h (desorption) with phosphate added at 0h, 24h and/or 48h, or never added, depending on the treatment (Table 4.1). For treatments *n,n,n* and *n,n,P,* a 0.01M KCl solution (8 mL) was added to soil at 0h and no phosphate was added. For treatments *P,n,n* and *P,n,P,* the added 0.01M KCl solution also contained phosphate while, for treatment *n,P,P,* the phosphate was added to the herbicide and antibiotic solutions. Radiolabelled glyphosate, MCPA or tetracycline in 0.01M KCl (2 mL) was always added at 24h. Radiolabelled chemical solutions contained 1 mg L^{-1} analytical-grade glyphosate, MCPA or tetracycline, with 6.67×10^5 Bq L^{-1} ^{14}C-labelled glyphosate, 3.83×10^5 Bq L^{-1} ^{14}C-labelled MCPA or 4.17×10^5 Bq L^{-1} ^{3}H-labelled tetracycline, respectively. The concentration 1 mg L^{-1} represented environmentally-relevant concentrations of herbicides and antibiotics detected in agricultural soils (Hu et al., 2012) or animal manure (Karcı and Balcıoğlu, 2009). Phosphate was added as potassium dihydrogen phosphate and always at a rate of 44 mg P kg^{-1}, corresponding to a concentration of 11 mg L^{-1} in the added solution. This rate is equivalent to an estimated 80 P kg ha^{-1} when assuming the fertilizer is being incorporated in the top 15 cm layer of a soil with a bulk density of 1,200 kg m^{-3}.

At 48h, tubes were centrifuged at 10,000 rev min^{-1} for 10 min and subsamples (1 mL) of the supernatant (duplicates) were added to scintillation vials (7 mL) containing 5 mL 30% Scintisafe scintillation cocktail (Fisher Scientific, Fair Lawn, NJ). Radioactivity was quantified by Liquid Scintillation Counting (LSC) with automated quench correction (#H method) (LS 6500 Beckman Instruments, Fullerton, CA). The sorption distribution constant, Kd (L kg^{-1}), of glyphosate, MCPA or tetracycline was quantified by Cs/Ce, where Cs is the concentration of the organic chemical in soil at equilibrium (mg kg^{-1}) and Ce is the concentration of the organic chemical in the equilibrium solution (mg L^{-1}). The concentration of the organic chemical in soil was calculated by the difference between the radioactivity in the initial solution and the equilibrium solution. The soil organic carbon coefficient, Koc (L kg^{-1}) of glyphosate, MCPA or tetracycline was calculated by dividing the Kd value by 0.0281 which was the fraction of soil organic carbon in soil.

Following the subsampling (2 mL in total), an additional portion of supernatant (6 ml) was removed so that what was left in the tubes was a "slurry" consisting of soil mixed with the remaining supernatant. Not all supernatant was removed from the tubes to ensure that the same amount of supernatant was removed from each tube (8 mL in total) and replaced by a 0.01M KCl solution (8 mL) with (treatments *n,n,P,; P,n,P* and *n,P,P*) or without phosphate (treatments *n,n,n* and *P,n,n*) in this solution (Table 4.1). Tubes were again rotated and at 72h, tubes were centrifuged and subsampled as described above to determine radioactivity remaining in solution. The percentage of organic chemical (herbicide or antibiotic) desorbed from soil was quantified by dividing the mass of the organic chemical desorbed from the soil at 72h by the mass of the organic chemical in the soil at 48h and

multiplying by 100. The mass of the organic chemical desorbed from the soil at 72h was calculated as the mass of organic chemical in the supernatant at 72h minus the mass of the organic chemical in the 2 mL solution remaining at 48h (please see OECD guideline 106 for details on the calculation) (OECD 2000).

Table 4.1. Addition of phosphate during pre-incubation, sorption and desorption steps

Code	Pre-incubation from 0h to 24h	Sorption from 24h to 48h	Desorption from 48 to 72h
n,n,n	No P added	No P added	No P added
n,n,P	No P added	No P added	P added at 48h
P,n,n	P added at 0h	No P added	No P added
P,n,P	P added at 0h	No P added	P added at 48h
n,P,P	No P added	P added at 24h	P added at 48h

n = no phosphate added during pre-incubation, sorption and/or desorption step; P = phosphate added at time 0h at the start of the pre-incubation step or at time 24h at the start of the sorption step; or at time 48h at the start of the desorption step.

4.3.4. Impacts of MCPA and Tetracycline in Solution on Glyphosate Sorption and Desorption in the Presence and Absence of Fresh Phosphate

Experiments followed similar protocols as described for *n,n,n*; *n,n,P*; and *P,n,P* in Table 4.1 above and also added to soil (at 0h) were MCPA, tetracycline (Tetra), or their mixtures (M/T). MCPA, Tetra, and M/T were added at concentrations of 1 or 11 mg L^{-1}. Treatments were labeled as *MCPA-n,n,n*; *Tetra- n,n,n*; *M/T- n,n,n*; *MCPA- n,n,P*, etc. There was also a treatment labeled as *n,n,n* in which case neither phosphate nor MCPA, Tetra, or M/T was added. The glyphosate solution was always added at 24h and contained 1 mg L^{-1} analytical-grade glyphosate with 6.67×10^5 Bq L^{-1} ^{14}C-labelled glyphosate.

4.3.5. Effect of The Pre-Sorbed Phosphate on the Sorption of Glyphosate, MCPA and Tetracycline

This batch equilibrium experiment only used the soil samples obtained from the plots that had not received phosphate fertilizer applications. Potassium dihydrogen phosphate solutions were prepared in 0.01M KCl at concentrations of 0, 11, 22, 44 mg P L^{-1} and added (8 mL) to air-dried soil (2 g) in Teflon tubes. Soil slurries were rotated in the dark at 5°C for 24h and centrifuged at 10,000 revmin^{-1} for 10 min. Supernatant (8 mL) was removed and the concentrations of phosphate was determined colorimetrically by the molybdate blue method (Murphy and Riley, 1962) to calculate the amount of phosphate retained in soil. 0.01M KCl (8 mL) was added to the soil followed by the addition of radiolabeled glyphosate, MCPA or tetracycline in 0.01M KCl (2 mL). Radiolabelled glyphosate, MCPA or tetracycline solutions contained 1 mg L^{-1} analytical-grade glyphosate, MCPA or tetracycline, with 6.67×10^5 Bq L^{-1} ^{14}C-labelled glyphosate, 2.08×10^5 Bq L^{-1} ^{14}C-labelled MCPA and 5.00×10^5 Bq L^{-1} ^{3}H-labelled tetracycline, respectively. Soil slurries were again rotated for 24h, then centrifuged and subsampled as described above to calculate Kd values and determine the effect of the pre-sorbed phosphate concentrations on the sorption of glyphosate, MCPA and tetracycline.

4.3.6. Effect of the Pre-sorbed MCPA on Glyphosate Sorption

Experiments followed similar protocols as described for the pre-sorbed phosphate above, and thus MCPA was added in 0.01M KCl (8 mL) to soil (2 g) at concentrations of 0, 11, 22, 44 mg MCPA L^{-1}. In order to calculate the amount of MCPA sorbed by soil, one subset

of samples (duplicated) also contained 2.83×10^3, 5.83×10^4, and 1.67×10^5 Bq L^{-1} ^{14}C-labelled MCPA to measure the radioactivity in subsamples (1 mL) from the supernatant that was removed. The mass of MCPA in the soil at 24h was calculated by the difference between the added radioactivity at 0h and the radioactivity in the supernatant at 24h. For the other subset of samples (duplicated), the supernatant (8 mL) was removed at 24h and then replaced by 0.01M KCl (8 mL) plus radiolabeled glyphosate in 0.01M KCl (2 mL). The glyphosate solution contained 1 mg L^{-1} analytical-grade glyphosate with 6.67×10^5 Bq L^{-1} ^{14}C-labelled glyphosate.

4.3.7. Statistical Analysis

Statistical analyses were carried out using SAS software version 9.4 for Windows (SAS Institute Inc. 2002-2012). Prior to each analysis, data were examined for outliers, conformation of the residuals to the Gaussian distribution (Shapiro-Wilk test) and homogeneity of variances (quantile-quantile plot). For the Kd values, data were analysed using the normal error distribution and for the percent desorption, the beta distribution (link logit). Two-way ANOVAs (2×5 factorial layout) in PROC GLIMMIX was used to quantify the effect of field aged-P (0P, 80P) and fresh-P addition timing (*n,n,n; n,n,P; P,n,n; P,n,P; n,P,P*) on the sorption and percent desorption of MCPA, tetracycline, and glyphosate in soil. Field aged-P and addition of fresh-P timing and their interaction were considered as fixed effect and block as random effect. One-way ANOVA in PROC GLIMMIX was utilized to determine the effect of pre-sorbed phosphate in soil on glyphosate, MCPA and tetracycline sorption, and of pre-sorbed MCPA in soil on glyphosate sorption. Pre-sorbed phosphate was considered as fixed effect and block as random effect. Both in the presence

and absence of fresh phosphate, two-way ANOVAs (2×3 factorial layout) in PROC GLIMMIX were carried out separately to quantify the effect of field aged-P (0P, 80P) and of the concentrations (0, 1, 11 mg L^{-1}) of MCPA, tetracycline, or MCPA-tetracycline mixtures on glyphosate Kd values. Field aged-P, the concentrations of MCPA and their interaction were considered as fixed effect and block as random effect. For fresh phosphate added at 48h only, or at both 0h and 48h, and in the absence of fresh phosphate, two-way ANOVAs (2×3 factorial layout) in PROC GLIMMIX were carried out separately to quantify the effect of field aged-P (0P, 80P) and of the concentration (0, 1, 11 mg L^{-1}) of MCPA, tetracycline, or MCPA-tetracycline mixtures on the percent of glyphosate desorbed. Field aged-P, the concentrations of MCPA and their interaction were considered as fixed effect and block as random effect. For all ANOVAs, the separation of treatment means was performed using the Tukey's test (p<0.05).

4.4. Results

Kd values on average ranged from 209 to 596 L kg^{-1} for glyphosate (Figure 4.1), from 118 to 135 L kg^{-1} for tetracycline, and from 4.99 to 5.37 L kg^{-1} for MCPA (Table 4.2). Koc values ranged from 6105 to 25,496 L kg^{-1} for glyphosate, from 3,928 to 4,901 L kg^{-1} for tetracycline, and from 156 to 209 L kg^{-1} for MCPA. These results are within the ranges observed in previous studies of the sorption of glyphosate (Giesy et al., 2000; Kumari et al., 2016; Laitinen et al., 2008), tetracycline (Bao et al., 2009; Pils and Laird, 2007; Wang et al., 2010) and MCPA (Hiller et al., 2012; Jacobsen et al., 2008; Shang and Arshad, 1998) in soils. Glyphosate (< 2%) (Figure 4.1) and tetracycline (< 1%) desorption was always small but MCPA desorption ranged from 26 to 31 % (Table 4.2).

Table 4.2. Effect of phosphate fertilizer on MCPA and tetracycline sorption and desorption in soil. See Table 4.1 for an explanation of the treatment labels.

Treatment	Kd (L kg^{-1})		Desorption (%)	
	MCPA	Tetracycline	MCPA	Tetracycline
n,n,n	5.37 A	134.49 A	27.45 B	0.51 B
n,n,P	5.28 A	129.02 A	29.63 A	0.73 A
P,n,n	5.00 B	117.50 B	29.04 A	0.69 A
P,n,P	5.00 B	122.55 B	30.18 A	0.71 A
n,P,P	4.99 B	117.55 B	29.91 A	0.74 A

Phosphate significantly reduced glyphosate sorption in soil (Figure 4.1). Without laboratory-added phosphate, glyphosate Kd values were 50% smaller in soil containing 81 to 99 mg kg^{-1} Olsen P than in soil containing 13 to 20 mg kg^{-1} Olsen P. Regardless of whether MCPA, tetracycline or MCPA/tetracycline mixture were added to soils in the laboratory, field aged-P always significantly reduced glyphosate Kd values (Table 4.3). When phosphate was added to soil solution at either 0h or 24h, it had the same significant effect on reducing glyphosate sorption with glyphosate Kd values being reduced by 37-45% in field soils containing 13 to 20 mg kg^{-1}, and by 23-27% in field soils containing 81 to 99 mg P kg^{-1} (Figure 4.1). In the pre-sorbed phosphate experiment, the soil retained 9.8, 18.5 and 32.4 mg P kg^{-1} for the additions of 11, 22, 44 mg P L^{-1}, respectively, and glyphosate sorption was significantly reduced by 41% (11 mg P L^{-1}), 52% (22 mg P L^{-1}) and 65% (44 mg P L^{-1}) (Figure 4.2).

The amount of field aged-P in soil had no significant impact on MCPA and tetracycline sorption in soil (Table 4S1). However, fresh phosphate added to soil solution significantly reduced tetracycline Kd values by 8 to13% and MCPA Kd values by 7 to 8% (Table 4.2). The competitive effect of phosphate on MCPA and tetracycline sorption was not dependent on when the phosphate was added in the laboratory (either 0h or 24h) (Table 4.2). In the pre-sorbed phosphate experiment, phosphate significantly reduced MCPA sorption by 10% and tetracycline sorption by 8% for the addition of 44 mg P L^{-1} (Table 4.4, Figure 4.2). However, there was no impact on MCPA or tetracycline sorption when phosphate additions were 11 or 22 mg P L^{-1}.
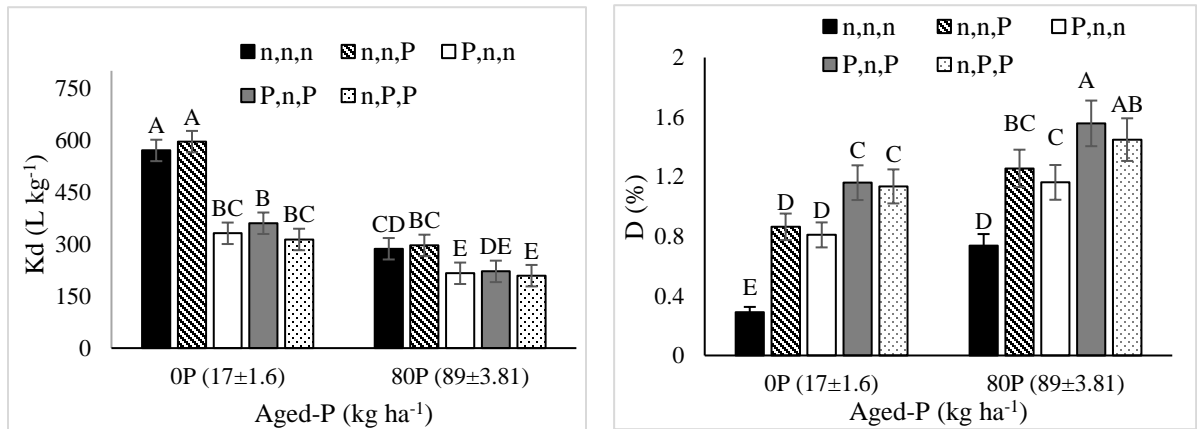
Figure 4.1. Effect of phosphate fertilizer on glyphosate sorption and desorption in soil. Potassium dihydrogen phosphate was added prior or during glyphosate addition for the sorption study and prior, during and/or post stage of glyphosate addition for the desorption study (see Table 4.1 for labels and details).

Glyphosate desorption was significantly greater in field soils containing 81 to 99 mg kg^{-1} Olsen P (0.74%) than in soils containing 13 to 20 mg kg^{-1} Olsen P (0.29%) (Figure 4.1, Table 4S1). Regardless of whether MCPA, tetracycline or MCPA/tetracycline mixture were added to soils in the laboratory, field aged-P always significantly increased

glyphosate desorption (Table 4S2). Fresh phosphate additions at 0h, 24h or/and 48h to soil

solutions in the laboratory also significantly increased glyphosate desorption by 0.52-

0.84% in soils containing 13 to 20 mg kg^{-1} Olsen P and by 0.52-0.82% in field soils

containing 81 to 99 mg kg^{-1} Olsen P (Figure 4.1). The amount of field aged-P in soil had

no significant impact on MCPA and tetracycline desorption in soil, but the addition of fresh

phosphate to soil solutions in the laboratory significantly increased desorption of MCPA

by 2 to 3% and tetracycline by 0.18 to 0.23% (Table 4.2). The competitive effect of

phosphate on MCPA, tetracycline and glyphosate desorption was not dependent when

phosphate was added to soil solution (either at 0h, 24h or 48h). The number of times that

phosphate was added had no significant effect on MCPA and tetracycline desorption (Table

4.2). However, glyphosate desorption was greater when phosphate was added twice (*P,n,P,*

or n,P,P) rather than once (*P,n,n or n,n,P*) but glyphosate desorption remained < 2% in all

cases (Figure 4.1).

Table 4.3: Effect of MCPA (0, 1, 11 mg L^{-1}), tetracycline (0, 1, 11 mg L^{-1}) and MCPA/tetracycline mixtures (0, 1, 11 mg L^{-1}) on sorption and desorption of glyphosate in soil in the presence and absence of phosphate

Chemicals	Concentration (mg L^{-1})	Kd (Lkg^{-1})	D (%)	Kd (Lkg^{-1})	D (%)	Kd (Lkg^{-1})	D (%)
		No P		P at 48h		P at 0h and 48h	
MCPA	0	428.48 A	0.52 A	445.99 A	1.10 A	290.80 A	1.38 A
	1	409.73 A	0.53 A	424.99 A	1.11 A	271.09 A	1.42 A
	11	370.88 B	0.60 B	382.32 B	1.16 A	278.44 A	1.43 A
Tetracycline	0	428.48 A	0.52 A	445.99 A	1.10 A	290.80 A	1.38 A
	1	415.64 A	0.54 A	426.02 A	1.04 A	283.50 A	1.36 A
	11	415.94 A	0.55 A	426.02 A	1.08 A	271.72 A	1.45 A
MCPA-tetracycline mixtures	0	428.48 A	0.52 A	445.99 A	1.10 A	290.80 A	1.38 A
	1	426.02 A	0.48 A	444.58 A	1.12 A	283.50 A	1.39 A
	11	318.05 B	0.66 B	386.72 B	1.15 A	290.51 A	1.44 A

Table 4.4: Effect of pre-sorbed phosphate (0, 11, 22, 44 mg L^{-1}) on glyphosate, MCPA and tetracycline sorption and pre-sorbed MCPA on glyphosate sorption (L kg^{-1}) in soil

Concentration (mg L^{-1})	Glyphosate	MCPA	Tetracycline	Glyphosate
0	544.60 A	5.48 A	108.22 A	544.6 A
11	321.78 B	5.09 AB	103.39 AB	540.8 AB
22	258.49 BC	5.05 AB	104.35 AB	518.25 AB
44	192.96 C	4.93 C	99.32 C	510.25 B
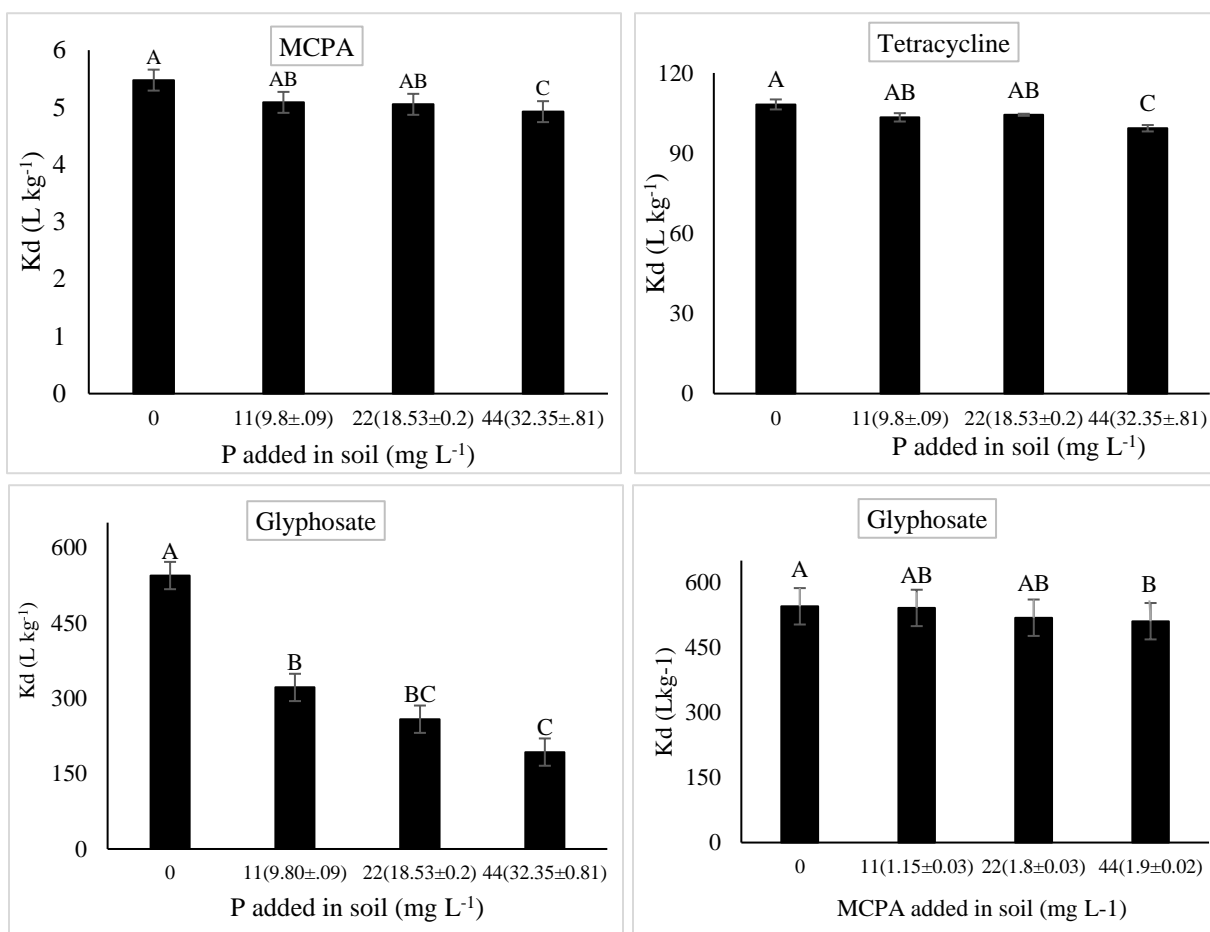
Figure 4.2. Effect of pre-sorbed phosphate concentrations on MCPA, tetracycline and glyphosate sorption, and of pre-sorbed MCPA concentrations on glyphosate sorption in soil. Numbers on x-axis in parenthesis refer to mean (+/-standard error) of measured pre-sorbed phosphate and MCPA
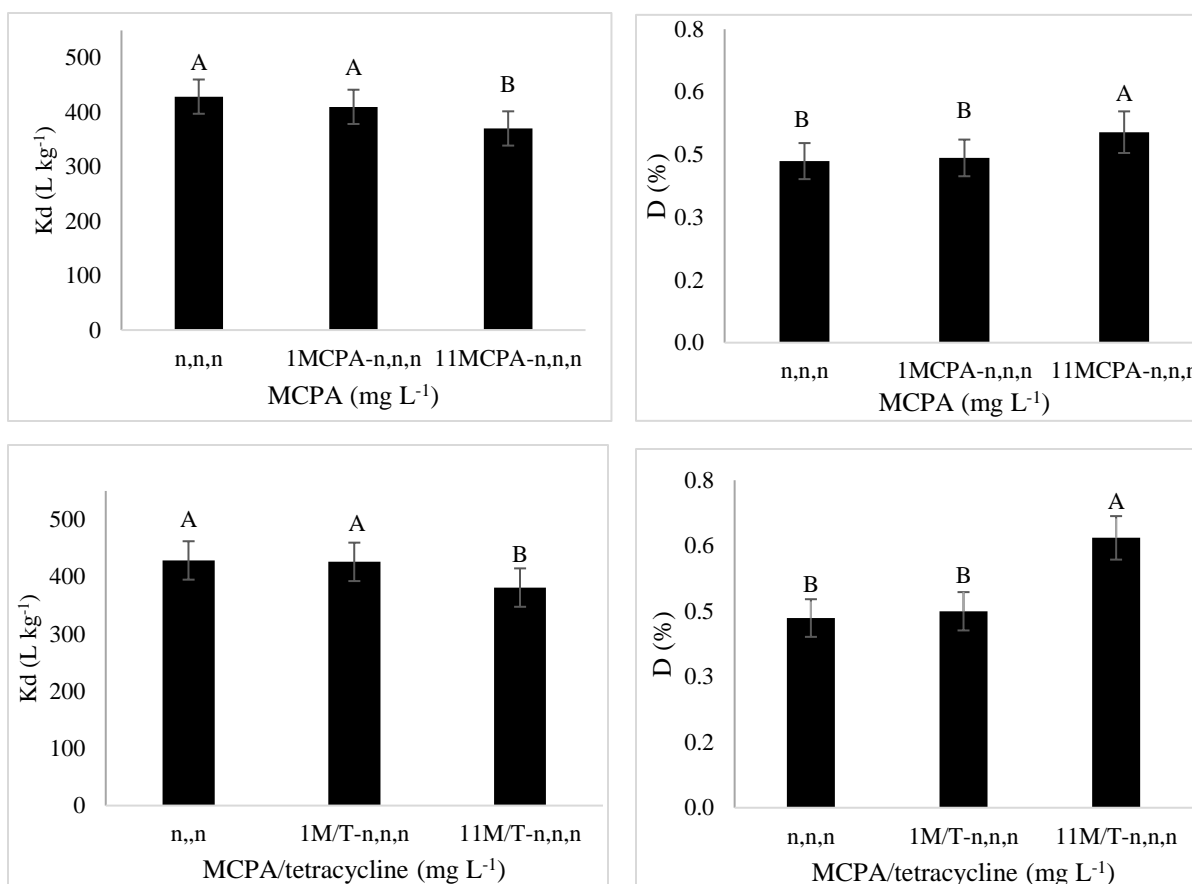
Figure 4.3. Effect of MCPA and MCPA/tetracycline mixtures on glyphosate sorption and desorption in soil. Potassium dihydrogen phosphate with MCPA or MCPA/tetracycline were added prior glyphosate for the sorption study and prior, or post stage of glyphosate addition for the desorption study: (see Table 4.1 for labels and details).

MCPA and MCPA/tetracycline mixtures added at 11 mg L^{-1} significantly reduced glyphosate Kd values and increased glyphosate desorption, but only when no phosphate was added to the soil solution (Figure 4.3). MCPA and MCPA/tetracycline mixtures added at 1 mg L^{-1} had no significant effect on glyphosate sorption and desorption. Tetracycline had no significant effect on glyphosate Kd values and desorption, regardless of whether it was added to soil at 1 or 11 mg L^{-1}, and whether or not phosphate was added to soil solution (Table 4.3). Thus, the effect of MCPA/tetracycline mixtures on glyphosate sorption and desorption was due to MCPA. MCPA addition significantly reduced glyphosate Kd values

by 14% (Figure 4.3) and glyphosate desorption by 0.1% (Figure 4.3). In the pre-sorbed MCPA experiment, the addition of 11, 22, 44 mg MCPA L^{-1} the soil retained 1.2, 1.8 and 1.9 mg MCPA kg^{-1}, respectively. The pre-sorbed MCPA significantly reduced glyphosate sorption by 6% for the addition of MCPA at 44 mg L^{-1}, but there was no impact on glyphosate sorption when additions were at 11 or 22 mg L^{-1} (Table 4.4, Figure 4.2).

4.5. Discussion

The addition of phosphate at either 0h or 24h yielded the same impact on glyphosate sorption (Figure 4.1), in agreement with the findings of Gimsing et al. (2004) who also reported that the timing of phosphate additions had no significant effect. Glyphosate and phosphate have been shown to compete for the same sorption sites in soil (Gimsing et al., 2007; Kanissery et al., 2015). Application of phosphate with glyphosate in solution reduced glyphosate sorption because phosphate is preferentially sorbed over glyphosate by available sorption sites (Gimsing and Borggaard, 2001).

Glyphosate Kd values were significantly lower in soils containing elevated Olsen P concentrations than in soils containing typical Olsen P concentrations. This elevated Olsen P concentrations resulted from eight years of annual phosphate application from 2002 to 2009, with soils being sampled for this study in 2013. These results indicate that phosphate persists in agricultural soils and occupies sorption sites that otherwise would be available sorption sites for glyphosate. In-addition, in the pre-sorbed phosphate experiment, glyphosate sorption was also reduced with increasing phosphate application to soil thus indicating that phosphate from recently fertilizer applications will also occupy sorption

sites otherwise available for glyphosate sorption. Given the moderately acidic conditions (soil pH 5), the sorption sites that phosphate ($H_2PO_4^{-1}$) occupies are positively charged Fe/Al-oxides. When phosphate ($H_2PO_4^{-1}$) is retained by Fe/Al-oxides, the Fe/Al-oxides will yield a net negative charge, leading to an electrostatic repulsion between the Fe/Al-oxides and glyphosate (H_2G^-) in soil (Gimsing et al., 2007; Laitinen et al., 2008). However, a portion of glyphosate molecules that were sorbed by available positively charged Fe/Al-oxides. The addition of phosphate after this sorption increased glyphosate desorption (Figure 4.1) possibly because phosphate is able to displace glyphosate bound to Fe/Al-oxides as the bonding forces between phosphate and Fe/Al-oxides are stronger than the bonding forces between glyphosate and Fe/Al-oxides (Gimsing and Borggaard, 2001).

Under the experimental conditions with the soil slurries being at a pH 5, the molecules of MCPA (pKa = 3.73, IUPAC, 2017) are predominantly negatively-charged. Given that tetracycline (pKa = 3.3, 7.7 and 9.7) (Tolls, 2001) is a zwitterion in moderately acidic to neutral soils   (Bao et al., 2009; Zhang et al., 2011) part of the tetracycline molecules are also deprotonated (Gu and Karthikeyan, 2005). Hence, phosphate ($H_2PO_4^-$), MCPA and tetracycline molecules may have competed for positively-charged Fe/Al-oxides in soil (Gu and Karthikeyan, 2005; Hiller et al., 2012; Wang et al., 2010). In the pre-sorbed phosphate experiment, an increasing addition of phosphate and sorption in soil increased the portion of Fe/Al-oxides with a net negative charge (Hiller et al., 2012; Wang et al., 2010). Of the three phosphate rates used in the pre-sorbed phosphate experiment (11, 22 and 44 mg P L^{-1}), MCPA and tetracycline sorption was only significantly reduced at the highest rate

because more Fe/Al-oxides were net negatively charged and repelling MCPA and tetracycline molecules.

The effect of phosphate on reducing sorption was less for MCPA and tetracycline than for glyphosate. Under moderately acidic conditions, Fe/Al-oxides are the dominant sorption sites for glyphosate and phosphate because both have a phosphonic acid group (Barja and Afonso, 2005; Gimsing et al., 2007, 2004; Kanissery et al., 2015; Sprankle et al., 1975). However, MCPA (i.e., carboxyl and phenyl groups) and tetracycline (i.e., tricarbonylamide, carbonyl, amine and hydroxyl groups) have other functional groups (Haberhauer et al., 2001; Sassman and Lee, 2005; Spadotto and Hornsby, 2003) and sorption sites for MCPA and tetracycline under moderately acidic conditions can include humic substances and clay minerals in addition to Fe/Al-oxides in soils (Gu and Karthikeyan, 2005; Wang et al., 2010; Werner et al., 2013).

MCPA no longer had a significant effect on glyphosate sorption when phosphate was added to the soil solution. The molecular size of phosphate (0.25 nm) is smaller than glyphosate (0.43 nm) and MCPA (0.77 nm) (Liu et al., 1999; Martin et al., 1999; Tylor et al., 2000). Therefore, it is possible that phosphate is preferentially sorbed over glyphosate and MCPA (Gimsing and Borggaard, 2001). Thus, when both phosphate and MCPA were added to the soil solution, phosphate occupied the sorption sites that may otherwise be available to MCPA and suppressed the effect of MCPA on glyphosate sorption. In the pre-sorbed experiment, in the absence of phosphate additions, MCPA reduced glyphosate sorption because pre-sorbed MCPA occupied some sorption sites which may otherwise be accessible to glyphosate.

MCPA was weakly retained with Koc values ranging from 156 to 209 L kg^{-1} while glyphosate and tetracycline were strongly retained with Koc values ranging from 6,105 to 25,496 and 3,928 to 4,901 L kg^{-1}, respectively. It has been reported that organic molecules are considered relatively mobile when Koc value ranges from 150 to 500 L kg^{-1} (Barcelo and Hennion, 1997). Thus, given these Koc values, MCPA is relatively mobile in soil because it is only weakly retained (Sørensen et al., 2006), unlike glyphosate and tetracycline. Thus, glyphosate is very strongly retained in soil and is less likely to be mobile in matrix flow than MCPA, regardless of the amounts of phosphate or MCPA that can compete with glyphosate for sorption sites in soil. In contrast, the presence of recent phosphate applications to agricultural soils may increase the mobility of MCPA to deeper depths but only when applied at relatively large phosphate fertilizer rates.

4.5. Conclusion

Field-aged phosphate had no significant effect on MCPA and tetracycline sorption and desorption but significantly reduced glyphosate sorption up to 50% and increased glyphosate desorption by 0.45%. Pre-sorbed phosphate had a greater impact on reducing glyphosate sorption than on reducing MCPA and tetracycline sorption. The addition of fresh phosphate in the laboratory also significantly decreased glyphosate sorption (up to 45%) and increased glyphosate desorption (up to 0.87%) and the impact on reducing MCPA and tetracycline sorption (< 13%) and increasing MCPA and tetracycline desorption (< 3%) was significant but smaller than the impact on glyphosate. Glyphosate and tetracycline were strongly retained in soil with Kd values > 100 L kg^{-1} and desorption

less than 2%. In contrast, MCPA was weakly retained in soil with Kd values < 6 L kg^{-1} and desorption was above 25%. Hence, even in soils with a large phosphate build-up, glyphosate will be less mobile in matrix flow than MCPA. MCPA but not tetracycline additions significantly decreased glyphosate sorption, but only when MCPA was present at concentrations ten times greater than typically detected in agricultural soils and there was no phosphate added to the herbicide solutions.

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5. OVERALL SYNTHESIS

5.1. Importance of the Research

The herbicides glyphosate and MCPA and the antibiotic tetracycline are detected in agricultural soils and have the potential to move to the broader environment including surface water and groundwater (Battaglin et al., 2014; Javid et al., 2016; Sanchís et al., 2012; Struger et al., 2015; Székács et al., 2015). The presence of individual organic chemicals or their mixtures in water is seen as a concern because herbicides and antibiotics can adversely impact aquatic organisms (Baier et al., 2016; Gonzá Lez-Pleiter et al., 2013; Le et al., 2010; Relyea, 2009). The sorption of chemicals by soil determines the pathway and likelihood of chemical transport to water (Iglesias et al., 2010; Zhang et al., 2010). Chemicals that are weakly-sorbed in soil have the potential to leach to groundwater or be transported to surface water by runoff, while strongly-sorbed chemicals have a greater risk to be transported to surface water by wind and water eroded sediments (Batie et al., 1993; Leonard, 1988; Sørensen et al., 2006; Xu et al., 2009). Sorption parameters such as Kd and Koc are used to express the relative strength of organic chemical sorption in soil. Sorption parameters are determined by batch equilibrium protocols in the laboratory and greater values of Kd or Koc indicate greater sorption (Wauchope et al., 2002). Kd or Koc is an important sensitive input parameter to chemical fate models used by policy makers and other stakeholders to assess the risk of organic chemical transport from agricultural soils to the broader environment (Dubus et al., 2003; Gagnon et al., 2014). Pesticide fate models are used to estimate the transport of pesticides or emerging organic chemical contaminants (e.g., antibiotics) from agricultural soils to surface water and groundwater. Pesticide fate models are of great interest to policy makers as these models can supplement pesticide

water monitoring programs in environmental exposure assessments (Farenhorst et al., 2014; Pullan et al., 2016).

When inorganic and organic chemicals are present as mixtures in soil they can compete for sorption sites (De Wilde et al., 2009; Farenhorst and Bowman, 1998; Hernández-Soriano et al., 2007; Leistra and Matser, 2004). Hence, the presence of other chemicals in soil can influence the values of Kd and Koc determined for an organic chemical in soil. Most of the previous studies focusing on such competitive interactions have examined the impact of phosphate on glyphosate Kd values (de Jonge et al., 2001; Gimsing et al., 2007; Kanissery et al., 2015; Sprankle et al., 1975), and only two studies have investigated the impact of phosphate on MCPA and tetracycline sorption (Hiller et al., 2012; Wang et al., 2010). Overall, the limitations of these previous studies have been: 1) the utilization of chemical concentrations larger than environmentally relevant when examining the impact of chemical mixtures on the sorption on individual chemicals in soil (Gimsing et al., 2007, 2004; Hiller et al., 2012; Zhou et al., 2004), 2) no studies on the impact of antibiotics on herbicide sorption in soil, 3) no studies on the impact of inorganic fertilizer on desorption of antibiotics in soil. To address some of limitations to date, the overall objective of this research was to examine the competitive sorption effects of various combinations of inorganic and organic chemicals in soil. The research included various scenarios of mixtures of phosphate, cadmium, glyphosate, MCPA and/or tetracycline at environmentally relevant and larger concentrations.

5.2. Summary of Research Findings

The application of phosphate fertilizers to agricultural land may lead to the build up of phosphate in soils, along with the build up of impurities present in these fertilizers such as cadmium. Utilizing samples from a repeated application of phosphate fertilizer field plots, this research examined the impact of field-aged phosphate and cadmium concentrations on glyphosate sorption. Cadmium concentrations ranged from 0.19 to 0.41 mg kg^{-1} within the range of typically detected cadmium concentration in agricultural soils. From chapter 2, batch equilibrium experiment showed that Cd concentrations in soil had no effect on glyphosate sorption in soil. Olsen P concentrations were from 13 to 99 mg kg^{-1} in sandy clay loam soil (chapter 2, 3, 4) and from 8 to 48 mg kg^{-1} in clay loam soil (chapter 3). Glyphosate Kd values in these soils ranged from 43 to 1173 L kg^{-1} (chapter 2), from 99 to 1173 L kg^{-1} (chapter 3) and from 172 to 716 L kg^{-1} (chapter 4). Phosphate concentrations from repeated application of phosphate fertilizer field plots always reduced glyphosate Kd values, by up to 57% in study 1 (chapter 2), up to 53% in study 2 (chapter 3) and up to 50% in study 3 (chapter 4). There were no significant differences in the experimental results when a glyphosate containing commercial formulated product was used or when analytical grade glyphosate was used. This indicates that other ingredients in this product had no effect on glyphosate sorption (chapter 2).

Given that phosphate and glyphosate have similar sorption through the phosphate group, and that phosphate concentrations decreased glyphosate sorption (study 1, 2, and 3), the research also examined the impact of phosphate and glyphosate concentration on phosphate sorption (chapter 3). Phosphate Kf values ranged from 3.2 to 68 L$^{1/n}$ mg $^{1-1/n}$ kg^{-1} in the

sandy clay loam soil and from 21 to 76 $L^{1/n}$ mg $^{1-1/n}$ kg^{-1} in the clay loam soil. Phosphate concentrations from repeated application of phosphate fertilizer field plots always significantly reduced phosphate Kf values up to 89% in sandy clay loam soil (chapter 3) and up to 52% in clay loam soil (chapter 3). Results showed that glyphosate application had no impact on phosphate sorption in either soil.

This PhD thesis was the first study to examine the combined impact of aged and fresh phosphate on glyphosate sorption. When fresh phosphate was added to soil solution glyphosate Kd values ranged from 219 to 813 L kg^{-1} in study 1 (chapter 2), from 84 to 703 L kg^{-1} in study 2 (chapter 3) and from 210 to 306 L kg^{-1} in study 3 (chapter 4). Fresh phosphate additions significantly reduced glyphosate sorption in soil by up to 54% in study 1 (chapter 2), by up to 53% in study 2 (chapter 3) and by up to 44% in study 3 (chapter 4).

The research also examined the impact of phosphate concentration on MCPA and tetracycline sorption, and the impact of MCPA, tetracycline and their mixture on glyphosate sorption (chapter 4). Kd values ranged from 4.42 to 5.87 L kg^{-1} for MCPA and from 110 to 138 L kg^{-1} for tetracycline. Field aged phosphate had no effect on MCPA and tetracycline sorption. However, fresh phosphate addition significantly reduced the sorption of MCPA by up to 8% and tetracycline by up to 13%. Tetracycline had no effect on glyphosate sorption. MCPA addition significantly reduced the sorption of glyphosate by up to 14% but only when MCPA was added at exceptionally high concentrations and no phosphate was added in soil solution.

Chapter 4 included a batch equilibrium desorption study to assess the impacts of phosphate concentrations on glyphosate, MCPA and tetracycline desorption and of MCPA, tetracycline and their mixture concentrations on glyphosate desorption in soil. Results showed that desorption of glyphosate and tetracycline was always very low (<2%) but desorption of MCPA ranged from 26 to 31%. Repeated application of phosphate fertilizer did not have significant effect on the desorption of MCPA and tetracycline but significantly increased desorption (<1%) of glyphosate. Fresh addition of phosphate in the laboratory also reduced increased desorption (<1%) of glyphosate in soil. Phosphate addition significantly increased desorption of MCPA (3%) and tetracycline (<1). Tetracycline had no effect on the desorption of glyphosate. MCPA significantly increased desorption (<1%) of glyphosate but only when exceptionally high concentrations of MCPA were added.

The results from this thesis also indicated that the sorption of glyphosate and phosphate was influenced by the pH and nature of the background electrolyte solutions used, as well as the Fe/Al oxides content and calcium carbonate content in soil. Glyphosate sorption decreased with increasing pH of the soil solution. Under very strong acidic conditions glyphosate Kd ranged from 160 to 1173 L kg^{-1}, but under moderately acidic to slightly alkaline conditions glyphosate Kd was always <100 L kg^{-1} (chapter 2). When 0.01M CaCl$_2$ was used as a background electrolyte solution, sorption was increased up to 11% for glyphosate and up to 40% for phosphate relative to when 0.01M KCl was used as a background electrolyte solution (chapter 3). Sorption of glyphosate was 80% greater in sandy clay loam than clay loam soil because sorption was enhanced by Fe/Al-oxides in sandy clay loam soil. Sorption of phosphate was 30% greater in clay loam soil than sandy

clay loam soil because sorption was enhanced by calcium carbonate in clay loam soil (chapter 3).

5.3. Practical Implications of the Research

Scientific studies are typically done with analytical grade active ingredients purchased from a company that supplies laboratory equipment and supplies to the scientific community. Farmers use herbicide products that are formulated to contain other ingredients in order for the active ingredient to provide improved pest control. This study showed that analytical-grade glyphosate and glyphosate in a formulated herbicide product yielded the same results for sorption. Hence, this indicates that studies done using analytical grade glyphosate provide useful information about the fate of glyphosate when applied to field soils in commercially available formulations.

Phosphate transport to surface water can lead to eutrophication. Recent concerns have been raised about glyphosate increasing the transport of phosphate to surface water (Barrera, 2016). This study showed that glyphosate containing products have no impact on phosphate sorption. Hence, this finding indicates that these recent concerns are not fully justified and that it is unlikely that phosphate transport to surface water is increased due to glyphosate application in agricultural crop field.

This PhD research generated important information on how sorption parameter values for a given chemical may change because of the presence of other chemicals in soil. The sorption parameter (e.g., Kd, Kf or Koc) is among the most sensitive input parameters used

in pesticide fate models (Dubus et al., 2003; Farenhorst et al., 2009; Luo et al., 2011). For example, for predicting the mass of pesticides transported to surface waters, predictions can be sensitive to the pesticide's Koc value up to a threshold value of 35,000 L kg^{-1} after which the prediction is independent of the pesticide's Koc value (Luo et al., 2011). Glyphosate Koc values ranged from 2700 to 41,753 L kg^{-1} in soils containing on average 17 mg P kg^{-1} and from 1530 to 12,498 L kg^{-1} in soils containing on average 89 mg P kg^{-1}. Phosphate concentrations decreased the glyphosate Koc value in a soil by as much as 70%, from a Koc value of 41,753 L kg^{-1} in a soil containing 13 mg P kg^{-1} to a Koc value of 12,498 L kg^{-1} in a soil containing 99 mg P kg^{-1}. Given the Koc values observed in this research, the amount of phosphate present in soil should be considered in setting sorption parameters for glyphosate in pesticide fate models. Olsen P concentrations ranged from 8 to 99 mg kg^{-1} in this research within the typical range of 8 to 114 mg kg^{-1} reported for soils in North America (McDowell et al., 2001). Hence, the findings from this PhD thesis about the competitive sorption effect of phosphate and glyphosate in soil would be applicable to a wider range of soils in North America.

In general, organic chemicals that have Koc values between 150 and 500 L kg^{-1} are considered moderately leachable in soil (Barcelo and Hennion, 1997). MCPA Koc values ranged from 174 to 208 L kg^{-1} in soils containing on average 17 mg P kg^{-1} and from 157 to 181 L kg^{-1} in soils containing on average 89 mg P kg^{-1}. Phosphate concentrations decreased the MCPA Koc value in a soil by as much as 25%, from a Koc value of 208 L kg^{-1} in a soil containing 13 mg P kg^{-1} to a Koc value of 157 L kg^{-1} in a soil containing 99 mg P kg^{-1}. Hence, the research results suggest that phosphate fertilizers should be applied at the

agronomic rates meeting crop requirements rather than in excess amounts as this can increase the movement of MCPA to groundwater.

5.4. Recommendations for Future Studies

In this study, the available phosphate concentrations in soils were measured by the Olsen P test because this test is most relevant to the North American Great Plains region. However, the concentration of phosphate in soil is also determined by other tests such as the Mehlich P, Kelowna P, Bray P, or other tests (Howard, 2006; McKenzie et al., 2003; Siebbsen and Sharpley, 1997). Because each study usually utilizes only one test to determine phosphate concentrations in soil, and because previous studies use different tests, it is difficult to compare the findings of this research directly to previous studies. Thus, in future studies examining the impact of phosphate concentrations on the sorption of inorganic and organic chemicals, it is best if these studies measure phosphate concentrations in different ways so it would be easier to better compare study results with the previous literature.

In this study, soil samples were collected from 0-15 cm depth which is the typical agronomic sampling depth. However, previous studies reported P stratification in the 0-5 cm depth after repeated application of phosphate fertilizer (Baker et al., 2017; Sharpley, 2003; Ziadi and Morel, 2017). Phosphate concentration decreases with the increase of soil depth (Sharpley, 2003). In a recent study, it has been shown that phosphate concentration was greater in 1 cm top soil than 4-5 cm surface soil because of P accumulation in soil (Ziadi and Morel, 2017). Hence, for the future studies it would be a good practice to collect

soil sample from different depth such as 0-5 cm, 5-10 cm or 10-15 cm etc. to examine the impact of phosphate concentrations on the sorption of organic and inorganic chemicals.

In this research, a series of sorption and desorption studies were conducted based on batch equilibrium procedures that are outlined in OECD guideline 106 (OECD, 2000). Batch equilibrium studies were utilized using five different background electrolyte solutions (0.01M HCl, 0.01M $CaCl_2$, 0.01M KCl, 0.01M KOH, and d.H_2O) with a soil/solution ratio of 1:5 to adjust the soil solution pH in study 1 (chapter 2). Among the five background electrolytes solutions utilized, glyphosate sorption was maximum when 0.01M $CaCl_2$ and 0.01M KCl were used as background electrolytes solutions. Therefore, 0.01M $CaCl_2$ and 0.01M KCl were chosen to examine the impact of background electrolyte solutions on sorption of glyphosate and phosphate in study 2 (chapter 3). Results from study 2, indicate that glyphosate and phosphate had a tendency to form complexes/precipitation with Ca^{2+}. Thus, glyphosate and phosphate were either adsorbed or precipitated (this phenomenon was particularly true for phosphate), but it is difficult to distinguish between these two processes. Hence, 0.01M KCl would be recommended to use as background electrolyte solution for future sorption studies.

Farmers apply formulated herbicide products to control weeds in crop production. This PhD thesis successfully identified that there is no difference in the sorption of analytical grade glyphosate in soil and glyphosate applied in the commercially available product Roundup Ultra2. However, only one pesticide formulation was examined in this research. There are now many different formulated products on the market that contain glyphosate

and hence these findings need to be examined for a wider range of glyphosate containing products. Also, studies should consider other active ingredients and their formulated products. For example a comparison of the sorption of analytical grade MCPA in soils with MCPA applied in a formulated product such as MCPA Ester 600, MCPA Amine 500 etc. (Crop Protection Guide, 2016). The herbicide, 2,4-D is also widely applied in Manitoba. Given that, MCPA influenced glyphosate sorption, hence, it would be worthwhile to include 2,4-D to examine the impact on glyphosate sorption in soil. In addition to analytical grade MCPA and 2,4-D, commercially available product such as 2,4-D amine 600, MCPA Ester 600 would be recommended to include for future studies to examine their impacts on sorption of commercially available glyphosate in soil.

Phosphate may not only build up because of repeated applications of phosphate inorganic fertilizers but particularly when livestock manures are applied to agricultural soils (Siebbsen and Sharpley, 1997). Manure application can impact other soil properties particularly soil organic carbon content and soil pH (Eghball, 2002; Khaleel et al., 1981). Given that, this study found a significant impact of phosphate concentrations on glyphosate sorption, additional studies should be carried out to examine the impact of manure applications on the sorption of glyphosate and other organic chemicals in soil.

This PhD research provides important information on the interaction between chemicals in batch equilibrium experiments that have a short duration such as 24h which is typically used in such experiments (Wauchope et al. 2002). However, sorption is influenced by the contact time between chemicals and soil and that sorption increases over time is known as

time dependent sorption (Mamy and Barriuso, 2007) or aging (Wauchope et al., 2002). Aged-sorption is important to consider in estimating sorption parameters particularly because it can reduce the risk of pesticide leaching to ground water and hence influence the outcome of exposure assessments as part of regulatory practices (Beulke and van Beinum, 2014). Therefore, it would be recommended to conduct time-dependent sorption studies when examining the competitive sorption effects of various combinations of inorganic and organic chemicals in soil.

This PhD thesis has successfully identified that the sorption of organic chemicals was influenced by the soil properties such as soil pH, Fe/Al-oxides, P content and Ca content. However, this study is limited to an acidic sandy clay loam and a calcareous clay loam soil in Prairies. Therefore, additional studies should be taken into consideration to collect soil samples from a wide range of agricultural fields in Canada (e.g., 100 or 200 fields) so that model equations account the influences of soil properties on the sorption of glyphosate or other organic compounds. This model would benefit policy makers and researchers to predict K_d, K_{oc} or K_f values of the organic compounds in soil without doing time-consuming sorption experiment.

This thesis does not provide any information about the sorption of pesticide metabolites. In some cases, metabolites have reduced sorption and greater leaching potential than parent products, for example, atrazine metabolite deethylatrazine is less strongly sorbed to soil (1.53 to 21.6 L kg^{-1}) than atrazine (2.56 to 39.6 L kg^{-1}) (Abate et al., 2004; Mersie and Seybold, 1996). In some other cases, metabolites have longer persistence than parent

products, for example, glyphosate metabolite AMPA has longer half-life (60 to 419 days) than glyphosate (30 to 197 days) in soil (Battaglin et al., 2014; Bergström et al., 2011; Giesy et al., 2000; IUPAC, 2017; Smith and Aubin, 1993). Also metabolites can be sorbed in a similar way as parent molecules because of similar functional groups (Báez et al., 2015; Sidoli et al., 2016) e.g., glyphosate and AMPA has a similar phosphonate group. Given that, phosphate and organic chemicals have competitive effect on sorption of glyphosate, additional studies should be carried out to examine the impact of the phosphate and organic chemical mixtures on the sorption of individual pesticide metabolites such as AMPA in soil.

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Supplementary Information: Chapter 2

Table 2S1. Effect of glyphosate formulation (Roundup Ultra2, analytical grade glyphosate) on glyphosate Kd values in batch equilibrium experiments utilizing either 0.01M HCl, 0.01M CaCl$_2$, 0.01M KCl, dH$_2$O and 0.01M KOH as a background liquid.

Effect	Num DF	Den DF	F Value	Pr > F
0.01M HCl				
Formulation	1	19	0.22	0.6472
0.01M CaCl$_2$				
Formulation	1	19	0.03	0.8682
0.01M KCl				
Formulation	1	19	0.01	0.9316
dH$_2$O				
Formulation	1	19	0.74	0.4002
0.01M KOH				
Formulation	1	19	0.16	0.6943

Table 2S2: Effect of Cd levels (low, medium, high) and phosphate levels (20P, 40P, 80P) on total Cd concentrations in soil

Effect	Num DF	Den DF	F Value	Pr > F
Cd levels	2	24	3.21	0.0581
Phosphate levels	2	24	0.95	0.4011
Cd*Phosphate levels	4	24	1.31	0.2951

Table 2S3. 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 CaCl₂ (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.H₂O (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

Table 2S4. 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

Results of regression analysis instead of ANOVA to data presented in Chapter 2-

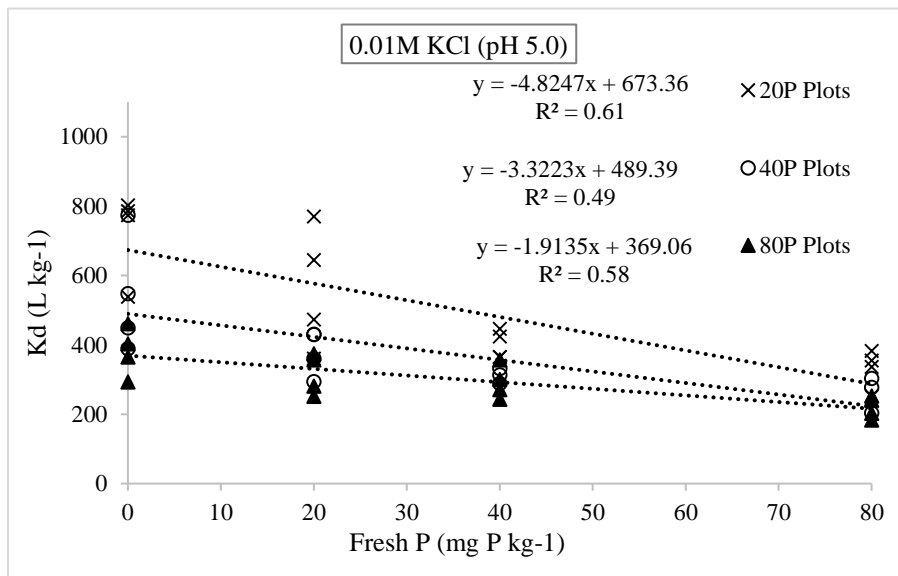**Phosphate fertilizer impacts on glyphosate sorption by soil figure 2.5**
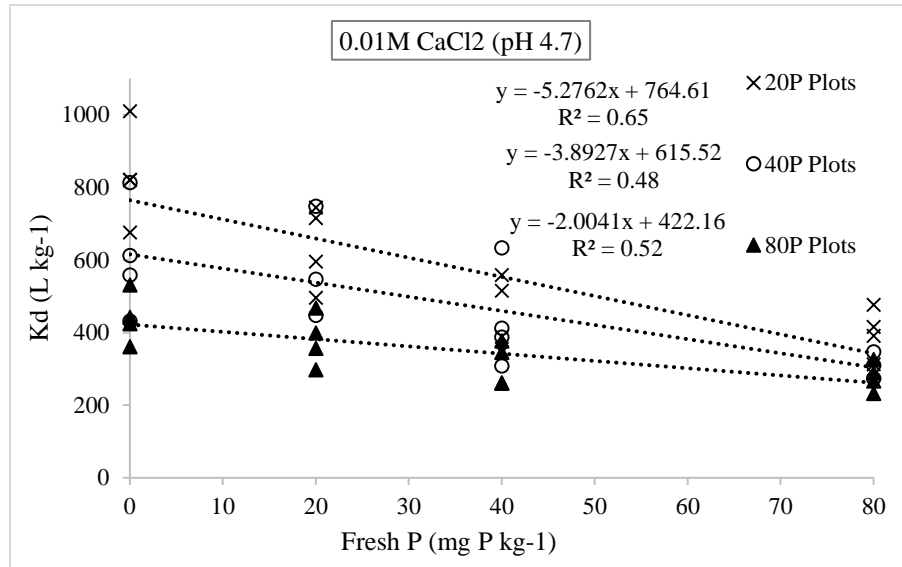
Figure S2.5. Effect of co-applying mono ammonium phosphate with glyphosate in solution, for batch equilibrium experiments using 0.01M CaCl₂ (pH 4.7) and 0.01M KCl (pH 5.0).

Table 3S1. Simple linear regression results using Olsen P as independent variables to predict phosphate, glyphosate sorption constant and phosphate equilibrium concentration in SCL-Fe$_2$O$_3$ and CL-CaCO$_3$ soils except phosphate equilibrium concentration in SCL-Fe$_2$O$_3$ soil was non-linear

Parameter	Soil	Electrolytes	Linear Model	R^2	P value
Phosphate Kf	SCL-Fe$_2$O$_3$	0.01M CaCl$_2$	Kf = - 0.59 Olsen P + 64.19	0.75	***
		0.01M KCl	Kf = - 0.46 Olsen P + 42.10	0.75	***
	CL-CaCO$_3$	0.01M CaCl$_2$	Kf = - 0.98 Olsen P + 77.17	0.77	***
		0.01M KCl	Kf = - 0.81 Olsen P + 55.08	0.85	***
Glyphosate Kd	SCL-Fe$_2$O$_3$	0.01M CaCl$_2$	Kd = - 6.85 Olsen P + 1001	0.69	***
		0.01M KCl	Kd = - 6.53 Olsen P + 916.07	0.67	***
	CL-CaCO$_3$	0.01M CaCl$_2$	Kd = - 0.55 Olsen P + 128.63	0.43	**
		0.01M KCl	Kd = - 0.60 Olsen P + 131.48	0.42	**
Phosphate EPCo	CL-CaCO$_3$	0.01M CaCl$_2$	EPCo = 0.0003 Olsen P – 0.0017	0.75	***
		0.01M KCl	EPCo = 0.0008 Olsen P – 0.0047	0.86	***

, and * denotes level of significance; p<0.01, and p<0.0001, respectively

Table 3S2: Parameters for the non-linear exponential model to predict phosphate equilibrium concentration using Olsen P as independent variable in SCL-Fe$_2$O$_3$ soil

Electrolytes	Non-linear model	P value
0.01M CaCl$_2$	EPCo = 0.0001e$^{0.06 \text{Olsen P}}$	***
0.01M KCl	EPCo = 0.0002e$^{0.05 \text{Olsen P}}$	***

*** denotes level of significance; p<0.0001

Table 3S3. Effect of Roundup Ultra2 (0, 100 mg L^{-1}) on phosphate sorption in SCL-Fe$_2$O$_3$ and CL-CaCO$_3$ soil

Effect	Num DF	Den DF	F Value	Pr > F
SCL-Fe$_2$O$_3$				
Roundup Ultra2	1	11	0.38	0.5519
CL-CaCO$_3$				
Roundup Ultra2	1	11	4.12	0.0672

Table 3S4. Simple linear regression results using phosphate sorption constant Kf to predict glyphosate sorption distribution coefficient Kd in SCL-Fe$_2$O$_3$ and CL-CaCO$_3$ soils.

Soil	Electrolytes	Linear Model	R^2	P value
SCL-Fe$_2$O$_3$	0.01M CaCl$_2$	Kd = 11.34 Kf + 267.30	0.85	***
	0.01M KCl	Kd = 14.19 Kf + 303.83	0.89	***
CL-CaCO$_3$	0.01M CaCl$_2$	Kd = 0.45 Kf + 91.12	0.37	*
	0.01M KCl	Kd = 0.58 Kf + 96.43	0.30	*

*, **, and *** denotes level of significance; p<0.05, p<0.01, and p<0.0001, respectively

Table 3S5. Simple linear regression results using potassium dihydrogen phosphate as independent variables to predict glyphosate sorption coefficient Kd (L kg^{-1}) SCL-Fe$_2$O$_3$ and CL-CaCO$_3$ soils

Soil	Plots	Electrolytes	Linear Model	R^2	P value
SCL-Fe$_2$O$_3$	0P	0.01M CaCl$_2$	Kd = - 10.12 Fresh P + 859	0.58	**
		0.01M KCl	Kd = - 10.30 Fresh P + 805	0.63	**
	80P	0.01M CaCl$_2$	Kd = - 2.73 Fresh P + 425	0.50	**
		0.01M KCl	Kd = - 2.42 Fresh P + 362	0.40	**
CL-CaCO$_3$	0P	0.01M CaCl$_2$	Kd = - 0.73 Fresh P + 116	0.87	***
		0.01M KCl	Kd = - 0.63 Fresh P + 118	0.94	***
	80P	0.01M CaCl$_2$	Kd = - 0.49 Fresh P + 105	0.74	***
		0.01M KCl	Kd = -0.39 Fresh P + 104	0.53	**

, and * denotes level of significance; p<0.01, and p<0.0001, respectively

Table 3S6. Effect of Olsen P concentrations on reducing phosphate (Kf) or glyphosate (Kd) sorption relatively to control plots in SCL-Fe$_2$O$_3$ and CL-CaCO$_3$ soils, and on the equilibrium phosphate concentration (EPCo) values in SCL-Fe$_2$O$_3$ and CL-CaCO$_3$ soils.

Background Electrolyte Solution	Treatment	Reduction of Kf (%)		Reduction of Kd (%)	
		SCL-Fe$_2$O$_3$	CL-CaCO$_3$	SCL-Fe$_2$O$_3$	CL-CaCO$_3$
0.01M KCl	Control	-	-	-	-
	20P	33	16	18	0
	40P	56	28	39	1
	80P	89	52	57	11
0.01M CaCl$_2$	Control	-	-	-	-
	20P	30	13	13	0
	40P	39	22	36	0
	80P	79	38	53	11

Table 3S7. Effect of potassium dihydrogen phosphate reducing glyphosate sorption (Kd) in SCL-Fe$_2$O$_3$ and CL-CaCO$_3$ soils, with sorption being determined by batch equilibrium experiments using 0.01M CaCl$_2$ or 0.01M KCl as background electrolyte solutions.

Solution	Treatment (mg L^{-1})	Reduction of Kd (%)		Reduction of Kd (%)	
		SCL- Fe$_2$O$_3$		CL-CaCO$_3$	
		0P	80P	0P	80P
0.01M KCl	0	-	-	-	-
	11	29	17	7	9
	22	42	21	14	8
	44	54	31	25	18
0.01M CaCl$_2$	0	-	-	-	-
	11	25	10	9	3
	22	42	23	13	10
	44	50	28	28	20

Fitting a linear regression equation to quadratic equation to the data presented in Chapter 3 – Phosphate and glyphosate sorption in soils following repeated application of phosphate, figure 3.1

Variable	Parameter Estimate	SE	t Value	Pr > |t|	R^2
SCL-Fe_2O_3-Phosphate Kf (0.01M $CaCl_2$)					
Intercept	58.11	7.10	8.18	<.0001	
Olsen P	-1.26	0.34	-3.68	0.0028	0.82
Quadratic term	0.007	0.003	2.38	0.0332	
SCL-Fe_2O_3-Phosphate Kf (0.01M KCl)					
Intercept	74.31193	9.79	7.59	<.0001	
Olsen P	-1.12824	0.47	-2.39	0.0326	0.79
Quadratic term	0.005	0.004	1.16	0.2683	
SCL-Fe_2O_3-Glyphosate Kd (0.01M $CaCl_2$)					
Intercept	1241.54	127.85	9.71	<.0001	
Olsen P	-19.60	6.16	-3.18	0.0072	0.77
Quadratic term	0.116	0.06	2.10	0.0553	
SCL-Fe_2O_3-Glyphosate Kd (0.01M KCl)					
Intercept	1141.46	127.45	8.96	<.0001	
Olsen P	-18.50	6.14	-3.01	0.0100	0.75
Quadratic term	0.11	0.055	1.98	0.0692	

Table 4S1: Effect of fresh-phosphate added at different time (*n,n,n; n,n,P; P,n,n; P,n,P; n,P,P*) and field aged-P (0P, 80P) on sorption and desorption of glyphosate, MCPA and tetracycline in soil

Effect	Num DF	Den DF	F Value	Pr > F	Num DF	Den DF	F Value	Pr > F
	MCPA, Kd				MCPA, %D			
Fresh-P	4	27	7.09	0.0005	4	27	8.17	0.0002
Aged-P	1	27	2.11	0.1578	1	27	0.91	0.3475
Fresh-P*Aged-P	4	27	0.39	0.8132	4	27	2.27	0.0874
	Tetracycline, Kd				Tetracycline, %D			
Fresh-P	4	27	24.69	<.0001	4	27	6.42	0.009
Aged-P	1	27	4.06	0.0541	1	27	1.28	0.2679
Fresh-P*Aged-P	4	27	0.57	0.6847	4	27	0.33	0.8525
	Glyphosate, Kd				Glyphosate, %D			
Fresh-P	4	27	80.78	<.0001	4	27	130.63	<.0001
Aged-P	1	27	461.56	<.0001	1	27	258.28	<.0001
Fresh-P*Aged-P	4	27	22.42	<.0001	4	27	12.40	<.0001

Table 4S2: Effect of MCPA (0, 1, or 11 mg L^{-1}) and field aged-P (0P, 80P); tetracycline (0, 1, or 11 mg L^{-1}) and field-aged-P (0P, 80P) MCPA-tetracycline mixture (0, 1, or 11 mg L^{-1}) and field-aged-P (0P, 80P) on sorption and desorption of glyphosate in soil

Effect	Num DF	Den DF	F Value	Pr > F	Num DF	Den DF	F Value	Pr > F
	Glyphosate, Kd				Glyphosate, %D			
MCPA	2	15	10.23	0.0016	2	15	15.06	0.0003
Aged-P	1	15	622.79	<.0001	1	15	1302.94	<.0001
MCPA*Aged-P	2	15	2.33	0.1317	2	15	0.77	0.4792
Tetracycline	2	15	3.14	0.0745	2	15	1.52	0.2512
Aged-P	1	15	820.69	<.0001	1	15	657.45	<.0001
Tetra*Aged-P	2	15	0.47	0.6369	2	15	0.05	0.9558
Mixture	2	15	6.37	0.0100	2	15	11.77	0.0011
Aged-P	1	15	560.14	<.0001	1	15	223.21	<.0001
Mixture*Aged-P	2	15	2.21	0.1442	2	15	1.53	0.2480

Table 4S3. Effect of pre-sorbed phosphate (0, 11, 22, 44 mg L^{-1}) on glyphosate, MCPA and tetracycline sorption and retained MCPA on glyphosate sorption (L kg^{-1}) in soil

Effect	Num DF	Den DF	F Value	Pr > F
		Glyphosate, Kd		
	3	9	71.14	<.0001
		MCPA, Kd		
Pre-sorbed P	3	9	5.72	0.0180
		Tetracycline, Kd		
	3	9	9.20	0.0042
Pre-sorbed MCPA		Glyphosate, Kd		
	3	9	5.03	0.0257