1. Information on the study

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Report author	Kjaer, J. et al.
Report year	2011
Report title	Transport modes and pathways of the strongly sorbing pesticides glyphosate and pendimethalin through structured drained soils
Document No	Chemosphere, (2011) Vol. 84, No. 4, pp. 471–9
Guidelines followed in study	None
Deviations from current test guideline	No
GLP/Officially recognised testing facilities	Yes, conducted at officially recognised testing facilities (Geological Survey of Denmark and Greenland)
Acceptability/Reliability:	Reliable with restrictions (Substance properties not sufficiently described, no evaluation of the residues in soil layers after finalization of the study was conducted, duration of the study too short)

2.Full summary of the study according to OECD format

Leaching of the strongly sorbing pesticides glyphosate and pendimethalin was evaluated in an 8–month field study focussing on preferential flow and particle–facilitated transport, both of which may enhance the leaching of such pesticides in structured soils. Glyphosate mainly sorbs to mineral sorption sites, while pendimethalin mainly sorbs to organic sorption sites. The two pesticides were applied in equal dosage to a structured, tile–drained soil, and the concentration of the pesticides was then measured in drainage water sampled flow–proportionally. The leaching pattern of glyphosate resembled that of pendimethalin, suggesting that the leaching potential of pesticides sorbed to either the inorganic or organic soil fractions is high in structured soils. Both glyphosate and pendimethalin leached from the root zone, with the average concentration in the drainage water being 3.5 and 2.7 µg/L, respectively. Particle–facilitated transport (particles >0.24 µm) accounted for only a small proportion of the observed leaching (13–16 % for glyphosate and 16–31 % for pendimethalin). Drain–connected macropores located above or in the vicinity of the drains facilitated very rapid transport of pesticide to the drains. That the concentration of glyphosate and pendimethalin in the drainage water remained high (>0.1 µg/L) for up to 7 d after a precipitation event indicates that macropores between the drains connected to underlying fractures were able to transport strongly sorbing pesticides in the dissolved phase. Lateral transport of dissolved pesticide via such discontinuities implies that strongly sorbing pesticides such as glyphosate and pendimethalin could potentially be present in high concentrations (>0.1 µg/L) in both water originating from the drainage system and the shallow groundwater located at the depth of the drainage system.

Materials and methods

Chemicals

Glyphosate [N–(phosphonomethyl)glycine] – the active ingredient in Roundup – is a broad–spectrum, post–emergence, non–selective herbicide that is one of the most used herbicides worldwide. In Denmark, glyphosate is the herbicide sold in the largest quantities; in 2003, glyphosate sales for agricultural purposes accounted for 44 % of all herbicide sales. By 2008, this had increased to 52 % (Danish Environmental Protection Agency, 2004, 2009). Pendimethalin [N–(1–ethylpropyl)–2,6–dinitro–3,4–xylidine] is a selective herbicide used to control most annual grasses and certain broadleaf weeds both pre–emergence (i.e. before weed seeds have sprouted) and early post–emergence. Pendimethalin ranks fourth among the herbicides used in Demark, accounting for 5 % of all herbicide sales for agricultural use in 2003 and 6 % in 2008 (Danish Environmental Protection Agency, 2004, 2009). Solubility of glyphosate is 10500 mg/L while that of pendimethalin is 0.33 mg/L pesticide properties database available at http://sitem.herts.ac.uk/aeru/footprint/index2.htm.

Site description

The study was conducted at the Estrup field research site in Denmark, a virtually flat systematically tile–drained loamy field located on glacial till with a cultivated area of 1.26 ha. The tile drains are located at an average depth of 1 m b.g.s., and the water table is relatively shallow, located 1–3 m b.g.s. The uppermost meter of the soil is heavily fractured and bioturbated, with plough layer containing 100–1000 biopores/m^2 (Lindhardt et al., 2001). The geological structure is complex, comprising a clay till core with deposits of different age and composition. Of three pedological profiles available for the site, one is classified as Aquic Argiudoll, one as Abruptic Argiudoll and one as Fragiaquic Glossudalf. Details on soil properties are reported in Table 1 and geological properties are further described in Lindhardt et al. (2001).

Agricultural management

After maize (Zea mays L.) had been harvested on 13 October 2005, glyphosate (1.44 kg/ha active ingredient; 4.0 L/ha Round–up Bio) and pendimethalin (1.44 kg/ha active ingredient; 3.6 L/ha Stomp) were applied simultaneously together with 30.0 kg/ha of potassium bromide as tracer on 9 November 2005. On 12 April 2006 the field was ploughed to a depth of 18 cm. Spring barley was sown on 27 April 2006. Whereas glyphosate had been applied previously (13 October 2000 and 2 September 2002) the field had been treated with pendimethalin 7 October 1997. The minor residues of glyphosate (0.01–0.03 µg/L) found in the drainage water before the current application of pesticides (Figure 2) is thus likely to derive from these previous treatment.

Monitoring and sample preparation

For a period of 8 months following application of the glyphosate and pendimethalin the concentration of the pesticides and bromide was measured on a weekly basis in drainage water sampled flow–proportionally. In addition, more intense sampling of drainage water was performed in connection with three flow events triggered by precipitation on 14 November 2005, 16 December 2005 and 11 January 2006 (Figure 1) in order to enable detailed description of the transport of water and pesticides. Sampling lasted for 2, 13 and 9 d, respectively. Flow events are characterised by an initial rapid rise in the hydrograph followed by a less rapid drop (tailing). During these events, drainage water subsamples were collected for every 2 mm of drainage runoff using a refrigerated Isco sampler (Teledyne Isco, Inc., US) containing eight 2–L borosilicate bottles. Within 24 h of the onset of the flow events, each bottle from the Isco sampler was shaken thoroughly to resuspend the sediment. The particles in the individual samples were then separated by centrifugation at 3500 rpm using Teflon vials. The time required for separation of particles ≥0.24 µm was calculated according to Gimbert et al. (2005). The supernatant was removed using a pipette, cleaned with 20 % HCl. The supernatant of samples to be analysed for pendimethalin was placed in glass bottles and preserved by adjusting to pH 2.0 with sulphuric acid. The pellets were flushed into a glass bottle using demineralised water and preserved using sulphuric acid. The samples to be analysed for pendimethalin were stored at 2°C until analysis. The supernatant of samples to be analysed for glyphosate and AMPA was pipetted into polypropylene (PP) bottles and adjusted to pH 2.0 with sulphuric acid. The pellets were flushed into PP bottles and adjusted to pH 2.0 with sulphuric acid. The latter two types of sample were stored at –18°C until analysis. As the flow event on 16 December 2005 occurred at a weekend, it was not possible to conduct particle separation on the samples. With all the samples collected on a weekly basis and the intensive samples collected following the flow event on 16 December 2005, pesticide concentrations were measured on the entire water sample. Thus the reported concentrations refer to the total concentration of both dissolved and particle–bound pesticide. With samples collected intensively following the flow events on 14 November 2005 and 11 January 2006, pesticide concentrations are reported for both particle–bound pesticide (concentration in the pellets) and dissolved pesticide (concentration in the supernatant). Furthermore, measurements of turbidity, chloride concentration and conductivity were conducted on all water samples obtained from the Isco sampler.

Table 1 Physical and chemical properties of the soil.

Profile[a]	Horizon	Depth	Clay[b] (%)	Silt[b] (%)	Sand[b] (%)	OM (%)	C/N	CEC (meq 100 g^{-1})	pH$_{CaCl2}$	Fe (mg kg^{-1})	Al (mg kg^{-1})
Estrup 2											
	Ap	0–26	13.8	12.7	70.8	2.7	13	12.1	6.5	2044	808
	Bt(g)	26–45	36.3	15.3	47.8	0.5	6	13.9	6.3	4144	1748
	Bt(g)2	45–121	33.0	15.9	50.9	0.2	4	16.8	6.6	2294	1034
	Cc[c]	121–150	31.1	24.9	7.5	0.5	6	19.7	7.5	2290	568
Estrup 3											
	Ap	0–28	9.9	7.1	77.5	5.5	17	15	7.0	1648	1024
	Bs/Bhs	28–58	8.8	4.7	85.7	0.8	12	10.2	6.6	1730	1340
	Bt(g)	58–115	12.2	4.3	83.1	0.4	8	9.2	4.2	1702	916
	2C	115–185	38.9	24.1	26.9	10.1	39	40.5	4.5	1576	2934

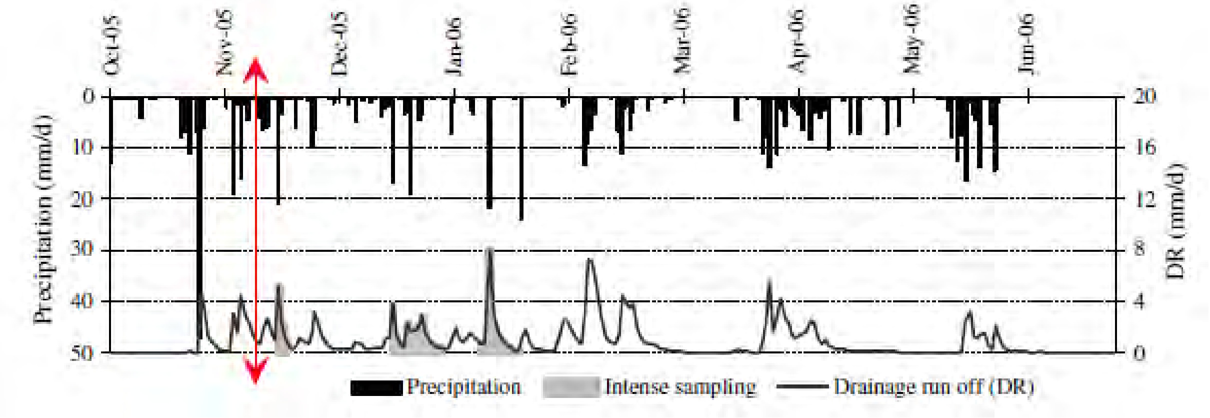
nd.: not determined; OM: organic matter determined as 1.72 total organic carbon; Fe and Al: oxalate extractable Fe and Al determined by the methods of McKeague and Day (1966).

a Profiles are classified as Abruptic Argiudoll (Estrup 2) and Fragiaquic lossudalf (Estrup 3).

b Clay: <2 µm; Silt: 2–20 µm; Sand: 20–2000 µm.

c Contains 36.1% $CaCO_3$. Contains 20.0% $CaCO_3$.

Figure 1. Precipitation (hanging bars on primary axis) and drainage runoff (solid line on secondary axis). The red vertical arrow indicates the date of application. The shaded grey area beneath the solid line indicates the flow events that were intensively monitored (see Section 2.4).



Methods of analysis

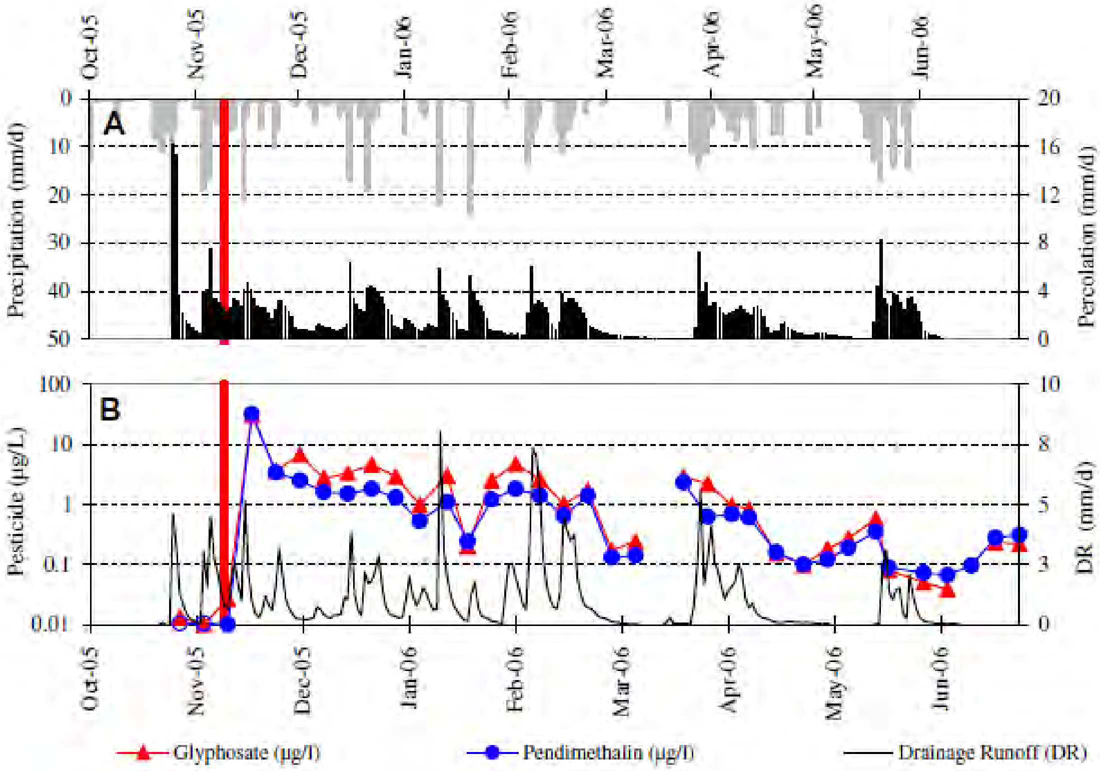
Glyphosate

The preserved water samples were first concentrated on a column of Chelex 100 resin, iron form 100–200 mesh from Bio–Rad. After washing with 0.1 M HCl, the analytes were eluted with 6 M HCl. The eluate was further cleaned on a column of AG 1–X8 resin, chloride form 200–400 mesh. The eluate was evaporated to dryness under nitrogen and redissolved in 200 µL of water–methanol–HCl (160:40:2.7). Derivatisation was carried out with 1 mL of trifluoroacetic anhydride–2,2,3,3,4,4,4–heptafluoro–1–butanol (2:1). The derivatives of glyphosate were measured by GC–MS using a 5 % phenyl methylsiloxane GC–column (HP–5) with the MS in electron impact (EI) mode. 2 µL sample was injected by splitless injection at 280°C with oven temperature at 65°C. After 2 min the oven temperature was raised to 310°C at 20°C min^{-1} and held at 310°C for 4 min. The glyphosate derivatives were identified by MS using m/z 612, 611 and 584. The calculations were made using the internal standard procedure with glyphosate–$^{13}C^{15}N$ as the internal standard. The LOD (limit of detection) was below 0.01 µg/L. The preserved pellet samples were treated with 1 M ammonia prior to analysis in order to extract the glyphosate from the solids. The extract was then diluted with water, adjusted to pH 2.0 with HCl, and analysed as described above for the water samples.

Inorganic analysis

The water samples were analysed for turbidity, conductivity and chloride concentration. Turbidity was measured with an infrared LED light source using a pHotoFlex Turb photometer (WTW Gmbh, Weilheim, Germany). Conductivity was measured using a Cond 340i conductivity pocket meter (WTW Gmbh, Weilheim, Germany). Chloride concentration was measured using a FIAstar™ 5000 flow injection analyser (Foss Analytical AB, Höganäs, Sweden).

Figure 2. Precipitation and simulated percolation (A) together with concentration of pendimethalin and glyphosate (B) in the drainage runoff (DR on secondary axis). The red vertical lines indicate the date of application. The open circles indicate concentrations below the LOD (0.01 µg/L).

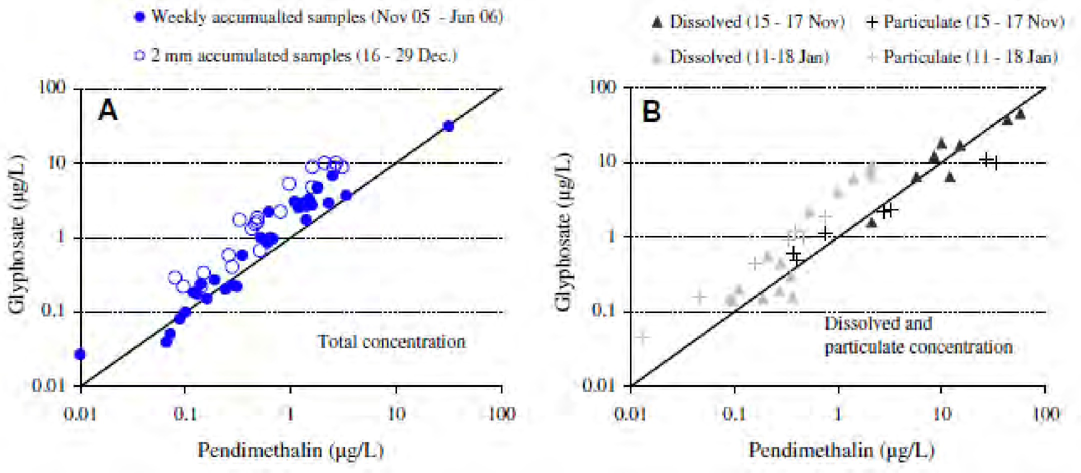


Results and Discussion

Leaching of glyphosate and pendimethalin

The leaching pattern of glyphosate resembled that of pendimethalin (Figure 2), thus suggesting (i) that the leaching potential of strongly bound pesticides from structured soil is high both with pesticides that bind to soil organic matter (e.g. pendimethalin) or to the inorganic fraction (e.g. glyphosate) and (ii) that the pathways governing the transport of these two pesticides are similar. Both glyphosate and pendimethalin leached from the root zone in average concentrations considerably exceeding the EU limit value for groundwater (0.1 µg/L) during the 8–month drainage flow period (Figure 2). The average concentration of glyphosate and pendimethalin in the drainage water was 3.5 and 2.7 µg/L, respectively. Both pesticides were found in all of the weekly drainage water samples. Among the 32 samples collected after pesticide application, the concentration exceeded 0.1 µg/L in 29 (Figure 2). The similarity of the leaching patterns of the two pesticides was reflected in the close correlation between the measured concentration of pendimethalin and glyphosate (Figure 3). R^2 for measured total concentration (both dissolved and particle–bound) in samples collected (i) on a weekly basis during the entire monitoring period (32 samples) and (ii) for every 2 mm of drainage runoff occurring during a 13–d period in December (20 samples) was 0.962 and 0.963, respectively (Figure 3A). A similar tendency was found when comparing the particulate and dissolved concentrations of pendimethalin and glyphosate measured during two individual flow events, R^2 being 0.943 for dissolved pesticide (19 samples) and 0.928 for particle–bound pesticide (10 samples) (Figure 3B). Pesticide leaching was governed by preferential transport, as evidenced by the soil hydraulic properties (Kjær et al., 2005) and fast solute transport. Piston flow through the low–permeable soil matrix would entail a transport time to the drainage system of about 98 d (Kjær et al., 2007). However, glyphosate and pendimethalin were detected in drainage water samples as early as 8 d after application. This finding is thus consistent with previous transport studies conducted at the Estrup site (Kjær et al., 2005, 2007), as well as other field studies demonstrating rapid macropore–mediated transport of pesticides (for a review see Jarvis (2007)). As both glyphosate and pendimethalin leached in high concentrations following the same transport pathways, the difference in sorption characteristics of glyphosate, which sorbs strongly to the inorganic soil fraction, and pendimethalin, which sorbs strongly to the soil organic fraction, had little impact on leaching in this structured soil. Our finding is in line with previous studies showing that differences in the leaching of pesticides that differ widely in sorption properties are significantly reduced in the presence of macropore flow (Larsson and Jarvis, 1999). Likewise, Flury (1996) concluded from the transport studies of Kladivko et al. (1991), Traub–Eberhard et al. (1995) and Flury et al. (1995) that part of the various pesticides applied simultaneously to the soil surface moved through structured soil in an identical manner irrespective of their chemical properties.

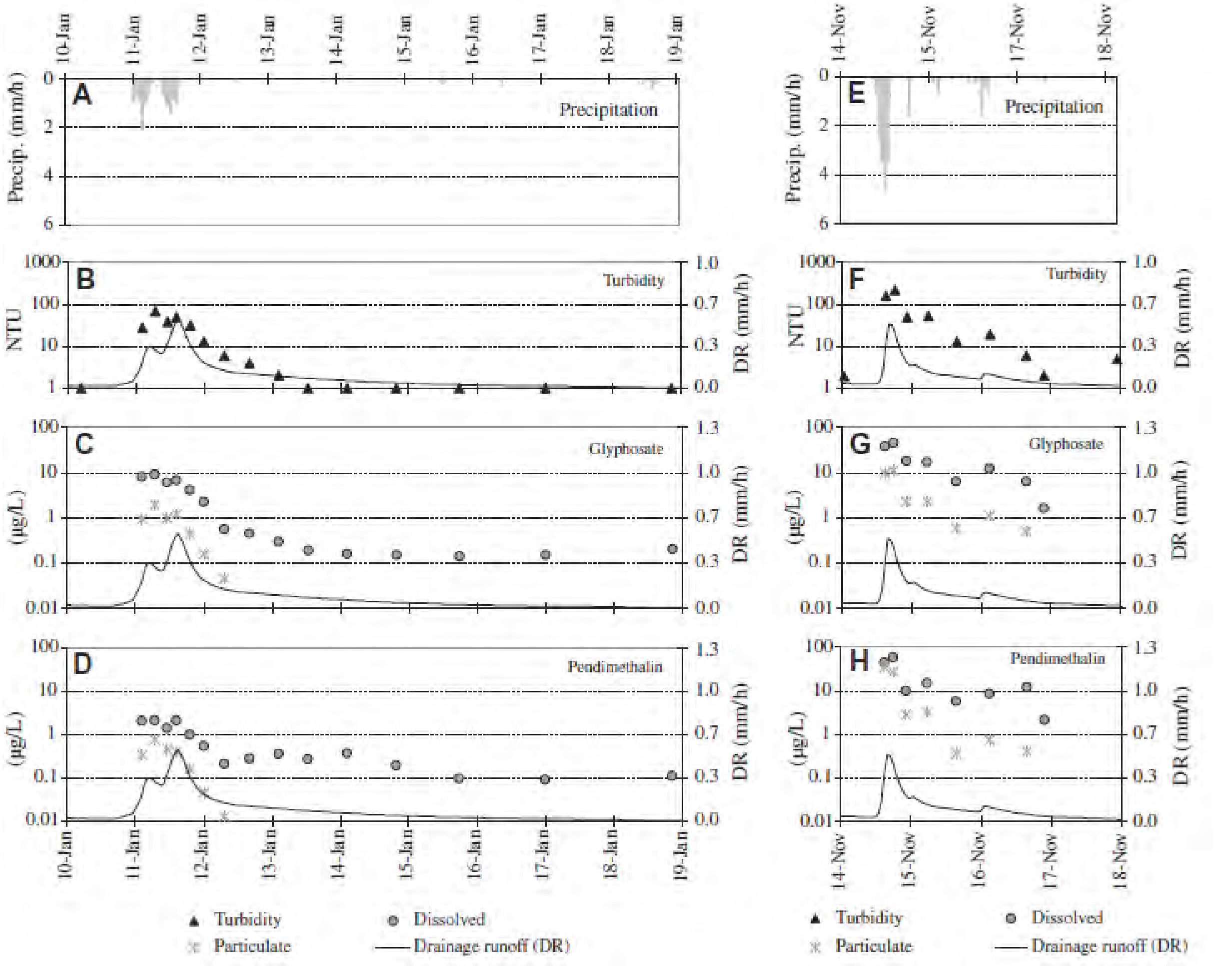
Figure 3. Measured concentration of glyphosate and pendimethalin in drainage water samples collected after pesticide application. A (left): Total concentration (both dissolved and particle–bound) in samples collected on either a weekly basis during the entire monitoring period (closed circles) or for every 2 mm of drainage runoff during a 13–d period in December 2005. B (right): Concentration of dissolved (triangles) and particle–bound (crosses) pesticide in samples collected for every 2 mm of drainage runoff during two selected flow events in November 2005 (black) and January 2006 (grey). Sampling periods are indicated in parentheses.



Quantitative impact of particle–facilitated transport on total leaching

Measured concentration of particle–bound pesticides were marked lower than that of dissolved pesticides, ratio between measured concentration of dissolved pesticides and particle–bound ranging between 4–14 and 1.2–30 for glyphosate and pendimethalin respectively. Intensive monitoring of two individual flow events (Figure 4) suggested that particle–facilitated transport (particles >0.24 µm) accounted for only a small proportion of the observed leaching (13–16 % of the leached mass of glyphosate and 16–31 % of the leached mass of pendimethalin). These values are in line with the few available field studies quantifying particle–facilitated transport of strongly sorbing pesticides. In Danish drainage water studies using a cut–off size of 0.7 µm, Petersen et al. (2003) found that 9 % of the leached pesticide was particle bound. Correspondingly, Vilholdt et al. (2000), using a cut–off size of 0.24 µm, found that 6 % of the leached pesticide was particle bound. In laboratory experiments with undisturbed 20–cm soil columns, de Jonge et al. (2000) found that particle–facilitated transport (particles >0.24 µm) accounted for <1–27 % of total glyphosate leaching. In a study by Gjettermann et al. (2009) using intact soil columns from ploughed and minimal tillage cultivation systems, colloid–facilitated glyphosate leaching (cut–off size >0.02 µm) accounted for 68 ± 10 % of total glyphosate leaching from the ploughed system as compared to only 17 ± 12 % from the minimal tillage system. That leaching of particle–bound glyphosate from the ploughed soil was markedly greater than that seen in our study and previous studies may be attributable to differences in experimental conditions, e.g. ploughing before or after pesticide application and precipitation intensity. In our field study the total amount of precipitation and maximum precipitation intensity were 12 mm within 11 h and 2.1 mm h^{-1} (11 January 2006) and 18 mm within 10 h and 4.6 mm h^{-1} (14 November 2005). In the study of Gjettermann et al. (2009) glyphosate was applied to the soil 1 d after the last of two rewettings and the soil then irrigated twice for 2 h using 15 mm h^{-1} on days 5, 8 and 12 following the last rewetting. In Denmark such high precipitation intensity is rare during the period relevant for autumn application of glyphosate (September–November). Analysis of precipitation data collected in a national grid of approximately 60 automatic climate stations run by the Danish Meteorological Institute revealed that there had only been 8 precipitation events exceeding 15 mm h^{-1} during the preceding 10 years (Birgit Sørensen, personal communication). The combination of wet, loose soil and very intensive precipitation shortly after the application of pesticide is likely to result in greater contact between pesticide and soil particles and enable greater mobilisation of soil particles. In soils having had time to consolidate, such as the minimal tillage soil studied by Gjettermann et al. (2009) and in the present study (ploughed 7 months before pesticide application) fewer particles will be available for contact with the pesticide.

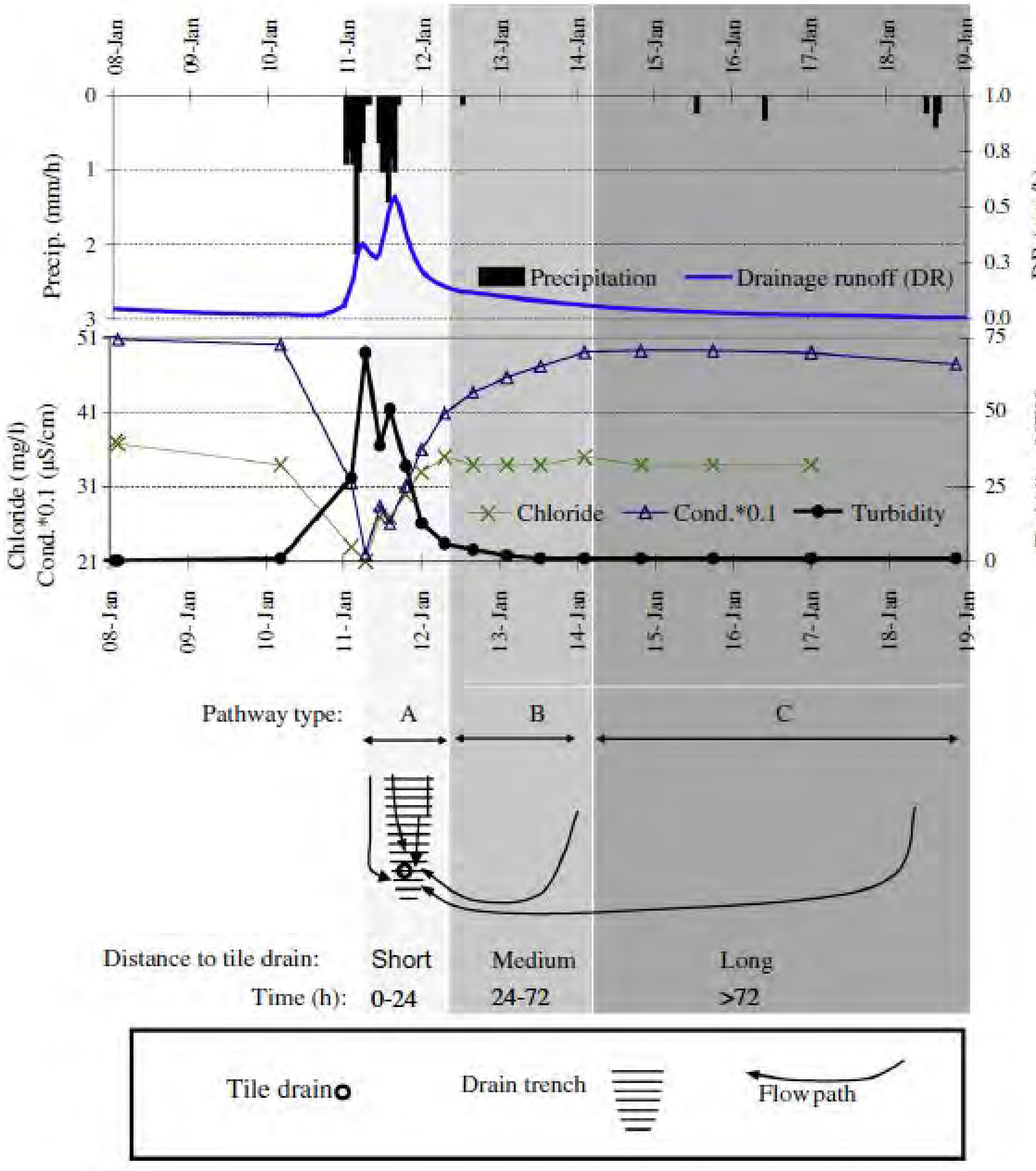
Figure 4. Hourly precipitation (grey hanging bars in A and E) together with turbidity (B and F), particulate (crosses) and dissolved (circles) glyphosate (C and G), particulate (crosses) and dissolved (circles) pendimethalin (D and H) in the drainage runoff (DR on the secondary axis) following flow events on 14 November 2005 (right) and 11 January 2006 (left).



Water flow pathways

Drainage water consists of a mixture of water of different origins, with the dominant flow pathways varying over the course of time (Jacobsen and Kjær, 2007). Knowledge of the dominant transport pathways is thus important for the interpretation of measured pesticide concentrations. The transport pathways during the flow event of 11 January 2006 are indicated by the measured turbidity, chloride concentration and conductivity (Figure 5). Thus the chloride concentration and conductivity decreased markedly during the first precipitation event, while the turbidity increased. This indicates rapid transport of precipitation with low chloride concentration and conductivity, probably through drain–connected macropores. During the 24–h period from the end of the first precipitation event the turbidity decreased, while the chloride concentration and conductivity increased. This indicates that water entered the drainage system not only from macropores connected directly to the drains but also from the vicinity of the drain pipe, i.e. the trench dug when installing the tile drain system. Transport pathways is likely also to involve a lateral component in the shallow saturated zone through natural macro pores aided by gradients generated by inter–drain mounding of the water table during the high–flow condition following the rain event (Figure 5). Water from here would have a relative short travelling distance before entering the drainage system. These transport pathways characterised by having a short flow path to the drain and being active during the first 24–h period are designated type A (Figure 5). From 24 to 72 h the turbidity remained low and below the detection level of 1 NTU (nephelometric turbidity units), the chloride concentration plateaued out and the conductivity continued to increase. That the chloride concentration returned to the ‘‘background level’’ indicates cessation of the rapid entrance of precipitation low in chloride. Instead the length of the pathway increased with drainage water entering from in between the drain trench, designated pathway B in Figure 5. During these longer transport pathways, the particles are filtered by the soil causing the turbidity to decrease below the detection limit, while the longer retention time allows the infiltrating water to interact with the soil matrix causing the conductivity to increase. By 72 h after the end of the first precipitation event the conductivity, chloride concentration and turbidity had returned to their background levels, and the drainage water is dominated by the longer transport pathways (designated type C). These transport pathways are likely to comprise precipitation that has infiltrated vertically some distance from the drain trench and subsequently been transported laterally to the drain via the saturated layer.

Figure 5. Hourly precipitation and drainage runoff together with measured chloride concentration, conductivity and turbidity (lower graph). The shaded areas indicate the dominant transport pathways (types A–C) feeding into the sampled drainage water during the flow event. While ‘‘time’’ and ‘‘pathway types’’ are classified directly from measured data, ‘‘distance to tile drain’’ and shown water flow pathways are indicative providing our interpretation of measured data. (see Section “*Through which pathways do strongly sorbing compounds enter the drainage system?*”).



Pesticide transportation pathway

During the 24–h period following cessation of the first precipitation event on 11 January 2006 the leaching pattern was similar for both particles, particle–bound pesticide and dissolved pesticide (Figure 4), thus indicating that all three follow the same transport pathways, presumably involving drain–connected macropores located above or in the vicinity of the drains or rapid lateral transport near the drain line (type A in Figure 5). Thereafter the leaching of particles and particle–bound pesticide ceased, whereas dissolved pesticide continued to leach in high concentrations (>0.1 µg/L) for up to 7 d after the precipitation had stopped (Figure 4). This ‘‘tailing’’ of dissolved pesticides indicates that the transport pathways involve transport through macropores between drains followed by lateral transport to the drains (types B and C; Figure 5). Moreover, it indicates that while particles (indicated by elevated turbidity) and particle–bound pesticide seems to be retained in the soil during the lateral transport in between the drain, dissolved pesticide can be transported laterally through the saturated zone to the drainage system. The leaching pattern following the flow event on 14 November 2005 (Figure 4) was very similar to that observed after the flow event on 11 January 2006, although sampling conditions precluded the recording of transport occurring through pathways B and C. The flow event on 11 January 2006 was characterised by high precipitation (12 mm) followed by 7 d virtually free of precipitation (1 mm in total). Such conditions are ideal for describing variation in flow pathways over time and capturing the transport involving all three pathways (A–C; Figure 5). In contrast, the flow event on 14 November 2005 was characterised by one major (18 mm on 14 November) and several minor precipitation events (6 mm in total), and sampling was performed for just 2 d (Figure 4. E–H). The conditions were ideal for descryibing transport pathway A, but inadequate for describing pathways B and C (Figure 5). The fact that turbidity remained high for a much longer period (approx. 24 h) during the November 2005 event than during the January 2006 event (Figure 4E–H) is attributable to the minor precipitation events on 16 and 17 November 2005 and resultant rapid preferential transport of leachate via pathway A (Figure 5). The direct transport from surface layers to drains via macropores (pathway A in Figure 5) reported here is in line with previous observations. Thus several studies report that the soil surface can be in direct contact with drains through macropores comprised of old root channels or earthworm burrows (Nielsen et al., 2010; Nuutinen and Butt, 2003; Shipitalo and Gibbs, 2000). The same pathways were also responsible for the leaching of colloid–size particles (Nielsen et al., 2011) and the strongly sorbing pesticides (both dissolved and particle–bound) pendimethalin (Petersen et al., 2003) and prochloraz (Vilholdt et al., 2000) on drained, loamy soils. The observed transport pathway involving transport through macropores located between drains followed by lateral transport to the drains (pathways B and C; Figure 5) is presumably attributable to connectivity between the vertical biopores and the three–dimensional fracture system in the soil, which enables rapid, lateral transport in the soil (Rosenbom et al., 2008; Nilsson et al., 2000, 2001; McKay et al., 1999). Studies of the transport of two fluorescent tracers in clayey till (Rosenbom et al., 2008) indicate that during periods of continuous drainage runoff the extent of rapid macropore transport in the soil between the drain lines is determined by the degree of connectivity between root zone biopores and high–permeability fractures. Evidence that such connectivity enables leaching of solutes from the surface of fractured till is also provided by forced gradient tracer experiments conducted at three different locations (Ringe, Avedøre and Lillebæk) in Denmark (Nilsson et al., 2000, 2001; McKay et al., 1999). These transport studies were all performed with conservative or slightly sorbing tracers (chloride, bromide, bacteriophage tracer PRD–1, colloidal tracer, sulforhodamine B, and acid yellow). Similar studies addressing the potential of pathways B and C (Figure 5) to transport strongly sorbing pesticides are very limited, however. Transport of the strongly sorbing pesticides pendimethalin and prochloraz in drained structured soil has been studied by Vilholdt et al. (2000) and Petersen et al. (2003). However, the study design, while suitable for describing vertical transport from the top soil to the vicinity of the drain line (pathways A and B), was unsuitable for describing transport involving vertical infiltration between the tile drains followed by a subsequent lateral transport to the drain (pathway C). In Vilholdt et al. (2000), pesticide sampling was performed 2.5 m either side of the drain trajectory up to 7.5 h following a precipitation event. The study of Petersen et al. (2003) was conducted on a very well drained soil with limited lateral water flow (drainage runoff accounting for only 2–10 % of total precipitation input during the sampling period) with most pesticide samples being collected within 36 h of the precipitation event. Under such conditions, lateral transport of pesticides (pathway C) is unlikely. The leaching pattern found in our study indicate that while particles (indicated by elevated turbidity) and particle–bound pesticides were retained in the soil during lateral transport, dissolved glyphosate and pendimethalin were transported through the saturated zone to the drainage system. Similar findings suggesting that dissolved, strongly sorbing pesticide can be transported over long distances via discontinuities are provided by Gooddy et al. (2007), who studied the concentration of dissolved and particle–bound diuron and its metabolites in chalk groundwater sampled 30 m b.g.s. Most of the pesticide–colloid complexes (particles >0.1 µm) formed in the soil were removed during migration of the water through the 30 m deep, unsaturated zone and/or the saturated zone, whereas pesticides in soluble form was detectable in the groundwater 30 m b.g.s. Moreover, in a study of the transport of brilliant blue, bromide and micropheres along macropores in sandy loam, Nielsen et al. (2011) found that while colloid–size particles were trapped in the bottom of the biopores, dissolved tracer (brilliant blue and bromide) migrated further into the soil.

Conclusion

Pesticides leaching from the unsaturated zone may eventually pose a risk to the aquatic environment. The present 8–month study of a loamy field demonstrates that:

Strongly bound pesticides, whether bound to the organic or inorganic soil fraction, may leach from the root zone and enter the aquatic environment in average concentrations exceeding 0.1 µg/L. Particle–facilitated transport (particles >0.24 µm) accounted for only a small proportion of observed leaching (13–16 % for glyphosate and 16–31 % for pendimethalin). The pathway by which these strongly sorbing compounds entered the drainage system involved transport through drain–connected macropores (above or in the vicinity of the drains) as well as the macropores situated between the drains and connected to underlying fractures. Particle–bound pesticide (particles >0.24 µm) was transported solely by vertical transport in macropores and rapid lateral transport occurring nearby the drain line, whereas dissolved pesticide was also transported laterally over larger distances through the saturated zone via discontinuities in the soil. This newly identified transport pathway whereby dissolved pesticides are transported laterally via discontinuities in the soil needs to be taken into account when assessing the risk posed by pesticides to the aquatic environment. Our findings imply that strongly sorbed pesticides such as glyphosate and pendimethalin may be present in high concentrations (>0.1 µg/L) in both the water flowing from the drainage system and in the shallow groundwater located at the depth of the drainage system.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The study describes a leaching experiment with glyphosate and pendimethalin in a Danish agricultural soil over eight months. The substance properties are sufficiently reported. With regard to the data requirement, the study is too short for a comprehensive evaluation of the leaching behavior. In addition, no residues were determined in different soil layers after finalization of the study, and sample storage stability prior to analysis was not established.

The study is therefore classified as reliable with restrictions (Category 2).

1. Information on the study

Data point:	KCA 7.1.3.1.1
Report author	Munira, S., et al.
Report year	2016
Report title	Phosphate fertilizer impacts on glyphosate sorption by soil
Document No	Chemosphere 153 (2016) 471-477
Guidelines followed in study	OECD 106 (2000)
Deviations from current test guideline	None
GLP/Officially recognised testing facilities	No, not conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Reliable with restrictions (no European climate and soil conditions, validity check for study could not finalized)

2. Full summary of the study according to OECD format

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 and from products containing 0.4, 70 or 210 mg Cd/kg as an impurity. A series of batch equilibrium experiments were carried out to quantify the glyphosate sorption distribution constant, Kd. Extractable Cd concentrations in soil had no significant effect on glyphosate sorption. Glyphosate Kd values significantly decreased with increasing Olsen-P concentrations in soil, regardless of the pH conditions studied. Experiments repeated with a commercially available glyphosate formulation showed statistically similar results as the experiments performed with analytical-grade glyphosate. Co-applications of MAP with glyphosate also reduced the available sorption sites to retain glyphosate, but less so when soils already contain large amounts of phosphate. Glyphosate Kd values in soils ranged from 173 to 939 L/kg under very strong to strongly acidic condition but the Kd was always <100 L/kg 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.

Materials and Methods

Experimental design and soil characteristics

Soil samples (0 -15 cm) with a sandy clay loam texture were collected in the spring 2013 from research plots situated under a durum wheat and flax rotation near Carman, Manitoba, Canada. The soil is classified as an Orthic Black Chernozem. The experimental plot was a randomized complete block design with 10 treatments and 4 replicates per treatment. In each of the forty plots, the composite soil sample consisted of ten samples collected in the plot using a Dutch augur. Treatments were a control (neither phosphate nor Cd applications), and plots receiving from 2002 to 2009 annual applications of mono ammonium phosphate (MAP) fertilizers that originated from three different phosphate rock sources containing 0.4, 70 or 210 mg Cd/kg, 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, or 20P, 40P and 80P, respectively. For all plots that received MAP, 20 kg P/ha 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 treatments, to avoid seedling toxicity, the additional MAP was broadcasted and then incorporated in soil. From 2010 to 2013, the rotation was continued but no phosphate or Cd was applied. Nitrogen fertilizer varied by year to optimize yields. The typical rate of N applied was 90 kg N/ha in durum wheat and 50 kg N/ha in flax. 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). Extractable Cd was extracted with diethylene triamine pentaacetic acid (DTPA) ICP. Various factors have been shown to influence the efficiency of micronutrient extraction by DTPA, including extraction temperature and shaking time. Available phosphate was extracted using Olsen ($NaHCO_3$) phosphorus test. Soil physical and chemical properties that are known to influence glyphosate and phosphate sorption by soil, but did not significant vary across the plots by treatment, were also determined. Soil organic carbon content was determined using combustion technique with a high temperature induction furnace. Extractable Fe_2O_3 and Al_2O_3 were extracted with DTPA and 0.01 M $CaCl_2$, respectively, and extracts were analyzed by ICP. Extractable Ca was also measured by ICP using ammonium acetate as an extractant. 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 (n = 40), extractable Al_2O_3: 6.4 ± 0.65 mg/kg (n = 16); and extractable Ca: 2252 ± 40.57 mg/kg (n = 16). Given that the study focused on Cd and P applications as treatments, the concentrations of extractable and total Cd, as well as Olsen-P in all plots were determined. We did not expect to see treatment differences for the other parameters that were measured (i.e., extractable Fe_2O_3, Al_2O_3, and Ca). Fe_2O_3 was also measured in all plots as previous studies have demonstrated that there is a strong positive association between Fe_2O_3 concentrations and phosphate or glyphosate sorption in soils. Since our results indicated no treatment differences induced by Cd and P applications on Fe_2O_3 concentrations extractable Al_2O_3, and Ca were quantified for 16 plots only (i.e., Control, 20P, 40P and 80P plots).

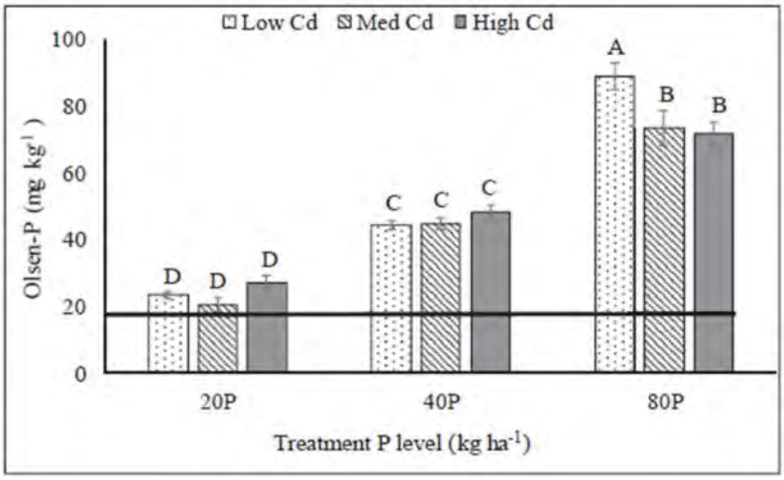


Fig. 1. Effect of phosphate fertilizers with different Cd levels on Olsen P concentrations in soil. The solid line indicates the concentration of Olsen P in control plots.

Sorption studies

Chemicals used in the sorption studies were: analytical grade ammonium phosphate monobasic (98% chemical purity) and glyphosate (99.9% purity), ^{14}C-labelled glyphosate [phosphonomethyl-^{14}C] (99% radiochemical purity; specific activity 50 µCi), and Roundup Ultra 2 (49% active). Active ingredient was potassium salt of N-(phosphonomethyl) glycine. Glyphosate sorption was determined by batch equilibrium with the initial solution containing 1 mg/L glyphosate and 6.67 x 10^4 Bq/L ^{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 was added to soil in centrifuge Teflon tubes (duplicates) and slurries were rotated in the dark for 24 h. A constant 5 ^{0}C temperature was utilized to minimize risks for biodegradation. Equilibrium solution was centrifuged and subsamples of supernatant were added in duplicated scintillation vials containing Scintisafe scintillation cocktail. 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). The glyphosate sorption distribution constant, Kd (L/kg) was calculated by Cs/Ce, whereby Cs = glyphosate sorption by soil at equilibrium (mg/kg), and Ce = glyphosate concentration of equilibrium solution (mg/L). 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_2O). For the control and high Cd 80P plots, the experiments were repeated but then using the Tier 2 parallel method with tubes being sampled at 1, 2, 4, 6, 8 and 24 h. The two subsequent experiments utilized soils from the plots labelled as low Cd and with 20P, 40P or 80P levels. In one experiment, for slurry pH conditions ranging from 3.6 to 7.3, batch equilibriums procedures were repeated but using Roundup Ultra 2 in 0.01 M HCl, 0.01 M $CaCl_2$, 0.01 M KCl, 0.01 M KOH or dH_2O to verify experimental results for a formulated product. In the other experiment, for slurry pH conditions range from 4.7 to 5.4, the effect of fresh phosphate additions on glyphosate sorption by soil was examined by adding analytical grade MAP to analytical glyphosate in 0.01 M $CaCl_2$, 0.01 M KCl and dH_2O solutions. The amounts of MAP added was equivalent to 11, 22 and 44 mg P/kg, or an estimated 20, 40 and 80 P kg/ha, respectively, assuming the fertilizer being present in the top 15 cm layer of a soil with a bulk density of 1200 kg/m^3.

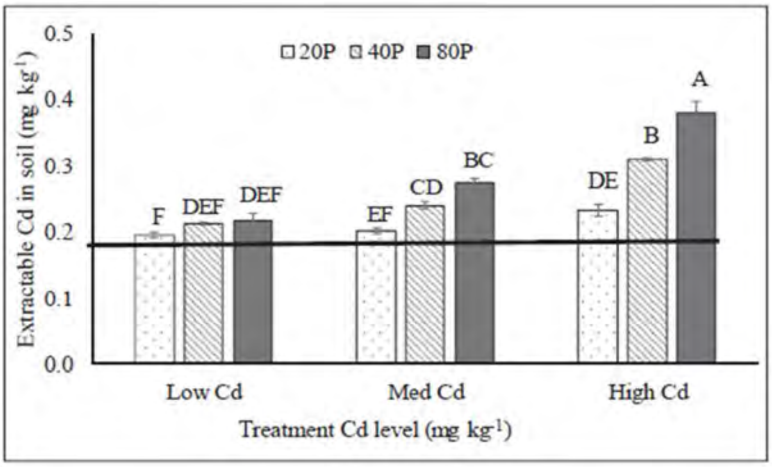


Fig. 2. Effect of phosphate fertilizers with different Cd levels on DTPA-extractable Cd in soil. The solid line indicates the concentration of extractable Cd in control plots.

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

Results and Discussion

Glyphosate Kd values ranged from 43 to 1173 L/kg which is in agreement with glyphosate Kd values reported in agricultural soils. There were no significant differences in glyphosate sorption by soil when using either Roundup Ultra 2 or analytical-grade glyphosate, suggesting that other ingredients in the commercial formulation had no impact on the sorption behaviour of the active ingredient glyphosate in soil. The additions of MAP fertilizers from 2002 to 2009 had a significant effect on phosphate concentrations in 2013 (Fig. 1). Olsen-P concentrations ranged from 13 to 99 mg/kg across plots and significantly decreased from 80P > 40 P > 20P plots. Total Cd concentrations in soil ranged from 0.42 to 0.98 mg/kg across plots but there were no significant treatment effects. Thus, the amount of Cd in the MAP fertilizers applied had no significant effect on the total Cd concentrations in 2013. DTPA-extractable Cd concentration ranged from 0.19 to 0.41 mg/kg, within the typical range of 0.1 - 0.5 mg/kg 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 (Fig. 2). For the 80P plots, extractable Cd concentrations significantly decreased in the order of high Cd > med Cd > low Cd. For the 40P plots, extractable Cd concentrations significantly decreased in the order of high Cd > (med Cd = low Cd). In 20P plots, only the high and low Cd treatments had significantly different extractable Cd concentrations. Despite these significant differences, extractable Cd concentrations in soil had no significant influence on glyphosate Kd values. The Cd concentrations in our field plots are those typically encountered in agricultural soils, but we recognize that in a batch equilibrium experiment, Zhou et al. (2004) demonstrated that the co-application of exceptionally large quantities of Cd to glyphosate solutions (i.e., 562 mg Cd/kg soil) can increase glyphosate sorption by approximately 1.6 times fold, relative to control soil. 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.

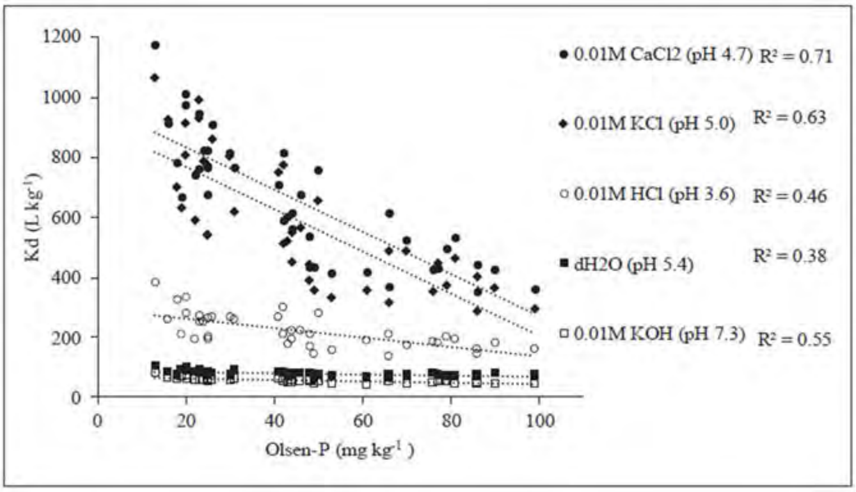


Fig. 3. Relation between Olsen-P concentrations in soil and the glyphosate sorption distribution constant, Kd, with soil slurries being under different pH conditions. All regression equations are significant at $P < 0.0001$

Regardless of the ionic solution used in the batch equilibrium experiments, increased Olsen P concentrations significantly decreased glyphosate sorption by soil (Fig. 3). A maximum reduction in glyphosate sorption occurred at a pH of 5 (0.01 M KCl solution) when the Olsen-P concentrations was on average 89 mg/kg Olsen P and the glyphosate Kd value was reduced by 57%, relative to the control plots that contained on average 18.75 mg/kg Olsen-P (Fig. 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. The iron oxides content of the Orthic Black Chernozem used is within the range of that observed in other Prairie soils in Canada suggesting the competitive effect of phosphate on glyphosate sorption could be applicable to a wider range of soils in the Prairie region of Canada particularly with low pH and high Fe content. At pH 5.4, in both 80P and control, time had no significant effect on glyphosate Kd values and sorption was always significantly smaller in 80P than control plots. For all other pH conditions, glyphosate sorption approached equilibrium at approximately 8 h because there were no significant differences in glyphosate Kd values between 8 and 24 h (Fig. 4). For these pH conditions, glyphosate sorption was almost always significantly smaller in 80P than control plots, regardless of the time, except for 0.5, 1 and 2 h under pH 3.6 and 0.5 h under pH 5.0 (Fig. 4). In general, longer shaking hours resulted in greater numerically differences in glyphosate Kd values between control and 80P plots. Regardless of the ionic solution used (Fig. 5), there was a significant interaction ($P < 0.01$) between field-aged and fresh phosphate on glyphosate sorption. In general, regardless of the amount of aged phosphate in soil, the addition of fresh MAP to the ionic solutions numerically reduced glyphosate Kd values, suggesting that phosphate and glyphosate compete for the same sorption sites in soil and that phosphate is preferentially sorbed when added with glyphosate to soil. Additions of 11 mg P/kg to the 0.01 M $CaCl_2$ solutions had no significant effect on glyphosate Kd values, except in the 20 P plots containing relatively small Olsen-P concentrations (Fig. 5). The addition of 22 or 44 mg P/kg to the 0.01 M $CaCl_2$ solutions always significantly reduced glyphosate Kd values, except the addition of 22 mg P/kg to 80 P plots (Fig. 5). For the largest co-application (44 mg P/kg), 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 to 0.01 M KCl solutions always significantly reduced glyphosate Kd values except for 80 P plots for which only the addition of 44 mg P/kg resulted in a significant reduction in glyphosate Kd values (Fig. 5).

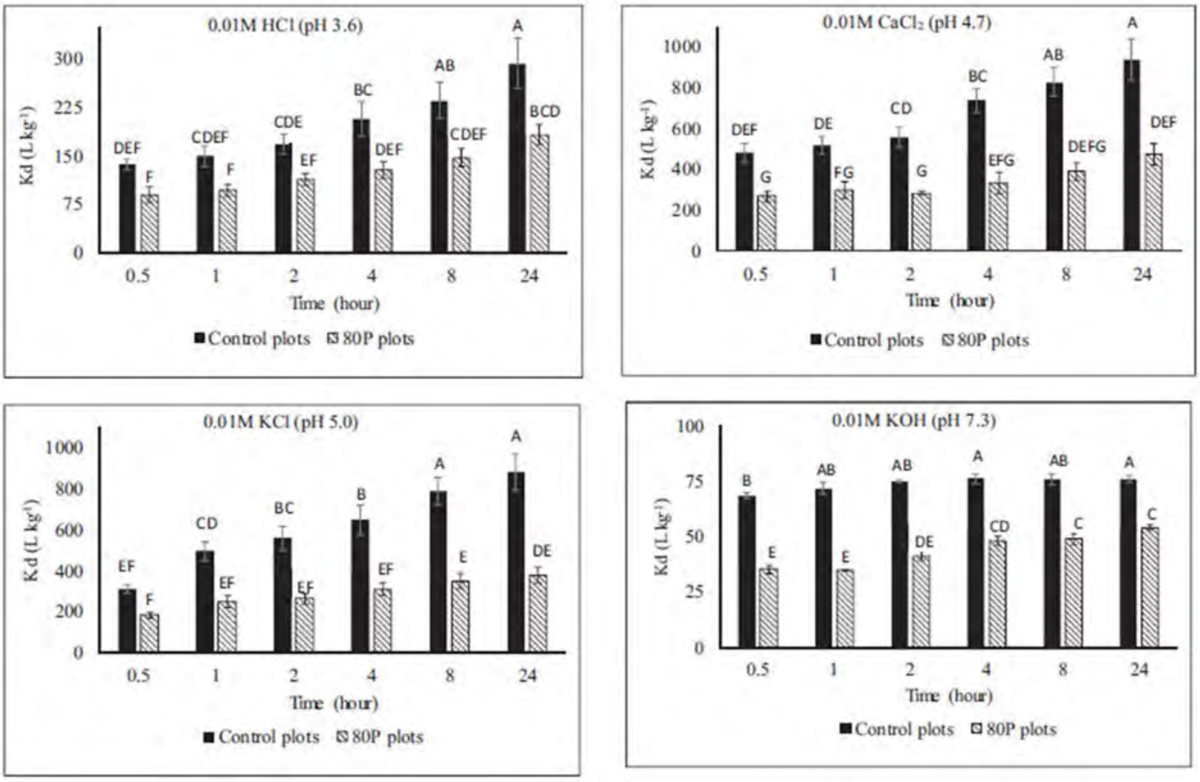


Fig. 4. Time dependent sorption study of glyphosate Kd values in control and 80P plots.

For the 44 mg P/kg 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 (0.01 M $CaCl_2$) 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%). The soil used in this study had already a relatively large Ca^{2+} content (2252 ± 40.57 mg kg^{-1}), and using 0.01 M KCl, would allow K^+ to replace Ca^{2+} on the exchange site of organic-clay complexes which may interact with glyphosate forming stable complexes. Glyphosate Kd values were greater at pH 3.6 (0.01 M HCl), than pH 5.4 (dH_2O) (Fig. 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%). 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 $H2G^-$ (~60%) and $HG2^-$ (~40%) at pH 5.4. The lowest sorption was observed at pH 7.3 (0.01 M KOH), as the negatively charged soil colloids increased and glyphosate molecules existed as $HG2^-$ (~100%).

Conclusion

Analytical-grade glyphosate showed similar results as a commercially-available glyphosate formulation. Long-term additions of phosphate fertilizers to soils will reduce the capacity of the soil to bind glyphosate under a wide range of pH conditions, but the impurities of Cd in these fertilizers have no impact on glyphosate sorption.

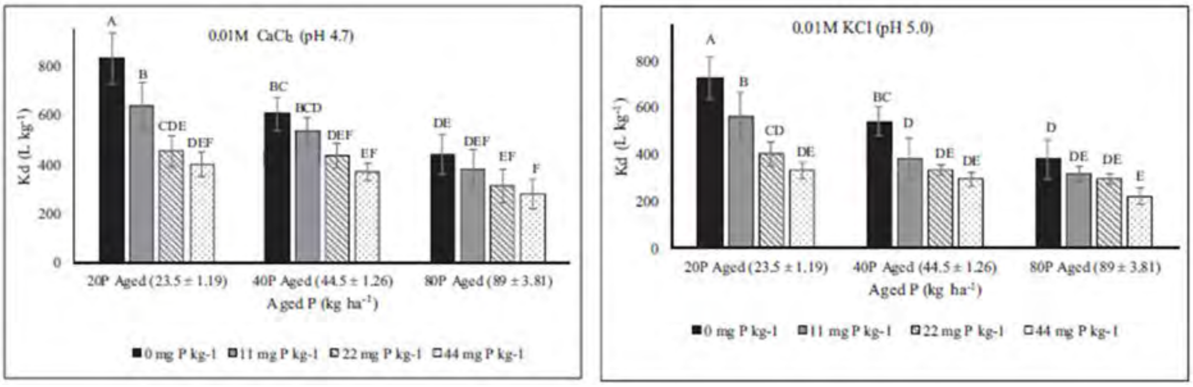


Fig. 5. Effect of co-applying mono ammonium phosphate with glyphosate in solution, for batch equilibrium experiments using 0.01 M $CaCl_2$ (pH 4.7) and 0.01 M KCl (pH 5.0).

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.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study describes a sorption experiment with glyphosate on a Canadian agricultural soil considering different treatments with phosphate fertilizer. Some information on soil and study design are not reported (soil characteristics, mass balances, amount of soil), so no final validity check is possible. The temperature of the experiment was set to 5°C.
The study is therefore classified as reliable with restrictions (Category 2).

1. Information on the study

Data point:	KCA 7.1.3.1.1
Report author	Sirajum Munira & Annemieke Farenhorst
Report year	2017
Report title	Sorption and desorption of glyphosate, MCPA and tetracycline and their mixtures in soil as influenced by phosphate
Document No	Journal of Environmental Science and Health, Part B 2017, VOL. 0, NO. 0, 1–9
Guidelines followed in study	OECD 106 (2000)
Deviations from current test guideline	Temperature: 5°C, 0.01 M KCl
GLP/Officially recognised testing facilities	No, not conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Reliable with restrictions (not valid against current guidelines)

2. Full summary of the study according to OECD format

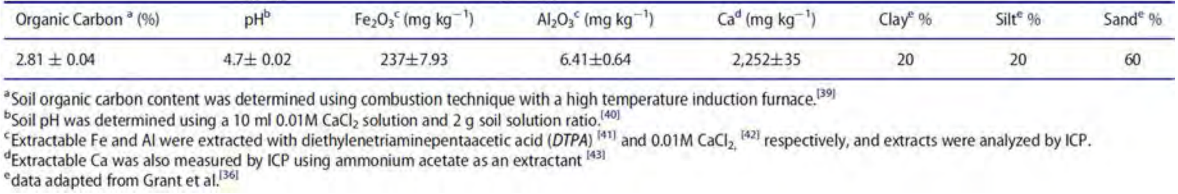
Phosphate fertilizers and herbicides such as glyphosate and MCPA are commonly applied to agricultural land, and antibiotics such as tetracycline have been detected in soils following the application of livestock manures and biosolids to agricultural land. Utilizing a range of batch equilibrium experiments, 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) and elevated (81 to 99 mg/kg) 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 was 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. The presence of chemical mixtures in soil solution has an overall greater impact on the sorption than desorption of individual organic chemicals in soil.

Materials and Methods

Chemicals

Analytical grade glyphosate (99.9 %), MCPA (99 %), tetracycline (98 %), potassium dihydrogen phosphate (KH_2PO_4), (99 %) and potassium chloride (100 %) were obtained commercially. Radioactive [phosphonomethyl–^{14}C] glyphosate (99 %; specific activity 50 mCi/mmol), [2–methyl–4–chlorophenoxyacetic acid ^{14}C] MCPA (98 %; specific activity 55 mCi/mmol) and [7–^{3}H (N)] tetracycline (98 % radiochemical purity; specific activity 20 Ci/mmol) were obtained commercially.

Table 1. Selected soil physical and chemical properties as mean with standard error.



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] %
2.81 ± 0.04	4.7± 0.02	237±7.93	6.41±0.64	2,252±35	20	20	60

[a] Soil organic carbon content was determined using combustion technique with a high temperature induction furnace.[39]
[b] Soil pH was determined using a 10 ml 0.01M $CaCl_2$ solution and 2 g soil solution ratio.[40]
[c] Extractable Fe and Al were extracted with diethylenetriaminepentaacetic acid (*DTPA*) [41] and 0.01M $CaCl_2$, [42] respectively, and extracts were analyzed by ICP.
[d] Extractable Ca was also measured by ICP using ammonium acetate as an extractant [43]
[e] data adapted from Grant et al.[36]

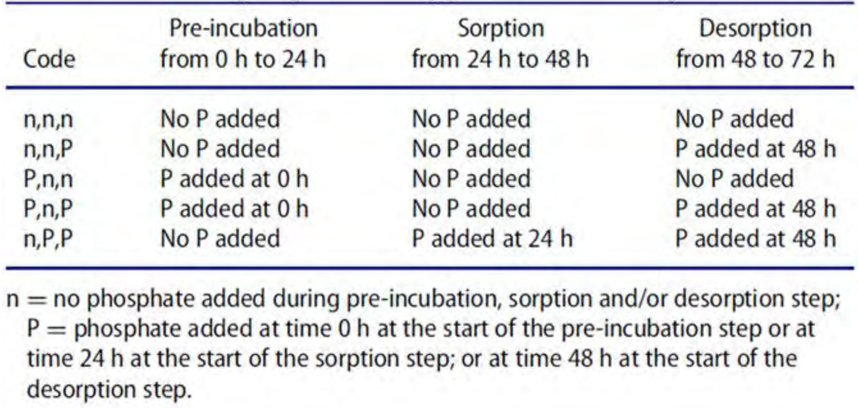
Soil characteristics and experimental design

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, 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, 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, as well as from control plots that did not receive MAP application during these years. 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. 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. Key soil properties are listed Table 1.

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 with air–dried soil (2 g) and a soil/solution ratio of 1:5 with 0.01 M KCl as the background electrolyte Soil slurries were rotated in the dark at 5°C from 0 to 24 h (pre–incubation), from 24 to 48 h (sorption) and from 48 to 72 h (desorption) with phosphate added at 0 h, 24 h and/or 48 h, or never added, depending on the treatment (Table 2). Radiolabelled chemical solutions contained 1 mg/L analytical–grade glyphosate, MCPA or tetracycline, with 6.67×10^5 Bq/L ^{14}C–labelled glyphosate, 3.83×10^5 Bq/L ^{14}C–labelled MCPA or 4.17×10^5 Bq/L ^3H–labelled tetracycline, respectively. The concentration 1 mg/L represented environmentally–relevant concentrations of herbicides and antibiotics detected in agricultural soils or animal manure. At 48 h, tubes were centrifuged at 10,000 rev/min 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). The sorption distribution constant, Kd (L/kg), 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) and Ce is the concentration of the organic chemical in the equilibrium solution (mg/L). 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) 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.

Table 2. Addition of phosphate during pre–incubation, sorption and desorption steps.



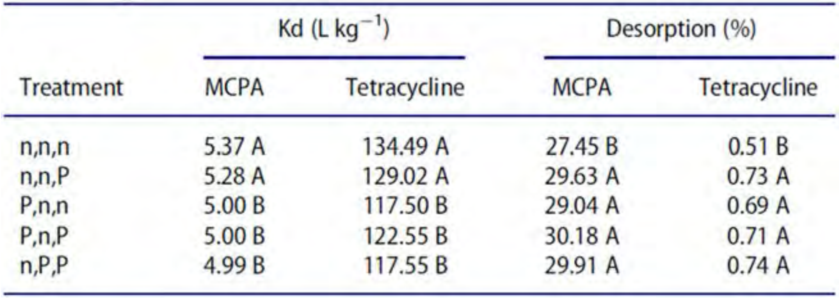
Code	Pre-incubation from 0 h to 24 h	Sorption from 24 h to 48 h	Desorption from 48 to 72 h
n,n,n	No P added	No P added	No P added
n,n,P	No P added	No P added	P added at 48 h
P,n,n	P added at 0 h	No P added	No P added
P,n,P	P added at 0 h	No P added	P added at 48 h
n,P,P	No P added	P added at 24 h	P added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

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

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 2 above and also added to soil (at 0 h) were MCPA, tetracycline (Tetra), or their mixtures (M/T). MCPA, Tetra, and M/T were added at concentrations of 1 or 11 mg/L. The glyphosate solution was always added at 24 h and contained 1 mg/L analytical–grade glyphosate with 6.67 x 10^5 Bq/L ^{14}C–labelled glyphosate.

Table 3. Effect of phosphate fertilizer on MCPA and tetracycline sorption and desorption in soil. See Table 2 for an explanation of the treatment labels.



	Kd (L kg^{-1})		Desorption (%)	
Treatment	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

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.

Effect of the pre–sorbed MCPA on glyphosate sorption

Experiments followed similar protocols as described for the pre–sorbed phosphate above. The glyphosate solution contained 1 mg/L analytical–grade glyphosate with 6.67 x 10^5 Bq/L ^{14}C–labelled glyphosate.

Statistical analysis

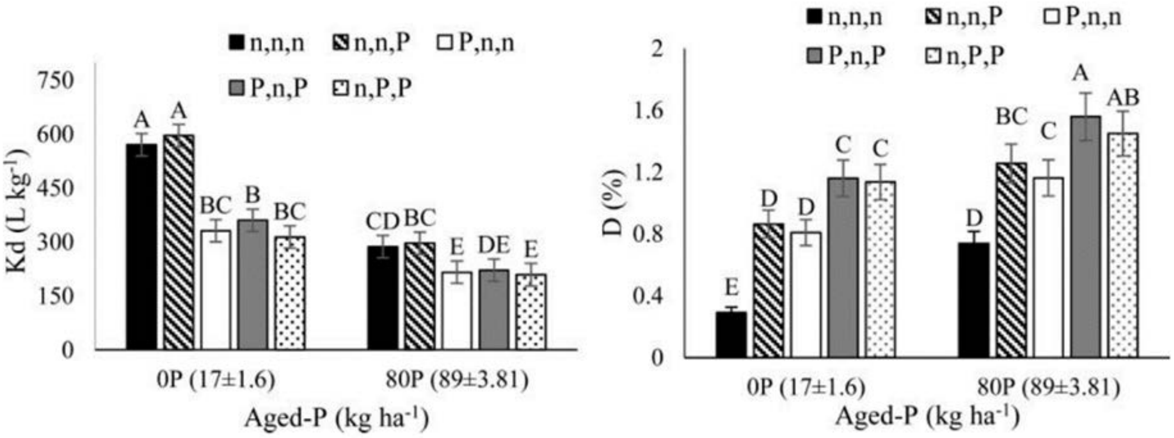
Statistical analyses were carried out using SAS software version 9.4 for Windows. Prior to each analysis, data sets were checked for outliers, normality of residuals and homogeneity of variances. Residuals were normally distributed and variances were homogeneous. For the Kd values, data were analyzed by using normal distribution and for the % desorption by beta distribution. Two–way ANOVA in PROC GLIMMIX was used to quantify the effect of field aged–P (0P, 80P) and fresh–P addition (0, 11 mg/L) on Kd values and % desorption of MCPA, tetracycline, and glyphosate in soil. One–way ANOVA in PROC GLIMMIX was utilized to determine the effect of retained phosphate in soil on glyphosate, MCPA and tetracycline sorption, and of retained MCPA in soil on glyphosate sorption. Both in the presence and absence of fresh phosphate, two–way ANOVAs in PROC GLIMMIX were carried out to quantify the effect of field aged–P (0P, 80P) and of the concentrations (0, 1, 11 mg/L) of MCPA, tetracycline, or MCPA–tetracycline mixtures on glyphosate Kd values. For fresh phosphate added at

48 h only, or at both 0 h and 48 h, and in the absence of fresh phosphate, two way ANOVAs in PROC GLIMMIX were carried out to quantify the effect of field aged–P (0P, 80P) and of the concentration (0, 1, 11 mg/L) of MCPA, tetracycline, or MCPA–tetracycline mixtures on the percent of glyphosate desorbed. For all ANOVAs, the separation of treatment means was performed using the Tukey's test ($p < 0.05$).

Results

Kd values on average ranged from 209 to 596 L/kg for glyphosate (Figure 1), from 118 to 135 L/kg for tetracycline, and from 4.99 to 5.37 L/kg for MCPA (Table 3). Koc values ranged from 6105 to 25,496 L/kg for glyphosate, from 3,928 to 4,901 L/kg for tetracycline, and from 156 to 209 L/kg for MCPA. These results are within the ranges observed in previous studies of the sorption of glyphosate, tetracycline and MCPA in soils. Glyphosate (<2 %) (Figure 1) and tetracycline (<1 %) desorption was always small but MCPA desorption ranged from 26 to 31 % (Table 3). Phosphate significantly reduced glyphosate sorption in soil (Figure 1). Without laboratory-added phosphate, glyphosate Kd values were 50 % smaller in soil containing 81 to 99 mg/kg Olsen P than in soil containing 13 to 20 mg/kg 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). When phosphate was added to soil solution at either 0 h or 24 h, 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 P/kg, and by 23–27 % in field soils containing 81 to 99 mg P/kg (Figure 1).

Figure 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 2 for labels and details).



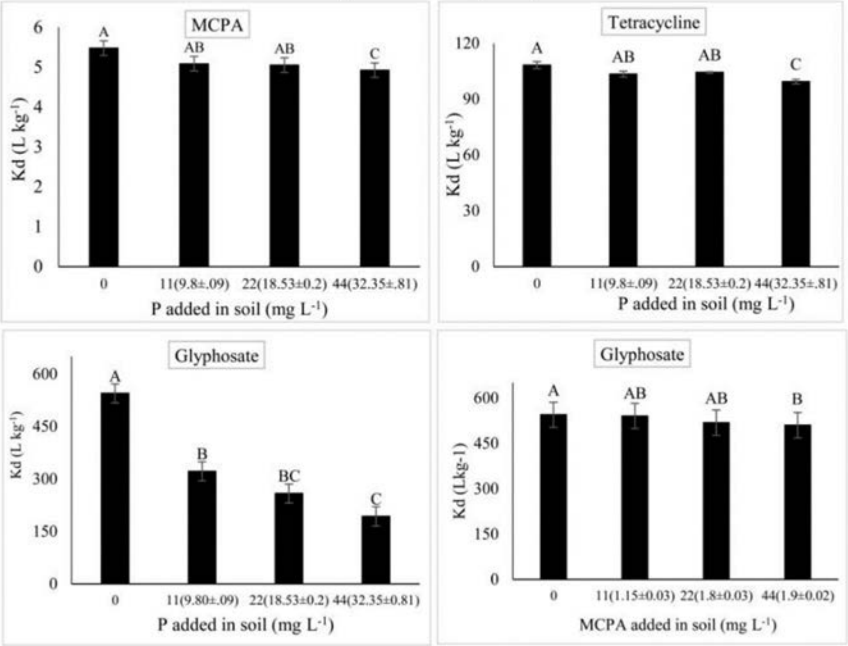
In the presorbed phosphate experiment, the soil retained 9.8, 18.5 and 32.4 mg P/kg for the additions of 11, 22, 44 mg P/L respectively, and glyphosate sorption was significantly reduced by 41 % (11 mg P/L), 52 % (22 mg P/L) and 65 % (44 mg P/L) (Figure 2).The amount of field aged–P in soil had no significant impact on MCPA and tetracycline sorption in soil. However, fresh phosphate added to soil solution significantly reduced tetracycline Kd values by 8–13 % and MCPA Kd values by 7–8 % (Table 3). The competitive effect of phosphate on MCPA and tetracycline sorption was not dependent on when the phosphate was added in the laboratory (either 0 h or 24 h) (Table 3). 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 (Table 5, or Figure 2). However, there was no impact on MCPA or tetracycline sorption when phosphate additions were 11 or 22 mg P/L. Glyphosate desorption was significantly greater in field soils containing 81 to 99 mg/kg Olsen P (0.74 %) than in soils containing 13 to 20 mg/kg Olsen P (0.29 %) (Figure 1). Regardless of whether MCPA, tetracycline or MCPA/tetracycline mixture were added to soils in the laboratory, field aged–P always significantly increased glyphosate desorption. Fresh phosphate additions at 0 h, 24 h or/and 48 h to soil solutions in the laboratory also significantly increased glyphosate desorption by 0.52–0.84 % in soils containing 13 to 20 mg/kg Olsen P and by 0.52–0.82 % in field soils containing 81 to 99 mg/kg Olsen P (Figure 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 2–3 % and tetracycline by 0.18–0.23 % (Table 3).

Table 4. Effect of MCPA (0, 1, 11 mg/L), tetracycline (0, 1, 11 mg/L) and MCPA/tetracycline mixtures (0, 1, 11 mg/L) on sorption and desorption of glyphosate in soil in the presence and absence of phosphate.

		No P		P at 48 h		P at 0 h and 48 h	
Chemicals	Concentration (mg L^{-1})	Kd (Lkg^{-1})	D (%)	Kd (Lkg^{-1})	D (%)	Kd (Lkg^{-1})	D (%)
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

The competitive effect of phosphate on MCPA, tetracycline and glyphosate desorption was not dependent when phosphate was added to soil solution (either at 0 h, 24 h or 48 h). The number of times that phosphate was added had no significant effect on MCPA and tetracycline desorption (Table 3). 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 1). MCPA and MCPA/tetracycline mixtures added at 11 mg/L significantly reduced glyphosate Kd values and increased glyphosate desorption, but only when no phosphate was added to the soil solution (Figure 3, Table 4). MCPA and MCPA/tetracycline mixtures added at 1 mg/L had no significant effect on glyphosate sorption and desorption (Table 4). 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, and whether or not phosphate was added to soil solution (Table 4). 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 3) and glyphosate desorption by 0.1 % (Figure 3). In the pre–sorbed MCPA experiment, the addition of 11, 22, 44 mg MCPA/L the soil retained 1.2, 1.8 and 1.9 mg MCPA/kg, respectively. The pre–sorbed MCPA significantly reduced glyphosate sorption by 6 % for the addition of MCPA at 44 mg/L, but there was no impact on glyphosate sorption when additions were at 11 or 22 mg/L (Table 5, Table 3 S, or Figure 2).

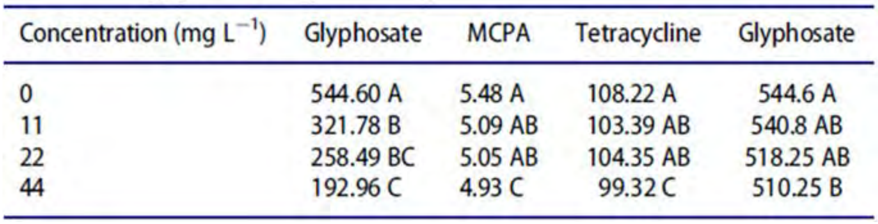
Figure 2. Effect of pre–sorbed phosphate concentrations on MCPA, tetracycline and glyphosate sorption, and of pre–sorbed MCPA concentrations on glyphosate sorption in soil. Numbers on x–axis in parenthesis refer to mean (± standard error) of measured pre–sorbed phosphate and MCPA.



Discussion

The addition of phosphate at either 0 h or 24 h yielded the same impact on glyphosate sorption (Figure 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 shown to compete for the same sorption sites in soil. Application of phosphate with glyphosate in solution reduced glyphosate sorption because phosphate is preferentially sorbed over glyphosate by available sorption sites. Glyphosate Kd values were significantly smaller 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 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. 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 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. Under the experimental conditions with the soil slurries being at a pH 5, the molecules of MCPA (pKa D 3.73) are predominantly negatively–charged.

Table 5. Effect of pre–sorbed phosphate (0, 11, 22, 44 mg/L) on glyphosate, MCPA and tetracycline sorption and pre–sorbed MCPA on glyphosate sorption (L/kg) in soil.

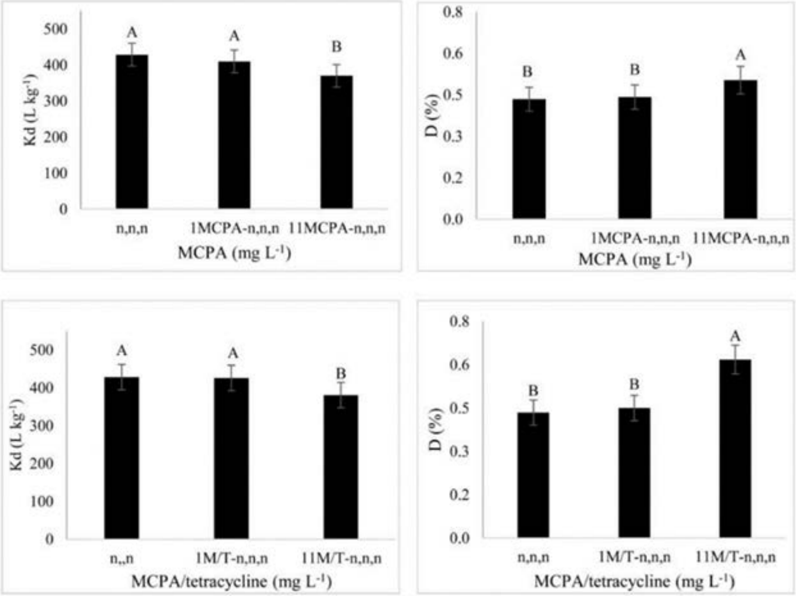


Concentration (mg L^{-1})	Glyphosate	MCPA	Tetracycline	Glyphosate
0	544.60 A	5.48 A	108.22 A	544.6 A
11	321.78 B	5.09 AB	103.39 AB	540.8 AB
22	258.49 BC	5.05 AB	104.35 AB	518.25 AB
44	192.96 C	4.93 C	99.32 C	510.25 B

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. However, MCPA (i.e., carboxyl and phenyl groups) and tetracycline (i.e., tricarbonylamide carbonyl, amine and hydroxyl groups) have other functional groups and sorption sites for MCPA and tetracycline can include under moderately acidic conditions humic substances and clay minerals in addition to Fe/Al–oxides in soils. MCPA had no longer 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). Therefore, it is possible that phosphate is preferentially sorbed over glyphosate and MCPA. 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 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, respectively. It has been reported that organic molecules are considered relatively mobile when Koc value ranges from 150 to 500 L/kg. Thus, given these Koc values, MCPA is relatively mobile in soil because it is only weakly retained, unlike glyphosate and tetracycline. 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.

Figure 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 2 for labels and details).



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 and desorption less than 2 %. In contrast, MCPA was weakly retained in soil with Kd values <6 L/kg 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.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The article describes an OECD 106 experiment with glyphosate on a Canadian soil considering the influence of phosphate additions. The article shows some deviations from the validity criteria for EU guidelines (Temperature, usage of 0.01 M KCl instead of 0.01 M $CaCl_2$).

The article is therefore classified as reliable with restrictions (Category 2).

1. Information on the study

Data point:	KCA 7.1.3.1.1
Report author	Munira, S., et al.
Report year	2018
Report title	Phosphate and glyphosate sorption in soils following long-term phosphate applications
Document No	Geoderma 313 (2018) 146–153
Guidelines followed in study	OECD 106 (2000)
Deviations from current test guideline	No
GLP/Officially recognised testing facilities	No, not conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Reliable with restrictions (No all validity criteria met for OECD 106, e.g. Temperature)

2. Full summary of the study according to OECD format

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 (K_f) 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 in the SCL-Fe_2O_3 but only 99 to 141 L/kg in the CL-$CaCO_3$. Glyphosate K_d and phosphate K_f values decreased significantly with increasing Olsen P concentrations in both soils. Glyphosate K_d values were further significantly reduced when phosphate was added to the slurry solutions, but phosphate K_f 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.

Methods

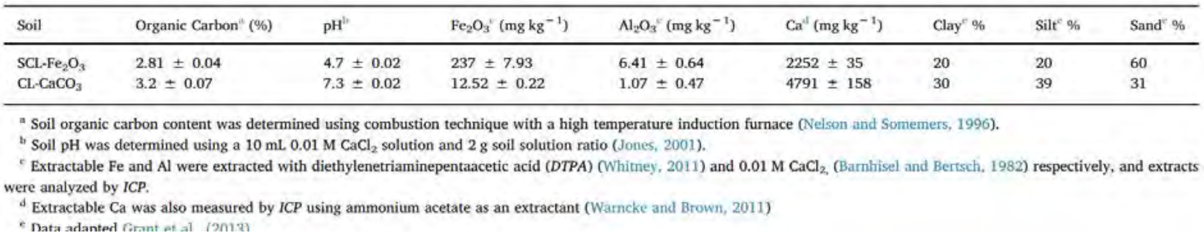
Chemicals

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, dehydrate (> 95% chemical purity) from Fisher Scientific, Fair Lawn, NJ.

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. 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 or 20P, 40P, and 80P, respectively, from 2002 to 2009. For all plots that received mono ammonium phosphate, 20 kg P/ha 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 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 and flax 50 kg N/ha· 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_2O_3), whereas the Forrest soil has a clay loam texture and is relatively high in calcium carbonates (CL- $CaCO_3$) (Table 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 0.5 N $NaHCO_3$ solution was mixed in a 50 mL Erlenmeyer flask. Flasks (duplicates) were shaken horizontally (200 excursions/min). Equilibrium solutions were filtered through Whatman No. 2 filter paper and phosphate concentrations were determined colorimetrically.

Table 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 0.01 M $CaCl_2$ solution and 2 g soil solution ratio (Jones, 2001).

[c] Extractable Fe and Al were extracted with diethylenetriaminepentaacetic acid (*DTPA*) (Whitney, 2011) and 0.01 M $CaCl_2$, (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 Grant et al., (2013)

Phosphate sorption

Phosphate sorption was determined by batch equilibrium using either 0.01 M $CaCl_2$ or 0.01 M KCl as the background electrolyte. Batch equilibrium procedures followed standard protocols using a soil/solution ratio of 1:10 and an equilibrium time of 24 h. 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.

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 were added to air-dried soil (2 g) in 50-mL centrifuge tubes (duplicates) and shaken horizontally (120 excursions/min) at room temperature (23 ± 2 °C) for 24 h. Equilibrium solution was centrifuged (6100 G for 10 min) and filtered (0.45 µm). Phosphate concentration was determined colorimetrically by the molybdate blue method. Linearized Freundlich isotherm has been specified as: The phosphate sorption coefficient, K_f ($L^{1/n}$ $mg^{1-1/n}$ kg^{-1}), was calculated using the linearized form of Freundlich equation: $\log q = \log K_f + 1/n \log C$. Where q represents phosphate sorption in soil at equilibrium (mg/kg), C represents phosphate concentration of equilibrium solution (mg/L), 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. EPCo levels above 0.025 mg/L suggest an increased risk of eutrophication because of P transport in soluble form.

Effect of glyphosate formulation on sorption of phosphate

In the second experiment, stock solutions of 150 mg P/L were prepared with and without 100 mg/L Roundup Ultra2 in the solution. The 100 mg/L Roundup Ultra 2 was equivalent to 378 mg glyphosate/kg soil. The 150 mg P/L 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. Batch equilibrium procedures were carried out as described above. The phosphate sorption coefficient, Kd (L/kg), was calculated by q/C, where q represents phosphate sorption by soil at equilibrium (mg/kg) and C represents phosphate concentration of equilibrium solution (mg/L).

Glyphosate sorption

Glyphosate sorption was determined by batch equilibrium with the initial glyphosate solution containing 1 mg/L analytical-grade glyphosate and 6.67 × 104 Bq/L ^{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.

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 24 h and 0.01 M $CaCl_2$ or 0.01 M KCl as background electrolyte. 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 24 h. Equilibrium solution was centrifuged (6100 G 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), was calculated by Cs/Ce, where Cs represents glyphosate sorption by soil at equilibrium (mg/kg) and Ce represents glyphosate con- centration of equilibrium solution (mg/L). The difference between the added radioactivity and radioactivity in the supernatant was assumed to be the proportion of glyphosate having been sorbed.

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 soil, or an estimated 20, 40 and 80 P kg/ha, respectively, when assuming the fertilizer being present in the top 15-cm layer of a soil with a bulk density of 1200 kg/m^3.

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 sets were checked for outliers, normality of residuals and homogeneity of variances. Residuals were normally distributed and variances were homogeneous. The paired *t*-test ($P < 0.05$) was used to test for the effect of background electrolyte solution (0.01 M $CaCl_2$ versus 0.01 M KCl) on glyphosate K_d or phosphate K_f and EPCo. For both background electrolyte solutions and at each site, simple linear regression analyses ($P < 0.05$) were carried out to estimate glyphosate K_d and phosphate K_f values using Olsen P concentration as the independent variable. In each of the glyphosate K_d and phosphate K_f figures, the slopes of regression lines developed for SCL-Fe_2O_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 K_d values by using the added fresh phosphate concentration as an 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 K_d and phosphate K_f values by using K_f 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

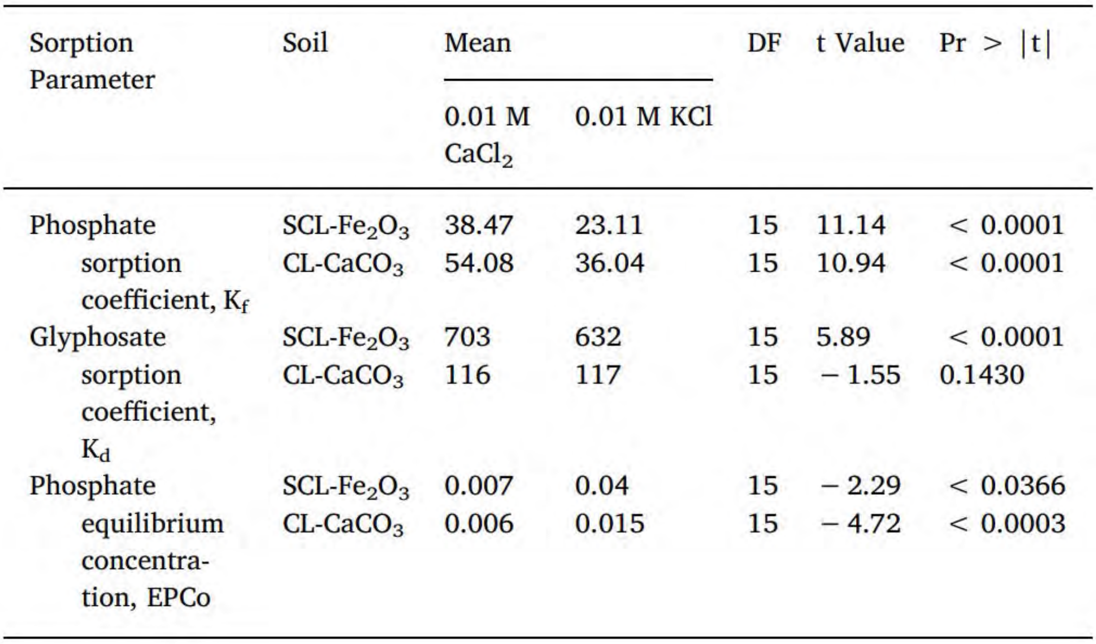
EPCo as a function of Olsen P showed that data did not fit well with simple linear regression for the SCL-Fe_2O_3 soil.

Results

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 2). Phosphate K_f values in both soils were significantly greater in experiments with 0.01 M $CaCl_2$ than experiments with 0.01 M KCl (Table 2).

Table 2. Statistical parameters (Paired t-tests) on the effect of background electrolyte solution (0.01 M $CaCl_2$ versus 0.01 M KCl) on glyphosate (L/kg) and phosphate sorption coefficient ($L^{1/n}$ mg $^{1-1/n}$ kg^{-1}) in soils.



Sorption Parameter	Soil	Mean		DF	t Value	Pr > |t|
		0.01 M $CaCl_2$	0.01 M KCl			
Phosphate sorption coefficient, K_f	SCL-Fe_2O_3	38.47	23.11	15	11.14	< 0.0001
	CL-$CaCO_3$	54.08	36.04	15	10.94	< 0.0001
Glyphosate sorption coefficient, K_d	SCL-Fe_2O_3	703	632	15	5.89	< 0.0001
	CL-$CaCO_3$	116	117	15	− 1.55	0.1430
Phosphate equilibrium concentration, EPCo	SCL-Fe_2O_3	0.007	0.04	15	− 2.29	< 0.0366
	CL-$CaCO_3$	0.006	0.015	15	− 4.72	< 0.0003

Phosphate K_f 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 L1/n mg $^{1-1/n}$ kg^{-1} in CL-$CaCO_3$ and 23 $L^{1/n}$ mg $^{1-1/n}$ kg^{-1} SCL-Fe_2O_3 with KCl. Thus, when 0.01 M $CaCl_2$ 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. In batch equilibrium experiments with 0.01 M $CaCl_2$, precipitation with Ca^{2+} occurs more readily for phosphate than glyphosate. For glyphosate sorption, K_d values were on average 116 L/kg in CL-$CaCO_3$ and 703 L/kg SCL- Fe_2O_3 with $CaCl_2$, and on average 117 L/kg in CL-$CaCO_3$ and 632 L/kg 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 and, because of the already high free calcium content in the CL-$CaCO_3$ soil, the addition of Ca with 0.01 M $CaCl_2$ solution had no impact on glyphosate sorption. For the SCL-Fe_2O_3 soil, glyphosate sorption was greater with 0.01 M $CaCl_2$ than 0.01 M KCl, suggesting that glyphosate was able to form complexes with Ca^{2+} in solution for enhanced sorption.

Effect of field-aged phosphate concentrations on sorption of phosphate

Despite being exposed to similar long-term phosphate fertilizer treatments, Olsen P ranged from 13 to 99 mg/kg in the acidic SCL-Fe_2O_3 soil but only from 8 to 48 mg/kg 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 in the SCL- Fe_2O_3 soil and 13 (control), 18 (20P), 24 (40P) and 41 (80P) mg/kg 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, such as the SCL-Fe_2O_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 1 M HCl. Olsen P concentrations ranged from 8 to 99 mg/kg in this research which is within the typical range of 8 to 114 mg/kg that has been reported for soils in North America. 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 K_f values significantly decreased with the increasing concentrations of Olsen P in soil (Fig. 1).

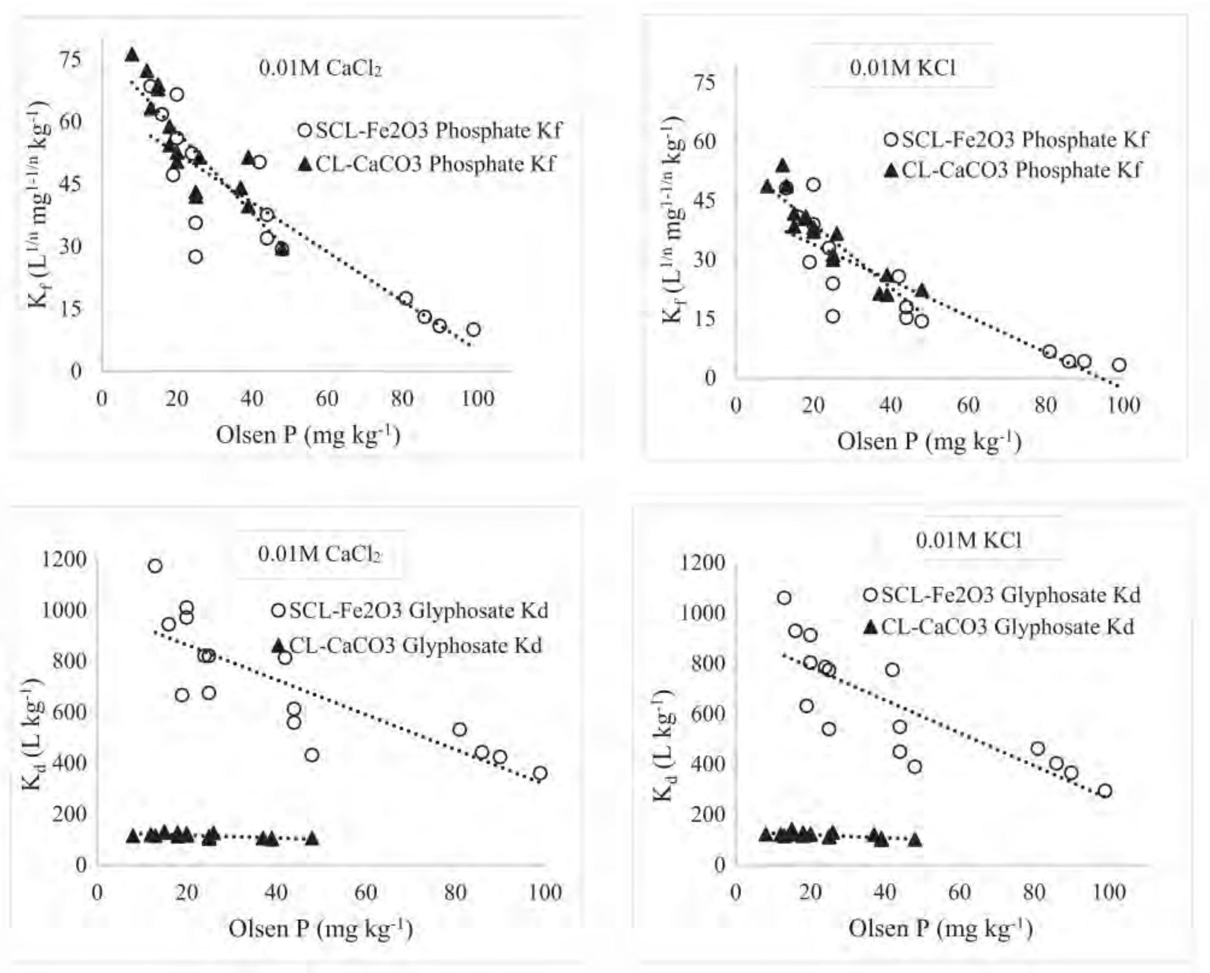


Fig. 1. Effect of Olsen P concentrations in soil on glyphosate and phosphate sorption in SCL-Fe_2O_3 and CL-$CaCO_3$ soils, as determined by batch equilibrium experiments using 0.01 M $CaCl_2$ or 0.01 M KCl as background electrolyte solutions. All regression equations are significant at $P < 0.05$.

The SCL- Fe_2O_3 and CL-$CaCO_3$ soils showed relatively similar phosphate sorption (Fig. 1). Phosphate K_f 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 and 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 and 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 K_f value was observed in SCL- Fe_2O_3 soil. The phosphate K_f value in SCL- Fe_2O_3 was reduced by 95% in soil containing 99 mg/kg Olsen P relative to soil containing 13 mg/kg Olsen P. Thus, P accumulation in soil reduced the capacity of soil to hold Wang et al. (2015) also reported that sorption of P decreased with the in- creasing concentrations of Olsen P because long-term application of P fertilizer leads to the accumulation of P in soil. In their study, they showed that long-term (5 to 15 years) application of phosphate significantly reduced phosphate sorption by 56% in soil containing 53 mg/kg Olsen P relative to soil containing 15 mg/kg Olsen P. Olsen P concentrations significantly predicted phosphate K_f (Fig. 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-$CaCO_3$. For the phosphate K_f, the regression slopes were significantly different between the soils in case of 0.01 M KCl but not with 0.01 M $CaCl_2$ because the presence of Ca in solution led to the possibility of precipitation of phosphate-Ca^{2+} complexes in both soils. Generally, in calcareous soil, Ca forms precipitation with the added phosphate in soil solution. For 0.01 M KCl, the CL- $CaCO_3$ showed a significantly steeper slope than SCL- Fe_2O_3 (Fig. 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.

EPCo significantly increased with increasing concentrations of Olsen P in both SCL- Fe_2O_3 and CL-$CaCO_3$ (Fig. 2). EPCo values ranged from 0 to 0.281 mg/L, depending on the background electrolyte solution and soil (Fig. 2). EPCo values in both soils were significantly greater in the experiments with 0.01 M KCl than experiments with 0.01 M $CaCl_2$ (Table 2) because of the formation of Ca^{2+}-phosphate complexes in both soils with 0.01 M $CaCl_2$. All EPCo levels were below the threshold value of 0.025 mg/L except in the 80P plots. The average calculated EPCo values for the four replicated 80P plots was 0.031 mg/L for CL-$CaCO_3$ and 0.190 mg/L for SCL- Fe_2O_3 with 0.01 M KCl, and 0.025 mg/L for SCL- Fe_2O_3 with 0.01 M $CaCl_2$. 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. Phosphate can be transported from the agricultural soil when phosphate fertilizer is applied in excess of crop requirements and also from plant residues during snow melt.

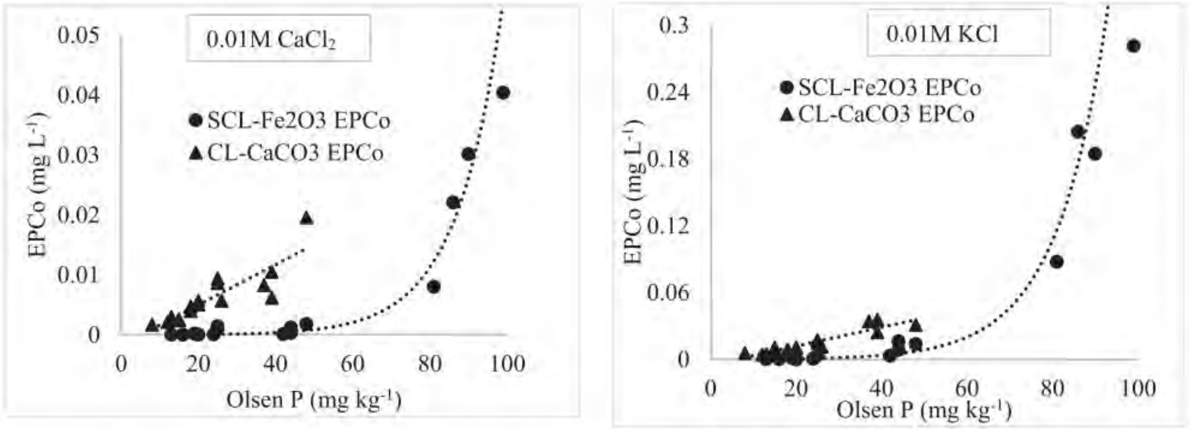


Fig. 2. Effect of Olsen P concentrations in soil on the phosphate equilibrium concentration, (EPCo) in SCL-Fe_2O_3 and CL-$CaCO_3$ soils determined by batch equilibrium experiments using 0.01 M $CaCl_2$ or 0.01 M KCl as background electrolyte solutions. Olsen P All regression equations are significant at $P < 0.05$.

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. Gimsing and Borggaard (2001) also found that, when glyphosate was added following phosphate additions to goethite, glyphosate did not displace the sorbed phosphate. In a recent article that was published in the magazine "No-Till Farmer", a statement was made that "20-25 percent of the dissolved reactive phosphorus in runoff is caused by glyphosate [use]" because of the assumption that glyphosate residues in soil decreases phosphate retention in soil. However, in our batch-equilibrium study that utilized very high rates of Roundup Ultra2, there was no significant difference in phosphate sorption between treatments with and without Roundup Ultra2 additions to soil slurries. Thus, given our findings, the recent concerns stated in Barrera (2016) are unlikely to be applicable to the Prairie soils that were included in our studies.

Effect of field-aged phosphate on sorption of glyphosate

Glyphosate K_d values significantly decreased with the increasing concentrations of Olsen P in both SCL-Fe_2O_3 and CL-$CaCO_3$ (Fig. 1). Glyphosate K_d values ranged from 293 to 1173 L/kg in the acidic SCL-Fe_2O_3 soil and from only 99 to 141 L/kg in the calcareous CL- $CaCO_3$ soil (Fig. 1), and these values are within the range of other studies (Farenhorst et al., 2008; Kumari et al., 2016; Sørensen et al., 2006). Long-term application of phosphate fertilizer in soil reduced glyphosate sorption because pre-sorbed phosphate occupied the sorption sites that would otherwise be available to glyphosate. A maximum reduction in glyphosate K_d value was observed in SCL- Fe_2O_3 soil. The K_d value was reduced by 75% in soil containing 99 mg/kg Olsen P relative to soil containing 13 mg/kg Olsen P in SCL- Fe_2O_3. Thus, results indicate 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 Olsen P relative to soil containing 6 mg/kg Olsen P.

Olsen P concentrations significantly predicted glyphosate K_d (Fig. 1) in both SCL- Fe_2O_3 and CL-$CaCO_3$. With both 0.01 M $CaCl_2$ and 0.01 M KCl, the slopes of the regressions predicting glyphosate K_d were significantly different between soils with the SCL- Fe_2O_3 showing steeper slopes than CL-$CaCO_3$ (Fig. 1). Regardless of the solution used, the sorption of glyphosate was greater in SCL- Fe_2O_3 than CL-$CaCO_3$ be- cause of the importance of Fe_2O_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. The SCL- Fe_2O_3 soil contained 94% more Fe-oxides and 83% more Al-oxides than the CL-$CaCO_3$ soil (Table 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. 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. 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%) (net negative charge of glyphosate is 2^-) at pH 7.3–7.5, and soil colloid deprotonation increases with soil colloids having a net negative charge in Prairie soils when soil pH > 6. Hence, regardless of the background electrolyte solutions, the sorption of glyphosate was always relatively low in the CL-$CaCO_3$ soil (Fig. 1). Thus, the effect of Olsen P concentrations on reducing glyphosate sorption was more pronounced for SCL-Fe_2O_3 than CL-$CaCO_3$. For example, with 0.01 M KCl, glyphosate K_d was reduced by 39% when the phosphate concentration increased from 17 mg/kg (control) to 44 mg/kg (40P plots) in SCL-Fe_2O_3 but by only 11% when the phosphate concentration increased from 13 mg/kg (control) to 41 mg/kg (80P plots) in CL-$CaCO_3$.

Association between glyphosate K_d and phosphate K_f in relation to field-aged phosphate

Phosphate K_f and glyphosate K_d values were positively correlated (Fig. 3). Thus, agreeing with previous studies suggesting phosphate and glyphosate have similar sorption pattern in soil. However, regardless of the background electrolyte solution, phosphate K_f and glyphosate K_d were more strongly correlated in SCL-Fe_2O_3 than CL-$CaCO_3$. 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. Thus, long-term application of phosphate fertilizer has an overall greater impact on reducing phosphate sorption than glyphosate sorption.

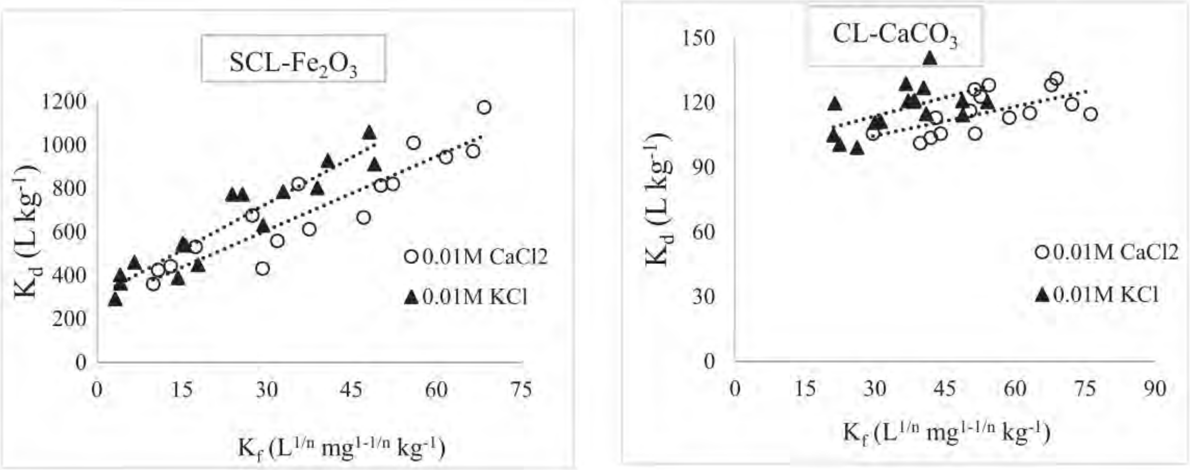


Fig. 3. Association between glyphosate K_d and Phosphate K_f in SCL-Fe_2O_3 and CL-$CaCO_3$ soils with sorption being determined by batch equilibrium experiments using 0.01 M $CaCl_2$ or 0.01 M KCl as background electrolyte solutions. All regression equations are significant at $P < 0.05$.

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 K_d values (Fig. 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 (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.

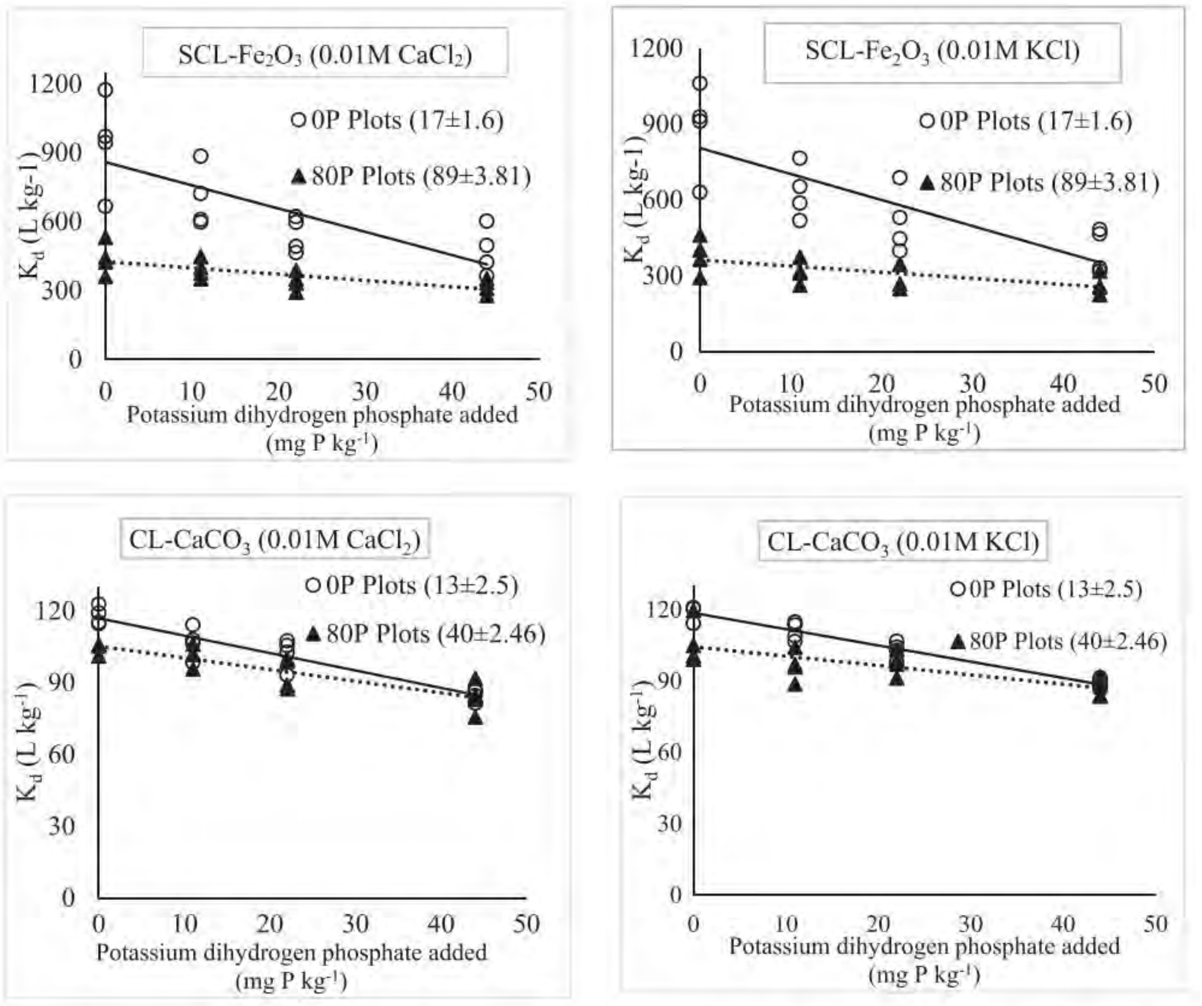


Fig. 4. Effect of potassium dihydrogen phosphate concentrations on glyphosate sorption in SCL-Fe_2O_3 and CL-$CaCO_3$ soils with low (0P) or high (80P) Olsen P concentrations. Potassium dihydrogen phosphate was added to glyphosate in soil sluries during batch equilibrium experiments using 0.01 M $CaCl_2$ and 0.01 M KCl. All regression equations are significant at $P < 0.05$. The values in parentheses in each legend represent mean values of Olsen P and standard error.

Fresh phosphate significantly predicted glyphosate K_d (Fig. 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 (Fig. 4). Thus, the effect of potassium dihydrogen phosphate addition in reducing glyphosate K_d 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-$CaCO_3$ because in CL-$CaCO_3$ soil at pH 7.3–7.5, glyphosate molecule existed as HG^{2-} (~ 100%) leading to less sorption, both in the presence and absence of fresh phosphate. 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.

Conclusion

The sorption of phosphate and glyphosate was reduced due to the long-term addition 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 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. Assessment and conclusion

Assessment and conclusion by applicant:

The study describes a sorption experiment with phosphate and glyphosate to Canadian agricultural soils. Some validation criteria of the underlying OECD 106 study protocol were not met, or insufficient information is reported.

The study is therefore classified as reliable with restrictions (Category 2).

1. Information on the study

Data point:	KCA 7.1.4.2
Report author	Marco Napoli; Stefano Cecchi; Camillo A. Zanchi; Simone Orlandini
Report year	2015
Report title	Leaching of Glyphosate and Aminomethylphosphonic Acid through Silty Clay Soil Columns under Outdoor Conditions
Document No	Journal of Environmental Quality 44:1667–1673 (2015)
Guidelines followed in study	None
Deviations from current test guideline	None
GLP/Officially recognised testing facilities	No, not conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Reliable with restrictions (Not sufficiently described to check validity against current guideline)

2. Full summary of the study according to OECD format

Glyphosate [*N*-(phosphono-methyl)-glycine] is the main herbicide used in the Chianti vineyards. Considering the pollution risk of the water table and that the vineyard tile drain may deliver this pollutant into nearby streams, the objective of the present study was to estimate the leaching losses of glyphosate under natural rainfall conditions in a silty clay soil in the Chianti area. The leaching of glyphosate and its metabolite (aminomethylphosphonic acid [AMPA]) through soils was studied in 1-m-deep soil columns under outdoor conditions over a 3-yr period. Glyphosate was detected in the leachates for up to 26 d after treatments at concentrations ranging between 0.5 and 13.5 µg/L. The final peak (0.28 µg/L) appeared in the leachates approximately 319 d after the first annual treatment. Aminomethylphosphonic acid first appeared (21.3 µg/L) in the soil leachate 6.8 d after the first annual treatment. Aminomethylphosphonic acid detection frequency and measured concentration in the leachates were more than that observed for the glyphosate. Aminomethylphosphonic acid was detected in 20% of the soil leachates at concentrations ranging from 1 to 24.9 µg/L. No extractable glyphosate was detected in the soil profile. However, the AMPA content in the lowest layer ranged from 13.4 to 21.1 mg/kg, and on the surface layer, it ranged from 86.7 to 94 mg/ kg. Overall, these results indicate that both glyphosate and AMPA leaching through a 1-m soil column may be potential groundwater contaminants.

Materials and methods

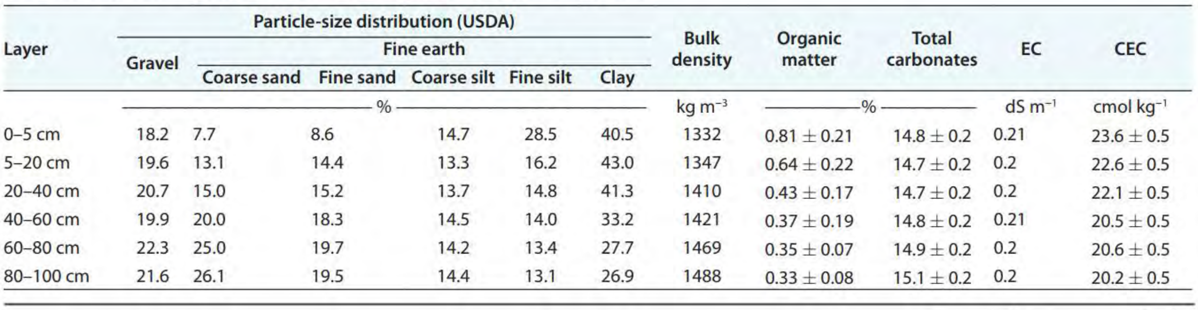
Borate buffer (0.05 M) was prepared by dissolving 1.9 g disodium-tetraborate-decahydrate in 100 mL ultra-pure water. The FMOC-Cl solutions (1 g/L) were prepared by dissolving 10 mg FMOC-C1 in 10 mL acetonitrile. Glyphosate and AMPA working standard (30 µg/L) were prepared by dissolving glyphosate and AMPA in ultra-pure water. Working standards were stored at 4°C for no more than 1 wk.

In summer 2006, three lysimeters were installed at a lysimeter station in Montepaldi, San Casciano Val di Pesa, Tuscany, Italy. Each lysimeter consisted of a cube-shape casing (1-m edge) made of 4-mm-thick stainless steel sheet. At the bottom end of each lysimeter, a polyethylene corrugated drainage pipe was installed to collect the leachate. During the summer of 2006, the containers were filled with a silty clay soil collected from a nearby Chianti vineyard that had been mechanically weeded over the previous 3 yr. The soil was taken from the 0- to 100-cm layer of three randomly selected vine interrows. The soil was then taken and placed in the lysimeter, taking care to maintain the profile's natural order of layers.
During the monitoring period, hourly temperature and rainfall data were measured by a meteorological station located 300 m from the experimental site. The annual mean temperature and precipitation at the study site were 14.6°C and 914 mm/yr, respectively.

The commercial formulations of glyphosate (360 g/ L a.i.) were applied in the study area at a dose of 2 L/ha per application. There were one to two spring applications along each vine row, covering a strip of ~1 m. This implies that along the treated strip, the concentration of the active ingredient ranged from 70 to 150 mg/m^2 depending on the number of spring applications. For the lysimeter study, the concentration data associated with the two spring applications was modeled. Therefore, in the middle of March and in the middle of May, glyphosate was applied to each lysimeters .An aqueous solution of herbicide was sprayed onto the surface of the soils to simulate an application rate of 0.72 kg/ha a.i.

Drainage water was collected after each rainfall event from 1 Mar. 2007 to 28 Feb. 2010. To ensure limited degradation, leachate volumes were determined gravimetrically and then preserved in the dark at −20°C for a maximum of 25 d until analysis. On 26 Feb. 2007 and then at the end of each year (i.e., the last week of February), the soil was sampled in triplicate for each of the lysimeters, which were separated into six layers (0–5, 5–20, 20–40, 40–60, 60–80, and 80–100 cm), air-dried, weighed, and sieved. The chemical and physical analyses were performed on air-dried, 2-mm fractions taken from each layers. The soil characteristics are listed in Table 1.

Table 1. Principal chemical and physical properties of study soil; organic matter and carbonates in percentage of the weight of the 2-mm sieved soil; soil electric conductivity (EC) and cation-exchange capacity (CEC) are reported.



Layer	Particle-size distribution (USDA)						Bulk density	Organic matter	Total carbonates	EC	CEC
	Gravel	Fine earth									
		Coarse sand	Fine sand	Coarse silt	Fine silt	Clay					
	%						kg m^{-3}	%		dS m^{-1}	cmol kg^{-1}
0–5 cm	18.2	7.7	8.6	14.7	28.5	40.5	1332	0.81 ± 0.21	14.8 ± 0.2	0.21	23.6 ± 0.5
5–20 cm	19.6	13.1	14.4	13.3	16.2	43.0	1347	0.64 ± 0.22	14.7 ± 0.2	0.2	22.6 ± 0.5
20–40 cm	20.7	15.0	15.2	13.7	14.8	41.3	1410	0.43 ± 0.17	14.7 ± 0.2	0.2	22.1 ± 0.5
40–60 cm	19.9	20.0	18.3	14.5	14.0	33.2	1421	0.37 ± 0.19	14.8 ± 0.2	0.21	20.5 ± 0.5
60–80 cm	22.3	25.0	19.7	14.2	13.4	27.7	1469	0.35 ± 0.07	14.9 ± 0.2	0.2	20.6 ± 0.5
80–100 cm	21.6	26.1	19.5	14.4	13.1	26.9	1488	0.33 ± 0.08	15.1 ± 0.2	0.2	20.2 ± 0.5

Water samples were filtered through 1-mm glass-fiber filters. The liquid was immediately derivatized. The herbicide residues in the sediment, along with the residues in the soil samples, were extracted first by ultrasonic extraction in methanol after which the derivatization procedure was used. To reduce the sorption of glyphosate and AMPA from the methanol-extracted solutions onto glassware surfaces, water and soil samples were dispensed in parallel into plastic vials. Methanol (50 mL) was added to soil samples (50 mg) that had been dried and sieved. The soil suspension was mixed for 60 min and then left at 20°C for 24 h to allow complete solvent evaporation. Then, 15 g of soil was added to 40 mL of solvent and sonicated at 30 to 40 kHz for 30 min. Extracts were filtered through Whatman 40 filter paper, and the filtrate was evaporated on a rotary vacuum evaporator at 40°C to dryness. The residue of herbicide extract was dissolved in 5.0 mL of water and then collected in plastic vials for the derivatization procedure. Sediment extraction was performed as depicted for soil samples.

Following Le Bot et al. (2002), 3-mL samples were derivatized by adding 0.5 mL borate buffer and, after mixing, 500 µL FMOC-Cl solution. Then, samples were shaken for 1 h and incubated, allowing the reaction to take place for 15 h at room temperature (20°C). Derivatization was performed in the dark. The reaction was stopped by adding formic acid at about pH 3.0. The samples were washed with 2 mL diethyl ether to eliminate excess derivatization reagent.

The solid-phase extraction (SPE) was performed by means of a Dionex AutoTrace 280 SPE autosampler (Thermo Scientific). Glyphosate and AMPA were analyzed by liquid chromatography–electrospray ionization–tandem mass spectrometry (TSQ Vantage triple quadrupole mass spectrometer, Thermo Scientific), which comprise an analytical column (Syncronis C8, 2.1 by 150 mm, 5 mm, Thermo Scientific) and a column guard (Syncronis C8, 2.1 by 10 mm, 5 mm, Thermo Scientific). Each standard and sample (3 mL) were injected onto the analytical column and then eluted in gradient mode using a binary solvent mix comprising 99% 5 mM ammonium acetate and 1% acetonitrile (mobile phase A) and 99% acetonitrile and 1% 5 mM ammonium acetate (mobile phase B). The mobile phase flow rate was 0.3 mL/min. Analyses were performed in negative ionization mode with a spray voltage of 3.5 kV. The source temperature and the ion transfer tube temperature were 325°C and 250°C, respectively. The minimum detectable level (MDL) was 0.1 µg/L for glyphosate and AMPA in leachates and 10 µg/kg in soil.

Results

The daily rainfall and the glyphosate and AMPA concentrations in the leachates are presented in Fig. 1.

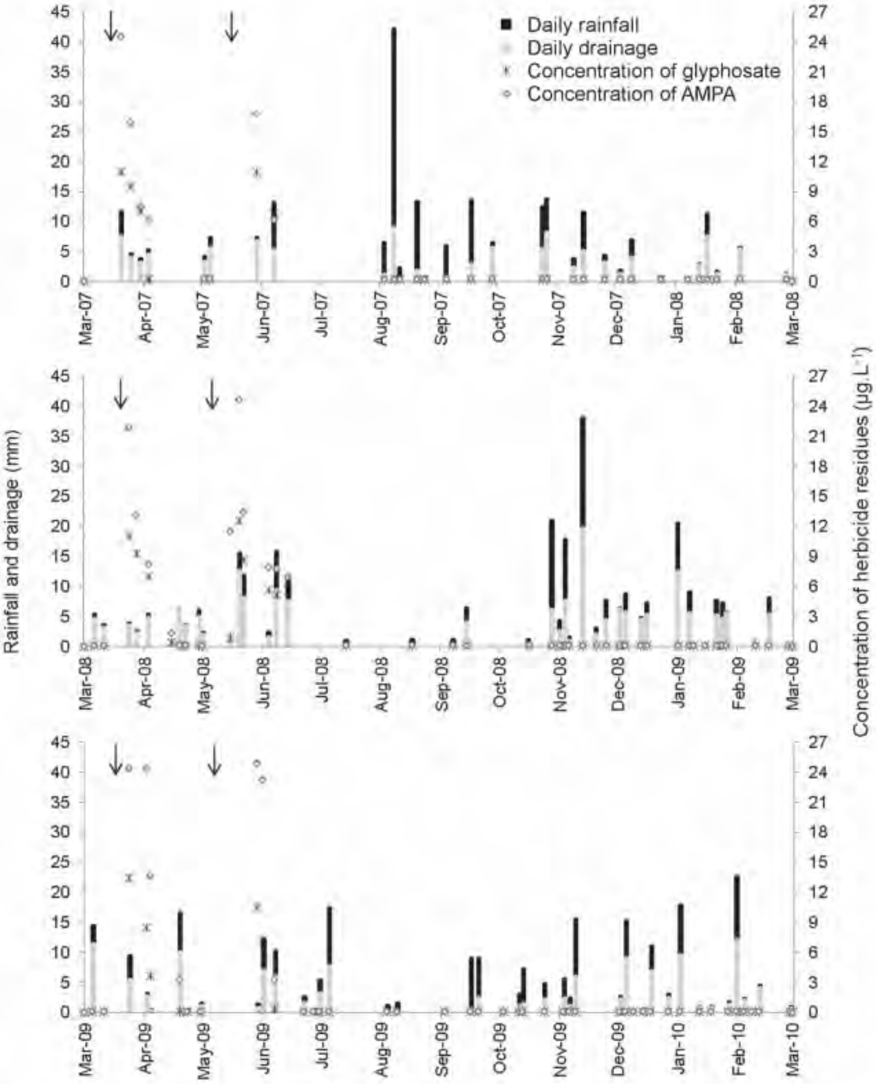


Fig. 1. Daily rainfall and drainage, and concentrations of glyphosate and AMPA measured in the leachates of the vineyard soil from March 2007 to March 2010. Herbicide dates of application are indicated with arrows.

The cumulative rainfall amounts for the period from 1 March to 28 February of the subsequent year were 524, 751, and 1429 mm during the first, second, and third year of the experiment, respectively. During the monitoring period, glyphosate was detected in 3% of the soil leachates at concentrations ranging from 0.2 to 1 µg/L and in 16% of the leachates at concentrations ranging from 1 to 13.47 µg/L. Glyphosate appeared at high concentrations (12.1 ± 1.3 µg/L) in the soil leachates 9.3 ± 4 d after each treatment.

Glyphosate was detected in the leachates for 25.8 ± 8.3 d after treatments at concentrations exceeding 0.5 µg/L. During the latter, average drainage of 15.5 ± 2.9 mm was measured, corresponding to 22.9 ± 6.7 mm of measured rainfall. Thereafter, the glyphosate concentration in leachates decreased to 0.1 µg/L. At the end of each trial year, the final glyphosate peaks appeared in the leachates between late January and early February (about 318.9 ± 8 d after the first annual treatment) at an average concentration of 0.3 µg/L.

Similar to the results for glyphosate, AMPA first appeared at an average concentration of 21.3 ± 6.2 µg/L in the soil leachate approximately 6.8 ± 1.2 d after each treatment. Aminomethylphosphonic acid was detected more frequently than glyphosate; it was detected in 13% of the leachates from soil at concentrations ranging from 0.2 to 1 µg/L and in 20% of the leachates from soil at concentrations ranging from 1 to 24.9 µg/L.

The amounts of water drained from the soil for the period from 1 March to 28 February of the following year, were 113.8, 187.4, and 130.5 mm, respectively, during the first, the second and the third year of the experiment. Approximately 0.19, 0.31, and 0.12% of the amount of glyphosate distributed in the first, second, and third year of the experiment, respectively, were recovered in the leachates as glyphosate, whereas 0.49, 0.78, and 0.48%, respectively, were recovered as AMPA.

On the basis of the analysis, the number of days from the treatment (DN) showed the highest negative correlation with the glyphosate and AMPA concentrations in leachate ($p \leq 0.001$). In contrast, the daily mean temperature (T_{med}) and the daily rainfall (R) showed a positive role in determining the herbicide concentration ($p \leq 0.05$). Since these variables were not autocorrelated, they were selected as independent variables X_1, X_2, and X_3, respectively, for the multiregressive model (Eq. [1]). The multiregression analysis led to the set up of Eq. [2] and [3] for the estimation of glyphosate and AMPA concentration in leachate, respectively:

$$Y_{\text{glyphosate}} = 0.0508T_{\text{med}} - 0.3445\text{DN} - 0.0179R + 13.2308 \qquad [2]$$

$$Y_{\text{AMPA}} = 0.1937T_{\text{med}} - 0.6727\text{DN} - 0.1412R + 25.2585 \qquad [3]$$

The glyphosate and AMPA concentrations were computed using data measured during the second and the third year of the experiment.

At least for the current study, climatic conditions and for this soil type, Eq. [4] and [5] can be used to determine the number of days free of rain (NR) necessary to ensure a safe threshold for distributing the herbicide.

$$\text{NR}_{\text{glyphosate}} = -2.9028Y_{\text{glyphosate}} + 0.1475T_{\text{med}} - 0.0519R + 38.4058 \qquad [4]$$

$$\text{NR}_{\text{AMPA}} = -1.4866Y_{\text{AMPA}} + 0.2879T_{\text{med}} - 0.2099R + 37.5479 \qquad [5]$$

No extractable glyphosate was detected in the soil profile. Aminomethylphosphonic acid was found as deep as 100 cm in the soil column. The concentration of AMPA increased with increasing depth, thus indicating a gradual accumulation of AMPA in the lower profile during the 3-yr experimental period. On the contrary, AMPA was distributed throughout the soil columns as shown in Fig. 2.

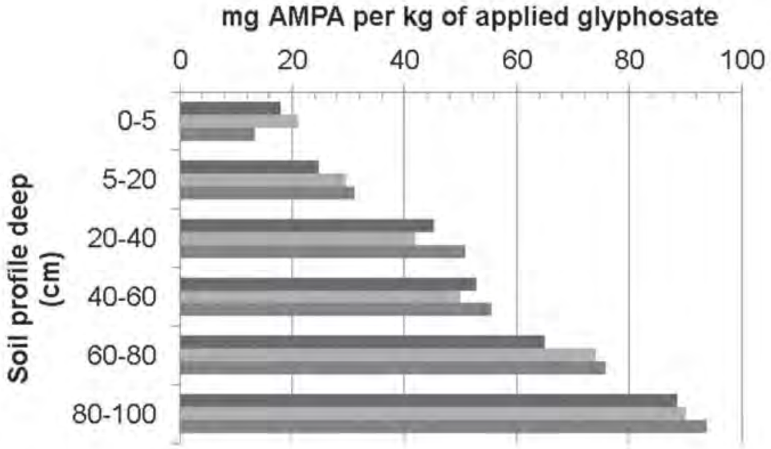
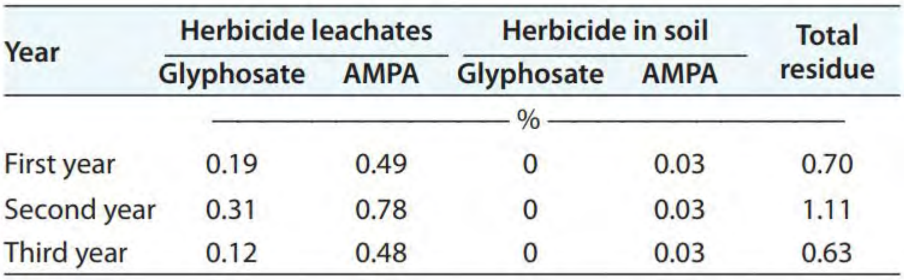


Fig. 2. Distribution profile of aminomethylphosphonic acid (AMPA) in the soil 1 year after the application of glyphosate for the first (dark gray), the second (light gray), and the third (medium gray) year of experiment.

The AMPA content in the surface soil layer ranged between 0.0013 and 0.0021%, based on the amount of glyphosate applied. The AMPA content in the lowest layer ranged between 0.0089 and 0.0094%, based on the amount of glyphosate applied. During the 3 yr, a continuous increase in the concentration of AMPA in the lower layers of the profile was measured; however, there are no statistical data to attribute this to an accumulation effect, but rather to different weather conditions. Finally, at the end of each year of experimentation, the total amount of AMPA recovered in the soil profiles was about 0.03%, based on the amount of glyphosate applied.

The amounts of glyphosate and AMPA, in terms of applied glyphosate, measured in the leachates and in the soil profiles were summed on a yearly basis (Table 2).

Table 2. Mass balance of glyphosate and aminomethylphosphonic acid (AMPA) in leachates and soil profles (in percentage based on the amount of glyphosate applied) for the three experimentation years.



Year	Herbicide leachates		Herbicide in soil		Total residue
	Glyphosate	AMPA	Glyphosate	AMPA	
			%		
First year	0.19	0.49	0	0.03	0.70
Second year	0.31	0.78	0	0.03	1.11
Third year	0.12	0.48	0	0.03	0.63

Conclusion

After a 3-yr experimental period under outdoor conditions, the present work has demonstrated that both glyphosate and AMPA may be transported in leachates through 100 cm of soil profile, thus confirming the high mobility of this herbicide. The mean annual percentage of glyphosate and AMPA, as a percentage of applied glyphosate, recovered in leachates were about 0.2 and 0.58%, respectively. Moreover, results suggested that preferential, flow along with rains that occurred within 2 wk after the treatment, can cause the leaching of glyphosate and AMPA in high concentration. At least in this environment and for this soil, a multiregressive equation was found to determine the number of days free of rain necessary to ensure a safe herbicide distribution. Soil analyses indicated that glyphosate was below detection in 1 yr. On the contrary, the total amount of AMPA, based on the amount of glyphosate applied, recovered in the soil profiles was around 0.03% at the end of each year of experimentation. Overall, these results suggest that when applied to shallow soils, herbicides can pose a risk of groundwater contamination, and, when applied to pipe-drained crops, contaminated leachate can be transported by the pipe drain to surface waters.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study describes a lysimeters study with glyphosate using three lysimeters from the Chianti region in Italy. The study is well described, however, there is some information missing to check the validity of the study against current guidelines.
The study is therefore classified as reliable with restrictions (Category 2).

1. Information on the study

Data point:	KCA 7.1.2.1.1
Report author	Nguyen, Nghia. K., et al.
Report year	2013
Report title	Soil properties governing biodegradation of the herbicide glyphosate in agricultural soils
Document No	24th Asian Pacific Weed Science Society pg 312-324
Guidelines followed in study	None
Deviations from current test guideline	None
GLP/Officially recognised testing facilities	No, not conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Reliable with restrictions (no sufficient data for evaluation of degradation behaviour reported, only mineralization after 32 days)

2. Full summary of the study according to OECD format

The relationships between soil properties and glyphosate biodegradation in different agricultural soils was investigated in this study. Soils differ hugely in soil texture, soil organic matter content. pH, oxalate extractable $A1^{3+}$ and Fe^{3+}. The biodegradation experiments were conducted under test conditions: water tension of -15 kPa as soil moisture, a soil density of 1.3 g/cm^3 and at 20 °C in the dark. The biodegradation experiments showed that the mineralization of glyphosate in 21 agricultural soils greatly varied. Between 7.6 to 68.7 % of the applied ^{14}C-glyphosate was mineralized to $^{14}C0_2$ in the 21 different soils within 32 days of incubation. The highest and lowest mineralized glyphosates were observed in Feldkirchen (68.7%) and Brejze soil sample (7.6%), respectively. Glyphosate was mineralized rapidly by the microorganisms in the soil solution and the highest mineralization rate was reached shortly after application. The mineralization of glyphosate in soils was individually regulated by exchangeable H^+, soil pH-$CaCl_2$, oxalate extractable AI^{3+} and bacterial cell numbers at the end of the experiments, but it was collectively controlled by exchangeable H^+, Ca^{2+} ions and plant available K. Moreover, soil textures, soil organic content, P_20_5, Cu^{2+}, oxalate extractable Fe^{3+} and CEC were found not to have any correlation with mineralization of glyphosate. The NaOH extractable residues were bioavailable for degradation whereas the bound residues of glyphosate in soils were mostly formed by microbial activity.

Materials and Methods

Soil

The experiment was conducted using 21 agricultural soils typical of Germany and Slovenia. There was a big variation in the different soil characteristics (soil textures, organic matter content, total N, C/N, plant available P, oxalate extractable Al^{3+}, Fe^{3+}, Cu^{2+}, CEC, pH-$CaCl_2$, water content at water potential of -15 kPa and heterotrophic bacteria). All soils were taken from the upper Ap layer of arable fields (0-30 cm), sieved (2 mm) after sampling, homogenized and stored at 4°C in the dark before use. At the beginning of the experiments all soils were conditioned and moistened to a water potential close to -15 kPa at room temperature (20 ± 2 °C) for 2 weeks and compacted to the soil density of 1.3 g/cm^3 to equilibrate the microbial processes and to make sure that all soils have the comparable conditions at the start of the experiments.

Table 1. Some characteristics of soil samples

Name of soil (site of origin)	Sand [%]	Silt [%]	Clay [%]	Water content -15 kPa (%)	pH ($CaCl_2$)	Organic mattter [%]	C [%]	N [%]	P_2O_5 (mg $100g^{-1}$)	K_2O (mg $100g^{-1}$)
1 (Ada-A02)	62.5	27.4	10.1	21.9	5.7	2.9	1.7	0.2	17	4.1
2 (Apace-njiva)	66.4	31.2	2.4	20.7	7.0	2.6	1.5	0.2	4	22.6
3 (Berta-A02)	46.4	39.4	14.2	28.1	5.7	2.5	1.5	0.2	8	12.5
4 (Brezje)	8.3	73.2	18.5	32.7	5.2	2.8	1.6	0.2	5	21.1
5 (Dunja - A06)	62.4	25.9	11.7	17.4	5.4	2.2	1.3	0.2	11	13.2
6 (Feldkirchen)	34.8	47.0	18.2	28.2	7.0	3.4	2.0	0.3	39	9.4
7 (Grace - A13)	50.3	41.3	8.4	21.0	5.4	2.6	1.5	0.2	12	9.6
8 (Hanna - A15)	62.3	24.2	13.5	18.4	5.2	1.7	1.0	0.1	7	8.2
9 (Hohenwart)	67.2	20.5	12.3	22.4	6.2	1.7	1.0	0.1	21	21.1
10 (Joy -A19)	31.6	45.6	22.8	31.9	5.9	2.7	1.6	0.2	34	43.2
11 (Kelheim)	76.2	15.5	8.3	12.5	6.5	1.2	0.7	0.1	23	17.0
12 (Konjise)	33.8	60.2	6.0	34.6	6.9	4.5	2.6	0.2	4	7.9
13 (Lamanose)	10.3	69.6	20.1	35.8	5.8	4.3	2.5	0.3	5	18.7
14 (Lea -A18)	18.9	66.8	14.3	28.9	5.2	1.9	1.1	0.2	6	23.8
15 (Lomanose)	21.9	60.2	17.9	25.8	5.8	1.7	1.0	0.2	11	16.8
16 (Neumark)	85.5	8.8	5.7	12.6	5.2	1.6	0.9	0.1	11	12.2
17 (Pearl - A20)	29.3	51.8	18.9	28.3	5.0	2.3	1.3	0.2	12	31.7
18 (Scheyern Lysi)	17.2	62.6	20.2	30.1	5.5	2.7	1.6	0.2	20	5.3
19 (Skrinjar)	67.5	27.0	5.5	19.2	7.1	1.6	0.9	0.1	21	24.7
20 (Zepovci)	41.3	43.1	15.6	24.0	5.7	2.9	1.7	0.2	11	24.7
21 (Zepovci (Plitv.))	11.8	72.2	16.0	27.4	5.2	1.9	1.1	0.2	8	20.2

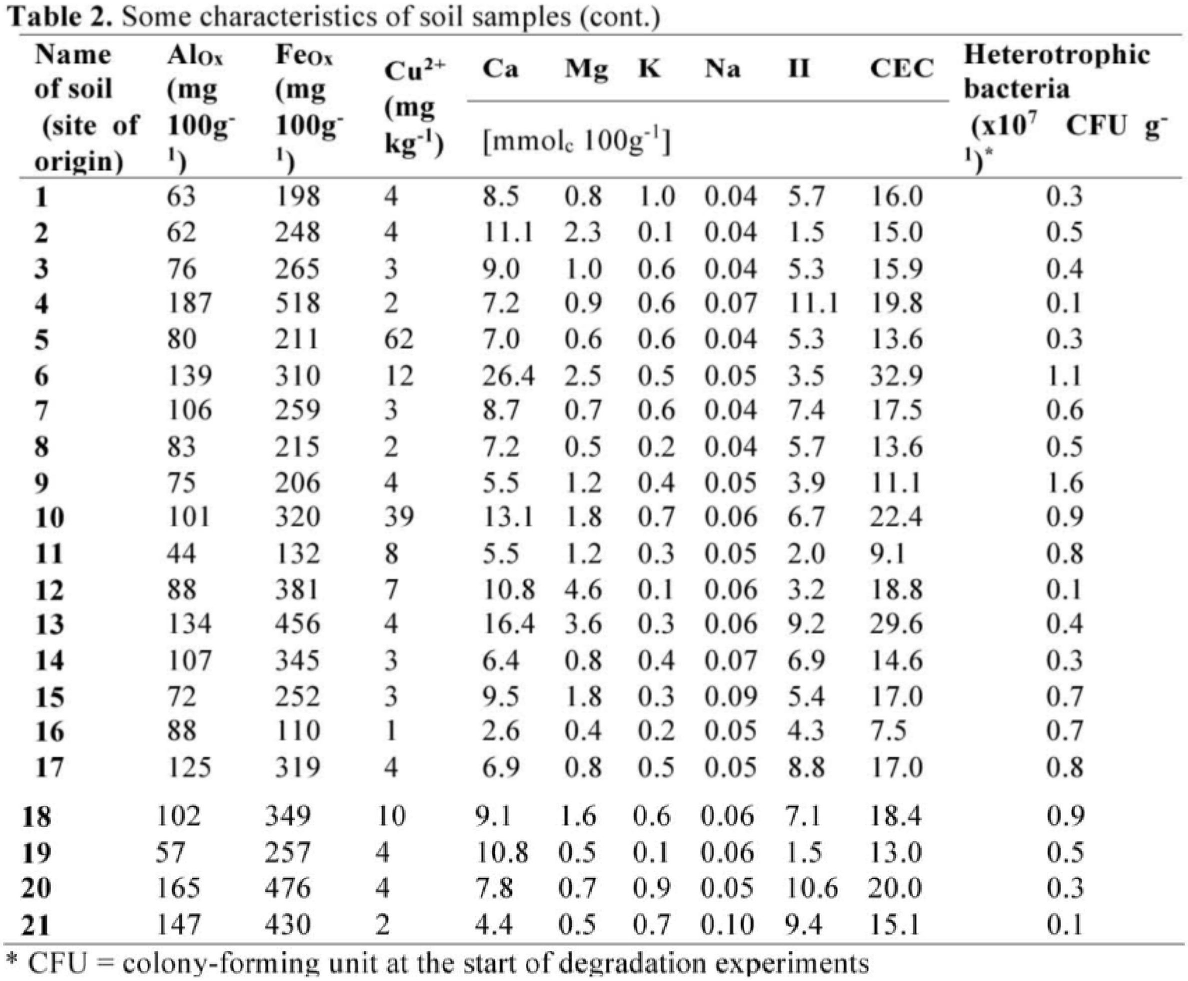


Table 2. Some characteristics of soil samples (cont.)

Name of soil (site of origin)	Al$_{ox}$ (mg 100g^{-1})	Fe$_{ox}$ (mg 100g^{-1})	Cu^{2+} (mg kg^{-1})	Ca	Mg	K	Na	II	CEC	Heterotrophic bacteria (x10^{7} CFU g^{-1})*
				[mmol$_c$ 100g^{-1}]						
1	63	198	4	8.5	0.8	1.0	0.04	5.7	16.0	0.3
2	62	248	4	11.1	2.3	0.1	0.04	1.5	15.0	0.5
3	76	265	3	9.0	1.0	0.6	0.04	5.3	15.9	0.4
4	187	518	2	7.2	0.9	0.6	0.07	11.1	19.8	0.1
5	80	211	62	7.0	0.6	0.6	0.04	5.3	13.6	0.3
6	139	310	12	26.4	2.5	0.5	0.05	3.5	32.9	1.1
7	106	259	3	8.7	0.7	0.6	0.04	7.4	17.5	0.6
8	83	215	2	7.2	0.5	0.2	0.04	5.7	13.6	0.5
9	75	206	4	5.5	1.2	0.4	0.05	3.9	11.1	1.6
10	101	320	39	13.1	1.8	0.7	0.06	6.7	22.4	0.9
11	44	132	8	5.5	1.2	0.3	0.05	2.0	9.1	0.8
12	88	381	7	10.8	4.6	0.1	0.06	3.2	18.8	0.1
13	134	456	4	16.4	3.6	0.3	0.06	9.2	29.6	0.4
14	107	345	3	6.4	0.8	0.4	0.07	6.9	14.6	0.3
15	72	252	3	9.5	1.8	0.3	0.09	5.4	17.0	0.7
16	88	110	1	2.6	0.4	0.2	0.05	4.3	7.5	0.7
17	125	319	4	6.9	0.8	0.5	0.05	8.8	17.0	0.8
18	102	349	10	9.1	1.6	0.6	0.06	7.1	18.4	0.9
19	57	257	4	10.8	0.5	0.1	0.06	1.5	13.0	0.5
20	165	476	4	7.8	0.7	0.9	0.05	10.6	20.0	0.3
21	147	430	2	4.4	0.5	0.7	0.10	9.4	15.1	0.1

* CFU = colony-forming unit at the start of degradation experiments

Chemicals

^{14}C-labelled glyphosate [N-(phosphonomethyl)glycine purity >97.0%.] was labelled on the phosphonomethyl group. ^{14}C-glyphosate was mixed with non-labelled glyphosate (purity 98%) resulting in a final specific radioactivity of 1.6 Bq/mg (for degradation experiments). Aminomethylphosphonic acid (AMPA) had the purity of 98 %. Sodium hydroxide (NaOH), monopotassium phosphate (KH_2P0_4), sodium chloride (NaCI), calcium chloride dihydrate ($CaCl_2x2H_20$), NH_4CI, NH_4NO_3, methanol (CH_40), diatomaceous earth, water for chromatography were purchased commercially.

Glyphosate mineralization experiments

Pesticide application procedure - All biodegradation experiments were performed in 4 replicates with 50 g soil (dry mass) for each replicate. ^{14}C -glyphosate was dissolved in autoclaved and distilled water and mixed with non-labeled glyphosate which was also dissolved in sterilized distilled water. This was the application standard solution with a concentration of a.i of 5.42 µg/L and a specific radioactivity of 166.70 Bq/µg. The application standard (0 .089 mL) was applied to an oven dried, pulverized soil and carefully stirred for 1 minute with a spatula. The spiked aliquot was transferred to another glass beaker containing the rest of equilibrated soils and mixed for another 2 min. The total concentration of glyphosate was 10 µg/g in each set corresponding to a total radioactivity of 83,000 Bq.

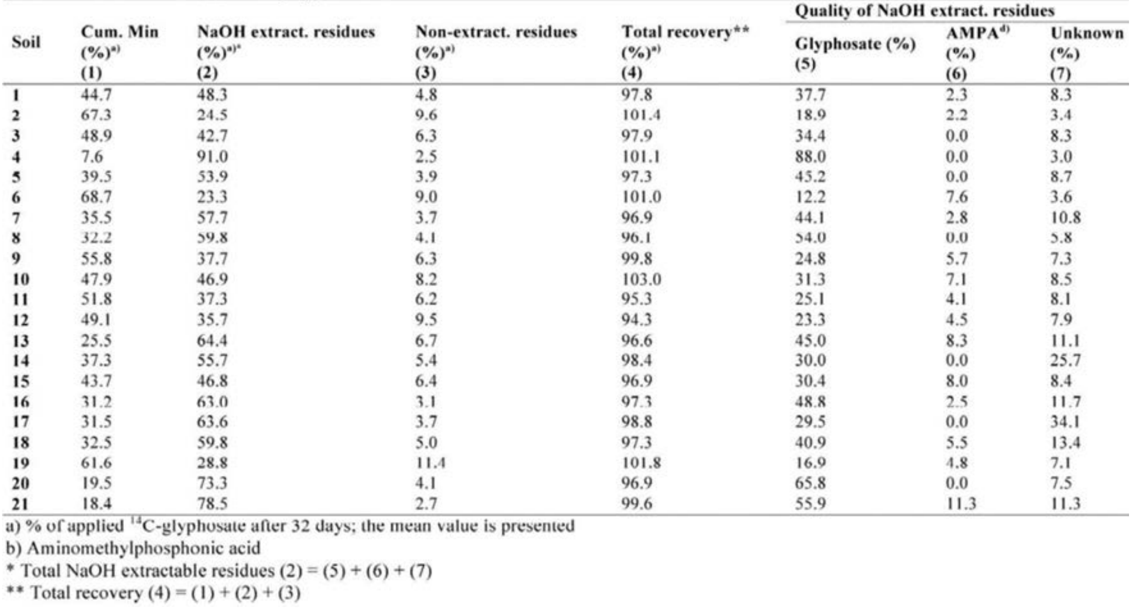
Test system, experimental conditions and samplings - The spiked soils were compacted to a soil density of 1.3 g/cm^3 and soil water was adjusted to a water potential of -15 kPa. The f1asks were covered with special rubber caps, and incubated at 20 ± 1°C in the dark for a period of 32 days. The soil humidity was controlled weekly. The rubber caps were equipped with an air inlet and outlet system as well as a facility to trap the evolved CO_2. The air exchange system should prevent unaerobiosis in the incubation flasks and consisted of a canal which was made of a stainless needle with a diameter of 1 mm. To eliminate CO_2 from the ambient air entering the flasks, a 12 mL plastic syringe filled with granular CO_2 absorber (soda lime) was connected to the canal at the top of the cap. Below the cap a small plastic beaker was placed containing 0.1 M NaOH solution to capture $^{14}CO_2$ released from glyphosate mineralization from the soil samples. The NaOH solution was exchanged three times per week and from the collected solution an aliquot of 2 mL was mixed with 3 mL of scintillation cocktail Ultim a Flo AF to determine $^{14}CO_2$ in a liquid scintillation counter. At the end of the experiment, 30 g of each soil sample (dry mass) were used for pore water extraction, 7 g of each soil sample were extracted with NaOH to determine the quantity and quality of the extractable residues as well as to quantify the non-extractable residues, while 1 g of each soil sample was used for cell counts.

NaOH extraction, clean up and HPLC analysis- For NaOH extraction, the method used by Gimsing et al. (2004a) was applied. At the end of the experiment, soil was extracted with 0.1 M NaOH by shaking on overhead shaker for 17 hours. The supernatant was collected after centrifuging for 10 min at 3020 rcf. Radioactivity of the filtered supernatant was measured by scintillation counting using 100 µl of supernatant aliquot and 5mL of scintillation cocktail Ultima Gold XR to quantify the NaOH extractable pesticide residues. Subsequently, extracts were concentrated and cleaned up before injecting to HPLC. Twenty µl of each sample (NaOH extract) were injected via an Auto Sampler AS50 to a HPLC system that was connected with a Radioflow detector LB 509. ^{14}C-glyphosate and its metabolites (AMPA, sarcorsine, glycine, methylamine) were identified by comparison of their retention times with standard substances. After each analysis the column was regenerated with Regenerant-RG019 at a flow velocity (isocratic) of 0.5 mL/min for 30 min.

Quantification of non-extractable ^{14}C-labelled residues - After extraction with 0.1 M NaOH, the rest of radioactivity remaining in the soil was considered as non-extractable residues. Soil material was intensively mixed and homogenized with diatomaceous earth for 2 min in a mortar. Four aliquots of each soil sample were weighed in combustion cups and mixed with 8 drops of saturated aqueous sugar solution to accelerate and ensure a complete oxidation of the ^{14}C. The oxidation step was done with an automatic sample-oxidizer 306. $^{14}CO_2$ from the combustion was trapped in Carbo-Sorb E and mixed with Permaflour E before scintillation counting. The extractable and non-extractable glyphosate residues were calculated after the combustion.

Bacterial cell counts- Bacterial cell counts were performed to count the cultivable and heterotrophic bacteria in the different soils. The method for bacterial cell counts was adapted from Ngigi et al. (2011). Soil bacteria were extracted from the soil by mixing soil with a buffer solution. Before use the buffer solution was autoclaved and shaken vigorously for 1 hour on a shaker at 150 rpm. The soil particles were allowed to sediment for 10 min. Then 0.1 mL of the supernatant was transferred to sterilized buffer solution for further dilution steps. A total of 4 dilutions (10^{-1} to 10^{-4}) were established. Finally, 0.1 mL of each dilution was spread in triplicates on Lysogeny broth (LB) agar media. This medium was also autoclaved before use. The number of CFU was determined after three days of incubation at 25 ^{0}C by counting.

Table 3. Behavior of ^{14}C-glyphosate in different soils



Soil	Cum. Min (%)$^{a)}$ (1)	NaOH extract. residues (%)$^{a)*}$ (2)	Non-extract. residues (%)$^{a)}$ (3)	Total recovery** (%)$^{a)}$ (4)	Quality of NaOH extract. residues Glyphosate (%) (5)	 AMPA$^{d)}$ (%) (6)	 Unknown (%) (7)
1	44.7	48.3	4.8	97.8	37.7	2.3	8.3
2	67.3	24.5	9.6	101.4	18.9	2.2	3.4
3	48.9	42.7	6.3	97.9	34.4	0.0	8.3
4	7.6	91.0	2.5	101.1	88.0	0.0	3.0
5	39.5	53.9	3.9	97.3	45.2	0.0	8.7
6	68.7	23.3	9.0	101.0	12.2	7.6	3.6
7	35.5	57.7	3.7	96.9	44.1	2.8	10.8
8	32.2	59.8	4.1	96.1	54.0	0.0	5.8
9	55.8	37.7	6.3	99.8	24.8	5.7	7.3
10	47.9	46.9	8.2	103.0	31.3	7.1	8.5
11	51.8	37.3	6.2	95.3	25.1	4.1	8.1
12	49.1	35.7	9.5	94.3	23.3	4.5	7.9
13	25.5	64.4	6.7	96.6	45.0	8.3	11.1
14	37.3	55.7	5.4	98.4	30.0	0.0	25.7
15	43.7	46.8	6.4	96.9	30.4	8.0	8.4
16	31.2	63.0	3.1	97.3	48.8	2.5	11.7
17	31.5	63.6	3.7	98.8	29.5	0.0	34.1
18	32.5	59.8	5.0	97.3	40.9	5.5	13.4
19	61.6	28.8	11.4	101.8	16.9	4.8	7.1
20	19.5	73.3	4.1	96.9	65.8	0.0	7.5
21	18.4	78.5	2.7	99.6	55.9	11.3	11.3

a) % of applied ^{14}C-glyphosate after 32 days; the mean value is presented
b) Aminomethylphosphonic acid
* Total NaOH extractable residues (2) = (5) + (6) + (7)
** Total recovery (4) = (1) + (2) + (3)

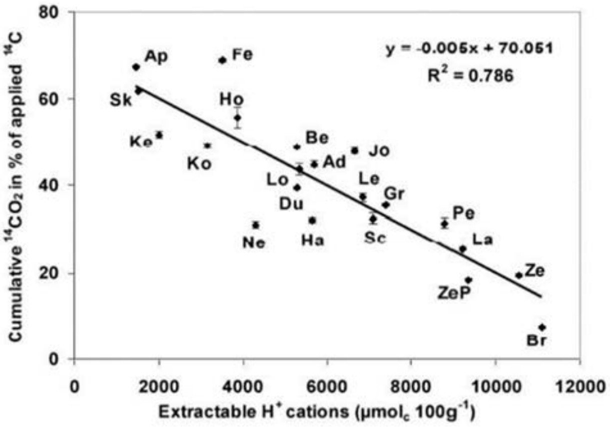


Fig. 1. Correlation between cumulative mineralization of glyphosate and extractable H^+ cations in soils (bars indicate standard deviation of 4 samples).

Statistical analysis

The data were statistically analysed using analysis of variance (ANOVA) and multiple regression analysis.

Results and Discussion

The main aim in this part of the study is to check the correlation between soil parameters and glyphosate mineralization. The selected soil parameters for correlation were exchangeable [H^+], silt, clay, soil organic matter, C, N, C/N, P_2O_5, Cu^{2+}, oxalate extractable Al^{3+}, oxalate extractable Fe^{3+}, K_2O, $CFU_{beginning}$ and CFU_{end}, Ca^{2+} , Mg^{2+} , K^+ , Na^+ , CEC, and Ph.

Mineralization of glyphosate

After 33 days of incubation a big variance of cumulative mineralization can be observed. Between 7.6 to 68.7 % of the applied ^{14}C -glyphosate was mineralized to $^{14}CO_2$ in the 21 different soil types (Table 3). Shortly after application, a high amount of glyphosate was mineralized. The lowest mineralization of ^{14}C-glyphosate was identified in Brezje soil while the highest mineralization of ^{14}C-glyphosate was obtained in Feldkirchen and Apace-njiva soils. Low mineralization of glyphosate was also observed in

Zepovci, Zepovci(Plitv.) and Lamanose soils. In these 3 soils less than 30% of the initial glyphosate was mineralized after 32 days. In contrast, other soils had a higher mineralization activity and $^{14}CO_2$ production after 32 days reached 31.2-68.7% of the initial glyphosate. A big difference in biomineralization of glyphosate among 21 soils indicates that agricultural soils have difference in ability to degrade glyphosate. The firstly rapid mineralization of glyphosate was observed for most soils during the first 4 days without a lag phase, but mineralization rates subsequently decreased over time, as found in other earlier studies (von Wiren-Lehr et al., 1997; Gimsing et al., 2004a). At the end of the biodegradation experiments, mass balances were established. Mass balances of ^{14}C-glyphosate are presented in Table 3. In all soils, the ^{14}C mass balances were quite good: over 94 % of the totally applied ^{14}C-glyphosate was recovered at the end of the biodegradation experiments.

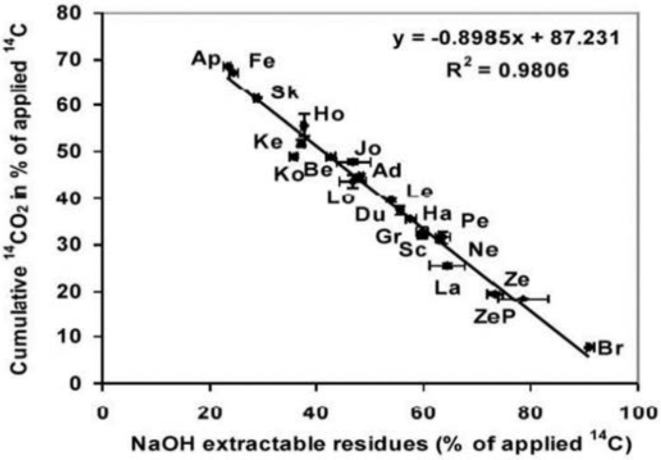


Fig. 2. Correlation between cumulative mineralization of glyphosate and NaOH extractable residues (bars indicate standard deviation of 4 samples).

Identification of the parameters governing mineralization of glyphosate

In order to identify the factors which govern glyphosate mineralization in the 21 soils, soil parameters, NaOH extractable residues, ^{14}C-glyphosate residues, non-extractable residues and the mineralized glyphosate were compared at the end of the biodegradation experiments and several significant correlations could be discovered.

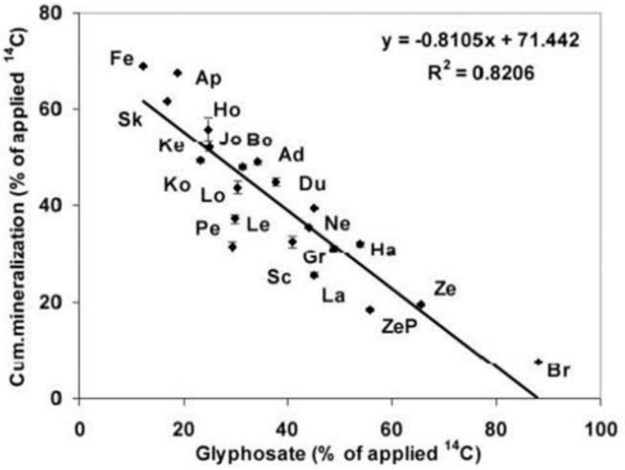


Fig. 3. Correlation between cumulative mineralization of glyphosate and glyphosate residues (from extractable residues) (bars indicate standard deviation of 4 samples).

Relationship between mineralized glyphosate and extractable acidity (extractable H^+ cations)

As can be seen in Fig. 1, according to univariate correlation analysis there was highly significant and negative correlation between the cumulative mineralization glyphosate and extractable H+ cations (p =

0.000). This illustrates that the extractable H+ cations interfered the mineralization process in soils. Therefore, the assessment of extractable H+ cations in soils appears suitable for ranking of soil according to the mineralization of the compound.

Relationship between mineralized glyphosate and NaOH extractable residues

NaOH extractable residues of the 21 investigated soils were performed after 32 days. The results show that the NaOH extractable fraction in all soils was relatively high and very various. Approximately between 23 and 91 % of initial glyphosate after 32 days incubation was extracted with NaOH 0.1 M (Table 3). Soils with higher mineralization had lower NaOH extractable fraction. A correlation was performed to check the relationship between mineralized glyphosate and NaOH extractable residues. Fig. 2 shows that there was a negative correlation between mineralized glyphosate within 32 days and NaOH extractable residues (p = 0.0000). This shows that NaOH extractable residues were non-available for microorganisms to be degraded.

Relationship between mineralized glyphosate and ^{14}C-glyphosate residues from extractable pool ^{14}C-glyphosate is the major component in the NaOH extract as compared to AMPA and unknown metabolites (Table 3). To test whether there is any relationship between the mineralized glyphosate and NaOH extractable residues, we calculated correlation between both values. There is exist significantly negative correlation between ^{14}C-glyphosate residues from extractable pool and mineralized glyphosate (p = 0.0000; Fig. 3). This indicates that in soils with low mineralization glyphosate is present in a high amount and that this glyphosate could not be degraded/mineralized because it was adsorbed to Al- or Fe-oxides.

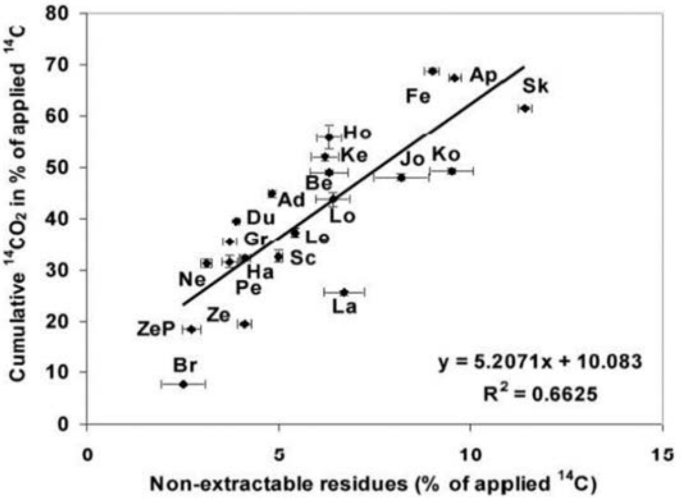


Fig. 4. Correlation between cumulative mineralization and non-extractable residues (bars indicate standard deviation of 4 samples).

Relationship between mineralized glyphosate and non-extractable residues The amount of non-extractable residues was relatively low. It varied between 2.5 % and 11.4 % of the initial glyphosate (Table 3). The non-extractable residues and mineralized glyphosate were compared together to see whether there is any relationship between both parameters. As can be seen from Fig. 4, a significant and positive correlation between mineralized glyphosate and non-extractable residues (p = 0.0000) was found. The high mineralization of glyphosate in soils coincided with non-extractable residues at the end of the experiment.

Relationship between mineralized glyphosate and bacterial cell counts

There was a significantly positive correlation between mineralization of glyphosate and bacterial cell counts (p = 0.003; Fig. 5). This shows that the mineralization of glyphosate in soils is limited not only by availability of glyphosate and its degradation products, but also by the bacterial activity. Therefore, it can be assumed that the bacterial cell numbers at the end of the experiment seemed to be the degrading microorganisms for glyphosate in soils and it was likely that microbes capable of degrading glyphosate aerobically exist in soils.

The interacting junctions of the different soil parameters on mineralized glyphosate

In order to investigate the interacting functions of the different soil parameters on cumulative glyphosate mineralization, a multiple regression analysis was used. The input parameters were extractable H^+ cations, silt, clay, soil organic matter, C, N, C/N, plant available P, Cu^{2+}, oxalate extractable AI^{3+}, oxalate extractable Fe^{3+}, plant available K, Ca^{2+}, Mg^{2+}, K^+, Na^+, CEC, pH , CFUs at beginning and CFUs at the end of the experiments. The result of multiple regression analysis reveals extractable H^+ cations, Ca^{2+} and plant available K as key parameters governing glyphosate mineralization in the 21 tested soils and Ca^{2+} and plant available K contributes additionally to extractable H^+ cations to the mineralization of glyphosate. In this multiple regress ion, extractable H+ cations has a negative correlation with mineralization of glyphosate, whereas exchangeable Ca^{2+} and plant available K have a positive correlation with cumulative mineralization of glyphosate. Once again, this result indicates that extractable H^+ cations is an important factor which reduces the bioavailability of glyphosate in soils, and as a consequence the mineralization of glyphosate is reduced. Regarding Ca^{2+} and plant available K, cumulative mineralization was found to be positively correlated with exchangeable Ca^{2+} and plant available K, respectively. Therefore, it is proposed in this study that a complexation between glyphosate with exchangeable Ca^{2+}/plant available K will not reduce the bioavailability and mineralization of glyphosate. In the contrary, Ca^{2+}-glyphosate complexes may be transported more efficiently across microbial cell walls than sole glyphosate compound as it has already been argued for Cu^{2+} complexes in literature (Kools et aI. , 2005). However, these mechanisms have not been documented and should be clarified.

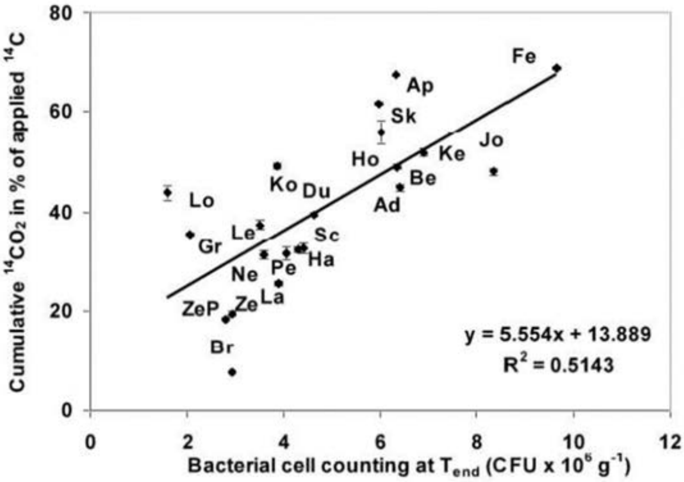


Fig. 5. Correlation between cumulative mineralization of glyphosate and bacterial cell counting (bars indicate standard deviation of 4 sample) .

Conclusions

Degradation of glyphosate in soils greatly varies. depending on soil properties. Mineralized glyphosate is affected by extractable acidity (H^+ cations) and bacterial cell counts. Sorption behavior and bioavailability of glyphosate in soil are important to regulate mineralization. extractable H^+ cations. Ca^{2+} ions and plant available K have been identified as important soil parameters that collectively control the mineralization of glyphosate in soil. Glyphosate that is absorbed by AI/Fe-oxides and extractable H^+ cations can be extractable with NaOH 0.1 M, but it is not available for degradation by soil microorganisms. Non-extractable residues of glyphosate which have been identified as a result of microbial activity.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The study describes the dissipation of glyphosate in agricultural soils in Europe. Experimental details were well described. However, insufficient data for evaluation of degradation behaviour are reported, only mineralization after 32 days.

The study is therefore classified as reliable with restrictions (Category 2).

1. Information on the study

Data point:	KCA 7.1.2.1.1
Report author	Norgaard, T., et al.
Report year	2015
Report title	Can Simple Soil Parameters Explain Field-Scale Variations in Glyphosate-, Bromoxyniloctanoate-, Diflufenican-, and Bentazone Mineralization?
Document No	Water, Air, & Soil Pollution (2015) 226: 262
Guidelines followed in study	None
Deviations from current test guideline	None
GLP/Officially recognised testing facilities	No, not conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Reliable with restrictions (not sufficient data provided to check validity against current guidelines)

2. Full summary of the study according to OECD format

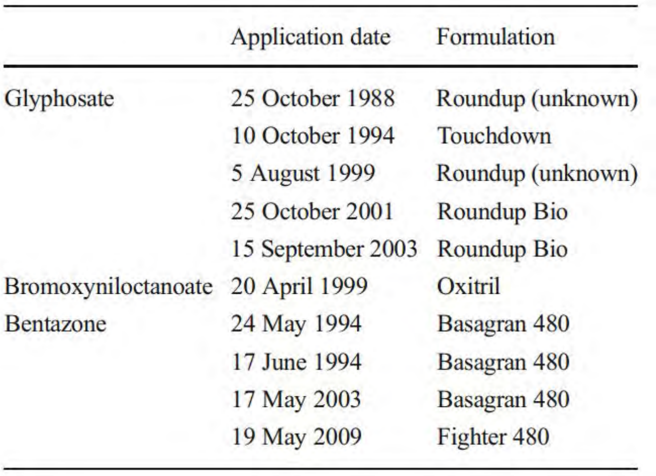
The large spatial heterogeneity in soil physico-chemical and microbial parameters challenges our ability to predict and model pesticide leaching from agricultural land. Microbial mineralization of pesticides is an important process with respect to pesticide leaching since mineralization is the major process for the complete degradation of pesticides without generation of metabolites. The aim of our study was to determine field-scale variation in the potential for mineralization of the herbicides glyphosate, bromoxyniloctanoate, diflufenican, and bentazone and to investigate whether this variation can be predicted by variations in basic soil parameters. Sixty-five soil samples were sampled from an agricultural, loamy field in Silstrup, Denmark, from a 60×165 m rectangular grid. The mineralization potential of the four pesticides was determined using a 96-well microplate ^{14}C-radiorespirometric method. Initial mineralization rates were determined using first-order kinetics for glyphosate and bromoxyniloctanoate and zero-order kinetics for diflufenican and bentazone. The mineralization rates of the four pesticides varied between the different pesticides and the different soil samples, but we could not establish correlations between the pesticide mineralization rates and the measured soil parameters. Only the glyphosate mineralization rates showed slightly increasing mineralization potentials towards the northern area of the field, with increasing clay and decreasing OC contents. The mineralization potentials for glyphosate and bentazone were compared with 9-years leaching data from two horizontal wells 3.5 m below the field. The field-scale leaching patterns, however, could not be explained by the pesticide mineralization data. Instead, field-scale pesticide leaching may have been governed by soil structure and preferential flow events.

Materials and Methods

Field Site

The agricultural test field (Silstrup, northwestern Jutland, Denmark) was a conventionally cultivated, loamy field with a cultivated area of 1.69 ha. The climate is coastal, cold temperate. The field has been cultivated as part of a routine agricultural practice with management and pesticide records dating back to 1983. Glyphosate was sprayed on the field five times since 1983, bentazone was sprayed four times, bromoxyniloctanoate only once, and diflufenican not at all. Application dates for the pesticides and their commercial formulations are shown in Table 1. Two horizontal wells, H1 and H2 (Fig. 1), are located 3.5 m below the surface, and each consists of three screen sections of 18 m. Water samples from the middle screen section of each well (H1.2 and H2.2) have been analyzed for pesticides every month, and the samples from the outer screen sections (H1.1, H1.3, H2.1, and H2.3) have been analyzed twice a year (Rosenbom et al. 2010). During 9 years screening (2000–2009), pesticides were detected in 44 % of the water samples from the middle section of the northern horizontal well (H1) whereas only 5 % of the water samples from the middle screen section of the southern horizontal well (H2) contained detectable pesticide concentrations. In the outer screen sections of the northern well (H1.1 and H1.3), pesticides were detected in 30 and 27 % of the water samples whereas there were no pesticide detections in the outer screen sections of H2 (Norgaard et al. 2012). Consequently, pesticides seem to leach only from the northern part of the field.

Table 1. Pesticide application history. There is no record of which Roundup formulation was applied in 1988 and 1999



	Application date	Formulation
Glyphosate	25 October 1988	Roundup (unknown)
	10 October 1994	Touchdown
	5 August 1999	Roundup (unknown)
	25 October 2001	Roundup Bio
	15 September 2003	Roundup Bio
Bromoxyniloctanoate	20 April 1999	Oxitril
Bentazone	24 May 1994	Basagran 480
	17 June 1994	Basagran 480
	17 May 2003	Basagran 480
	19 May 2009	Fighter 480

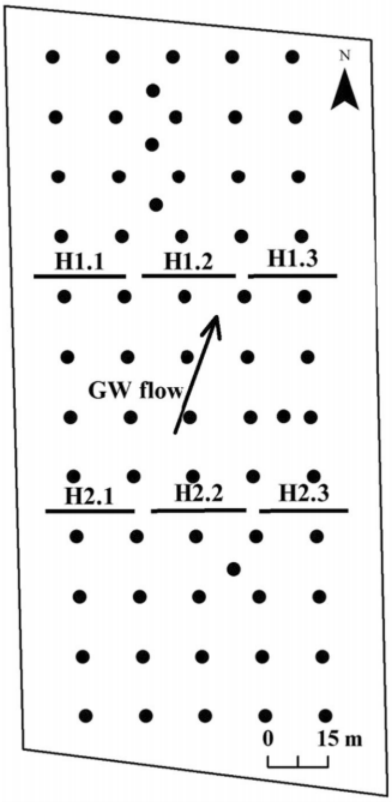


Fig. 1 Schematic presentation of the Silstrup field. Sample positions are indicated by the black dots. The horizontal wells (H1 and H2) and the screen sections in each well are indicated by the lines. The arrow indicates the groundwater flow direction

Soil Sampling

Sixty-five samples were sampled from a 60 × 165 m rectangular field with a distance of 15 m between sampling points (Fig. 1) on 6 December 2011. Soil was sampled from the plough layer at a depth of approximately 8–16 cm. First, the upper 8-cm top soil was removed and then a sample was taken by pounding a sterile 50-ml centrifuge tube (upside down) into the ground until the tube was almost full and then the tube was sealed. In the lab, each sample was homogenized by sieving twice through a sterile 4-mm mesh. The soil was further mixed thoroughly and stored at 2 °C for 1 week.

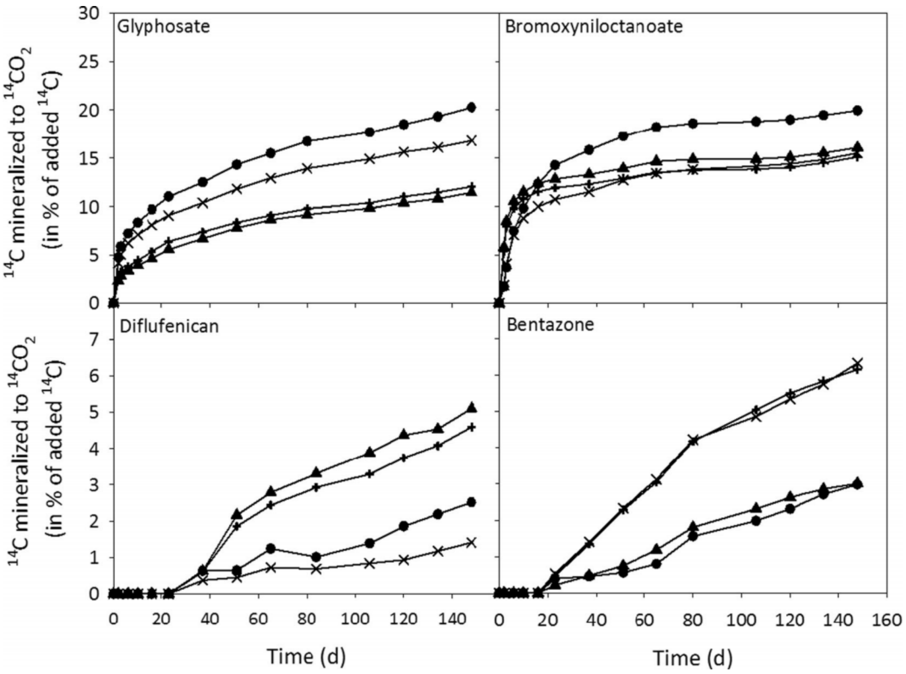


Fig. 2. Mineralization curves depicting the variation in mineralization within the field. The curves represent for each herbicide the two soil samples with the lowest initial mineralization rate and the two with the highest

Physical and Chemical Soil Analyses

Soil texture was determined according to Gee and Or (2002) using a combined sieve/hydrometer method. Organic carbon was determined on a LECO analyzer coupled with an infrared CO_2 detector. Bulk density was determined from weights of 20×20 cm intact soil columns after drying at 105 °C for 2 weeks. The soil pH was measured in a soil/water suspension of 1:4 (*v/v*), and the soil electrical conductivity (EC) was measured in a soil/water suspension of 1:9 (*v/v*). Oxalate-extractable iron, aluminum, and phosphorus were determined at AGROLAB GmbH, Germany, using the procedure described by Schoumans (2000). The Dexter index (Dexter *n*) for each soil sample was calculated as the ratio (*w/w*) between clay and organic carbon.

Table 2. Basic soil parameters. Minimum, maximum, mean, and coefficient of variation (CV) of soil texture, organic carbon (OC), Dexter n, bulk density, oxalate-extractable aluminum (Al), oxalate-extractable iron (Fe), and oxalate-extractable phosphorus (P), pH, and electrical conductivity (EC)

	Clay (<2 µm)	Silt (2–50 µm)	Sand (0.05–2 mm)	OC	Dexter *n*	Bulk density	Al	Fe	P	pH	EC
	kg kg^{-1}	kg kg^{-1}	kg kg^{-1}	kg kg^{-1}	–	g cm^{-3}	mmol kg^{-1}	mmol kg^{-1}	mmol kg^{-1}	–	mS cm^{-1}
Min. value	0.14	0.23	0.45	0.017	6.75	1.39	24	33	7.60	6.39	0.40
Max. value	0.19	0.33	0.59	0.022	10.43	1.60	36	53	15	7.45	0.71
Mean	0.16	0.30	0.51	0.02	8.11	1.50	28.3	43.3	10.6	6.75	0.47
CV	8.4	5.5	4.2	6.9	11.7	3.4	7.7	10.4	14.2	2.2	11.0

Mineralization Potentials

The mineralization potential of the four pesticides was tested using a modified version of a radiorespirometric microplate method. [P-methylene-^{14}C]glyphosate (>99 % radiochemical purity) was purchased from IZOTOP, Institute of Isotopes (Budapest, Hungary). Radioactive pesticide solutions (10 mg/mL, approximately 870 Bq/mL) were prepared by dissolving appropriate amounts of radioactive pesticide and the corresponding non-labeled pesticide in sterile water. For each of the 65 homogenized soil samples, subsamples of 0.5 g were transferred to microplate wells, one microplate for each of the four pesticides and one subsample per pesticide. The microplates were 96-well polypropylene microplates (Nunc 278752) with a well volume of 2.0 mL to minimize oxygen depletion. Fifty microliters of ^{14}C-labeled pesticide solution was added to all wells, corresponding to an initial pesticide concentration of 1 mg/kg soil. The microplates were sealed with PCR sealing tapes on which 96 $^{14}CO_2$ traps (Ca(OH)-amended filter paper discs) were placed in a pattern corresponding to the microplate wells. Polyurethane foam sheets (the size of a microplate lid) were placed on top of the sealing tapes, microplate lids were added, and the plates and lids were held tightly together with strong rubber bands. The sealing tapes were changed after approximately 2, 3, 6, 10 16, 23, 37, 51, 65, 80, 106, 120, 134, and 148 days of incubation at 10 °C. The trapped $^{14}CO_2$ from each well, captured on the $Ca(OH)_2$-impregnated filters, was quantified from a standard series of $NaH^{14}CO_3$ using digital autoradiography and subsequent digital image analysis as described by Hybholt et al. (2011).

Mineralization Kinetics

A two-parameter exponential model (first-order kinetics, Eq. 1) was used to fit the mineralization curves for glyphosate and bromoxyniloctanoate.

$$y = a \cdot (1 - e^{-bt}) \qquad (1)$$

where y is the accumulated $^{14}CO_2$ (% of added ^{14}C) released at time t (day), a is the maximum ^{14}C mineralized (% of added ^{14}C), and b is the mineralization rate constant (day^{-1}).

Since we were interested in estimating the in-situ mineralization potentials, we fitted only the first 23 days of mineralization, where the mineralization followed first-order kinetics. The initial rate at time zero was then calculated from the first derivative function (Eq. 2).

$$dy/dt_0 = ba \qquad (2)$$

A linear regression model (Eq. 3) was used to fit the mineralization curves for diflufenican and bentazone.

$$y = a + bt \qquad (3)$$

For diflufenican and bentazone, the models are based on the mineralization data from days 23–84 and 16–65, respectively. This was done in order to capture the initial, linear part of the mineralization curves from the first detection of mineralization in each of the two cases. The slope of the linear models was used as an estimate of the initial mineralization rate.

Two-Dimensional Interpolation and Statistical Analysis

The spatial, field-scale variation in soil texture, organic carbon content and the mineralization rates were mapped using minimum curvature interpolation with regularized spline interpolation in ArcMap 10.1. The number of points used in the calculation of each interpolated cell was set to 12 and the weight parameter to 0.1. The mineralization rates were correlated to soil physical and chemical parameters using the linear correlation coefficient (R^2), as it shows the fraction of the variation in the mineralization potentials that can be explained by the variation in the physical or chemical soil parameters. Coefficients of variation (CVs) for the pesticide mineralization rates and the soil parameters were calculated as the standard deviation divided by the mean and are given as percentage.

Most Probable Number of Pesticide Degraders

The most probable numbers (MPNs) of cultivable glyphosate-, bromoxyniloctanoate-, diflufenican-, and bentazone degraders were estimated by a modification of the above microplate radiotracer method. To represent the gradients in clay and organic carbon across the field, selected samples were pooled into groups with high clay and low organic carbon content, low clay and high organic carbon content, and intermediate clay and organic carbon content (five to seven subsamples for each group: group A with

17.6–18.9 % clay and 1.8– 1.9 % organic carbon, group B with 14.2–14.3 % clay and 2.0–2.1 % organic carbon, and group C with 16.1– 6.2 % clay and 1.9–2.0 % organic carbon).

A well was considered mineralization-positive if the accumulated amount of $^{14}CO_2$ at the end of the experiment (148 days at 10 °C) exceeded 5 % of the initially added ^{14}C-labeled pesticide. The MPNs were calculated according to Hurley and Roscoe (1983) from the distributions of positive and negative microplate wells. The lower detection limit was calculated by assuming only one mineralization-positive well at the lowest dilution (10 - fold), and the upper limit was calculated from only one mineralization-negative well at the highest dilution (21,870-fold).

Results

Pesticide Mineralization

The MPNs of the microbial degrader populations were for glyphosate and bromoxyniloctanoate above the detection limit of 6.6×10^4 cells/g soil, which indicates a large potential for microbial degradation of these pesticides. This was reflected in the rapid mineralization without any-lag phase of both glyphosate and bromoxyniloctanoate (Fig. 2). After a fast, immediate phase, the mineralization of glyphosate leveled off at 10–20 % and bromoxyniloctanoate at 13–26 %. Diflufenican and bentazone both showed slow linear mineralization with a lag-phase, and both pesticides reached very low mineralization levels (diflufenican 1–5 %, bentazone 3–7 %) within the 148 days of the experiment (Fig. 2). The first mineralization was detected on day 23 for bentazone and on day 37 for diflufenican. We did not detect any microorganisms that could utilize diflufenican or bentazone as a source of carbon and energy (MPN <4 cells/g soil), which probably explains the long lag-phases in the mineralization of these two herbicides. The mineralization of bromoxyniloctanoate showed the best model fits (R^2 = 0.980–0.996, average 0.992, Fig. 3), whereas the glyphosate mineralization was slightly underestimated within the first 3 days and slightly overestimated the following 13 days (R^2 = 0.933–0.987, average 0.968). The model fits for diflufenican mineralization (R^2 = 0.734–0.995, average 0.963) and bentazone mineralization (R^2 = 0.850–1.00, average 0.992) were more variable.

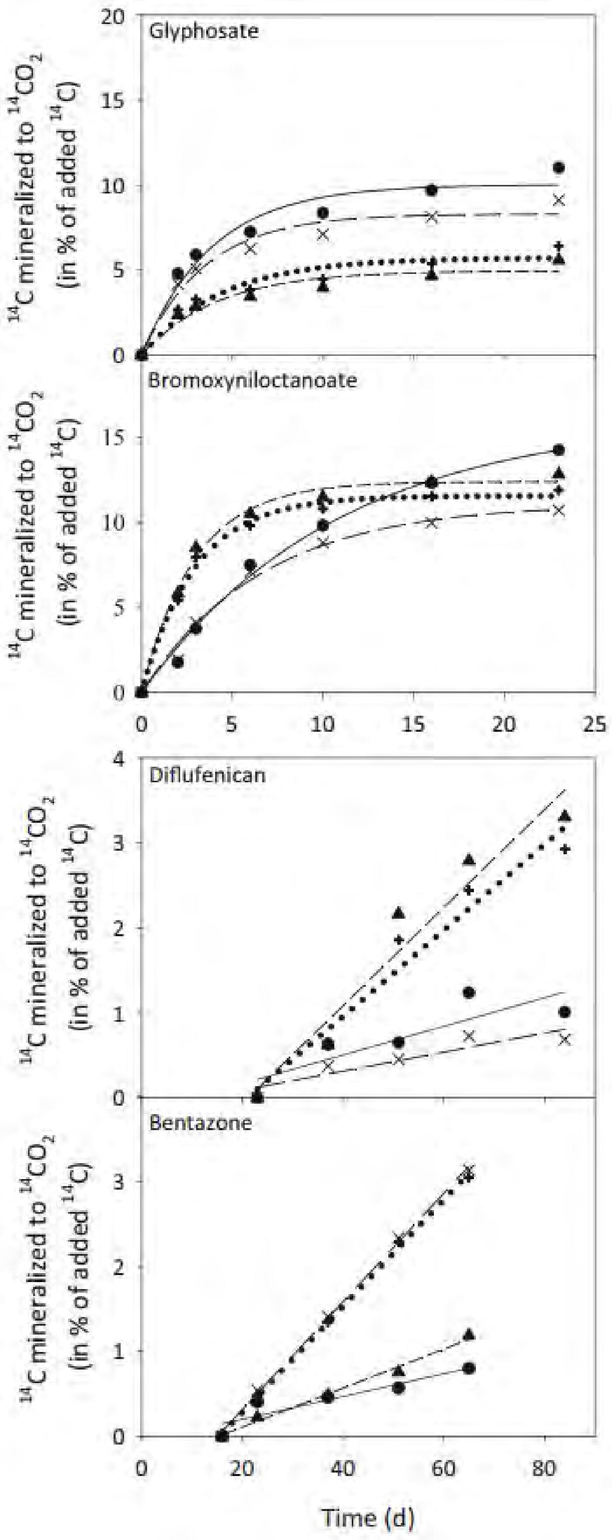


Fig. 3 Examples of herbicide mineralizations and the corresponding model fits. The data represent for each herbicide the two soil samples with the lowest initial mineralization rate and the two with the highest initial mineralization rate within the fitted time period.

Field-Scale Variation in Pesticide Mineralization Rates

The spatial variability of the initial mineralization rates, derived either from the initial rate at time zero for glyphosate and bromoxyniloctanoate or as the slope of the linear regression models for diflufenican and bentazone, is depicted in Fig. 4. Throughout the field, the initial glyphosate mineralization rates varied from 12.1 to 26.0 µg/(kg day) (average 17.1 µg/(kg day), CV=16.7 %), with a slight indication of lower mineralization in the southern part of the field. Bromoxyniloctanoate had the largest initial mineralization rates varying from 14.9 to 42.0 µg/(kg day) (average 29.6 µg/(kg day), CV=16.5 %). Diflufenican and bentazone showed very limited mineralization of only 0.11– 0.58 µg/(kg day) (average 0.32 µg/(kg day), CV= 24.7 %) and 0.13–0.64 µg/(kg day) (average 0.47 µg/(kg day), CV= 22.4 %).

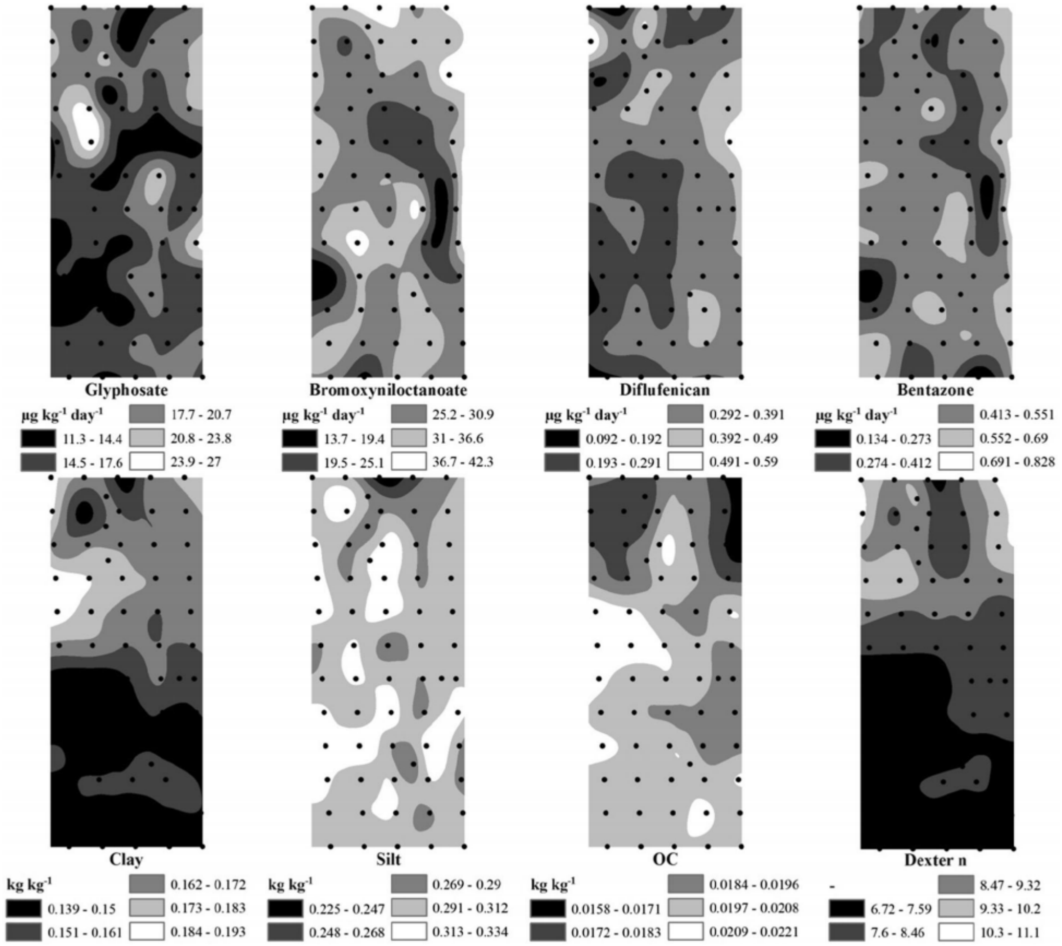


Fig. 4. Spatial distributions of herbicide mineralization rate, clay-, silt-, sand-, and organic carbon (OC) content, and Dexter n (clay/OC ratio). The dots denote the sampling points (n=65).

Table 3. The linear correlation coefficients (R^2) between the pesticide mineralization rates and the basic soil parameters

	Clay (<2 µm)	Silt (2–50 µm)	Sand (0.05–2 mm)	OC	Dexter *n*	Bulk density	Al	Fe	P	pH	EC
Glyphosate	0.09	0.01	0.00	0.11	0.17	0.06	0.06	0.03	0.10	0.00	0.00
Bromoxyniloctanoate	0.01	0.10	0.04	0.04	0.04	0.01	0.08	0.04	0.05	0.05	0.01
Diflufenican	0.06	0.02	0.00	0.06	0.10	0.03	0.01	0.01	0.07	0.00	0.00
Bentazone	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01

Mineralization Rates and Soil Characteristics

The range, mean, and CV for the measured soil parameters are reported in Table 2. Gradients in clay and organic carbon content run in opposite directions within the field. Thus, highest clay contents and lowest organic carbon contents were found in the northern part of the field and lowest clay and highest organic carbon contents were found in the southern part of the field (Fig. 4). The ratio between clay and organic carbon, Dexter *n*, therefore increased from south to north (Fig. 4). The mineralization rates for each of the four pesticides generally showed no correlation or very little correlation to the soil parameters

(Table 3). The highest correlation was between the glyphosate mineralization and the Dexter *n*, but this correlation was also weak (R^2=0.17). Linear correlations between the mineralization rates of the four pesticides are reported in Table 4. As in Table 3, the correlation coefficients are weak and the strongest correlation was between the mineralization rates of bromoxyniloctanoate and bentazone (R^2=0.16).

Table 4. The linear correlation coefficients (R^2) between the pesticide mineralization rates

	Glyphosate	Bromoxyniloctanoate	Diflufenican	Bentazone
Glyphosate	1.00	0.07	0.08	0.01
Bromoxyniloctanoate		1.00	0.02	0.16
Diflufenican			1.00	0.03
Bentazone				1.00

Table 5. Number of groundwater samples from H1 and H2 analyzed for glyphosate, aminomethylphosphonic acid (AMPA), bentazone, and 2-amino-N-isopropylbenzamide

	Number of samples, *n*					
	H1.1	H1.2	H1.3	H2.1	H2.2	H2.3
Glyphosate	8	48	8	10	46	8
AMPA	8	48	8	10	46	8
Bentazone	10	63	10	7	46	6
2-Amino-*N*-isopropylbenzamide	4	29	4	4	29	4

Field-Scale Leaching

Water from the two horizontal wells (H1 and H2, Fig. 1) was analyzed for glyphosate and bentazone and their main metabolites aminomethylphosphonic acid (AMPA) and 2-amino-N-isopropylbenzamide (Table 5). Glyphosate was applied five times on the field during the period from 1988 to soil sampling in 2011 with two applications within the monitoring period (2001 and 2003, Table 1). The glyphosate and AMPA contents in water from different subsections of H1 and H2 were analyzed from 2001 to 2005. Glyphosate was not detected in any of the samples. The glyphosate degradation product AMPA, however, was detected in 6.3 % of the analyzed samples from H1.2 and 25 % of the analyzed samples from H1.3 (Fig. 5). None of the AMPA concentrations exceeded the drinking water quality criterion of 0.1 µg/L. Bentazone was applied four times on the field from 1994 to 2011, and two of these applications were within the monitoring program (2003 and 2009, Table 1). Bentazone was analyzed for in the periods from 2003 to 2006 and from 2009 to 2011. The bentazone metabolite was analyzed for only in the period from 2003 to 2006. In total, bentazone was detected in 20 % of the samples from H1, 19.5 % of the H1.2 samples, and 10 % of the H1.3 samples (Fig. 5). One of the detections in H1.2 was above the criterion of 0.1 µg/L. The bentazone degradation product, 2-amino-N-isopropylbenzamide, was not detected in any of the analyzed samples. The metabolite, however, was analyzed for only in the period from 2003 to 2006, whereas bentazone was analyzed for in the periods from 2003 to 2006 and from 2009 to 2011. The horizontal monitoring well, H2, was suspended from 2009.

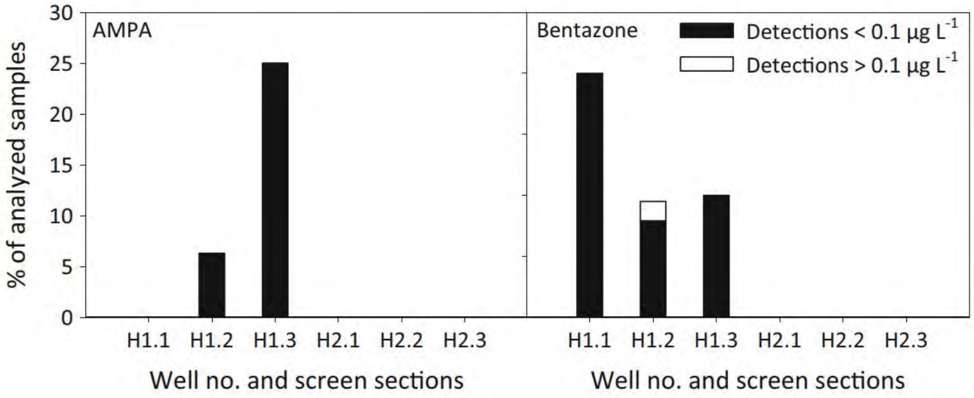


Fig. 5. Percentage of samples AMPA Bentazone from the two horizontal wells, H1 and H2 (Fig. 1), containing detectable levels of the glyphosate degradation product AMPA or bentazone. Water was collected monthly (H1.2 and H2.2, n=29–63) or half yearly (remaining filter sections, n=4–10)

Discussion

In this study, we have investigated the potential mineralization of four herbicides commonly used in agriculture (Miljøstyrelsen 2014). These herbicides represent different physico-chemical properties with very different literature reports on hydrophobicity and sorption. Glyphosate was an easily mineralized, hydrophilic compound with strong sorption to clay loam. Bromoxyniloctanoate was also easily mineralized and strongly sorbing, but hydrophobic. Diflufenican was difficult to mineralize, hydrophobic, and strongly sorbing, and bentazone was also difficult to mineralize, in spite of being hydrophilic with low sorption.

It is clear from the above that bioavailability, expressed as the soil/water distribution coefficient K_d, did not determine the different mineralization patterns between the four herbicides. One reason could be that we added the Tween-80 detergent to the solutions of bromoxyniloctanoate and diflufenican to be able to handle these compounds in aqueous solution. Also, bentazone was not mineralized to any great extent in spite of high bioavailability, which suggest a microbiological limitation rather than a physico-chemical limitation. Glyphosate, in contrast, was easily mineralized in spite of a high distribution coefficient and thus low bioavailability, indicating that sorption may be less important when degraders are very numerous in the soil.

We used first-order kinetics to quantify the mineralization of glyphosate and bromoxyniloctanoate for the first 23 days. Linear regression was used to quantify the mineralization of diflufenican and bentazone covering the time periods 23–84 and 16–65 days, respectively. The linear mineralization patterns indicate that these pesticides were probably mineralized by slow co-metabolic metabolism without growth of the degrader organisms, which is consistent with the absence of bacteria that could utilize them for growth. The 2–3-week delay in mineralization may imply that the degrader organisms were fungi.

The mineralization potentials of bromoxyniloctanoate, diflufenican, and bentazone did not correlate with the gradients in clay and organic carbon across the field or any other of the measured soil parameters. Only the glyphosate mineralization rates tended to increase towards the northern part of the field, correlating slightly with increasing clay and decreasing organic carbon contents (Table 3). The highest correlation was, however, between the glyphosate mineralization and Dexter *n*, so that it was the ratio between clay and organic carbon more than the total contents that influenced the glyphosate mineralization.

Our results indicate that the development of generally valid models for predicting pesticide mineralization across field sites, based on simple soil characteristics and in-vitro mineralization rates, may be unrealistic. Furthermore, if the mineralization of two or more of the herbicides were determined by the same soil parameters, we would have seen correlations between these herbicides, which were not the case (Table 4). It seems difficult to connect pesticide mineralization (or degradation) and specific topsoil parameters, but what about pesticide mineralization and leaching? Though included in the analyses, we did not detect glyphosate in the samples from the horizontal monitoring wells, but the glyphosate degradation product, AMPA, was detected. In contrast, only bentazone was detected, and not the degradation product, 2-amino-Nisopropylbenzamide. All detections of AMPA and bentazone were from the H1 well that collected water from the northern part of the field. Neither AMPA nor bentazone was detected in the samples from H2 which collected water from the southern part of the field. This pattern does not correspond well with the rather random distribution of mineralization potentials of the two herbicides (Fig. 4).

Conclusion

Glyphosate was an easily mineralized, hydrophilic compound with strong sorption to clay loam. Bromoxyniloctanoate was also easily mineralized and strongly sorbing, but hydrophobic. Diflufenican was difficult to mineralize, hydrophobic, and strongly sorbing, and bentazone was also difficult to mineralize, in spite of being hydrophilic with low sorption. It is clear from the above that bioavailability, expressed as the soil/water distribution coefficient K_d, did not determine the different mineralization patterns between the four herbicides. The linear mineralization patterns indicate that these pesticides were probably mineralized by slow co-metabolic metabolism without growth of the degrader organisms, which is consistent with the absence of bacteria that could utilize them for growth.

The mineralization potentials of bromoxyniloctanoate, diflufenican, and bentazone did not correlate with the gradients in clay and organic carbon across the field or any other of the measured soil parameters. Only the glyphosate mineralization rates tended to increase towards the northern part of the field, correlating slightly with increasing clay and decreasing organic carbon contents. Our results indicate that the development of generally valid models for predicting pesticide mineralization across field sites, based on simple soil characteristics and in-vitro mineralization rates, may be unrealistic. Furthermore, if the mineralization of two or more of the herbicides were determined by the same soil parameters, we would have seen correlations between these herbicides, which were not the case (Table 4). It seems difficult to connect pesticide mineralization (or degradation) and specific topsoil parameters.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The study reports the results from an aerobic soil mineralization experiment with glyphosate (among other pesticides) in the lab. In parallel the leaching behavior of the substances in the field where the soils used in the mineralization experiment come from, was investigated. The study does not follow guideline procedures. The provided data does not allow to check the validity of the study against current guidelines. Furthermore, no data on glyphosate content per sampling data and no half-lives were provided, i.e. no endpoint is available.

The study is therefore classified as reliable with restrictions (Category 2).