グリホサートカリウム塩

要旨及び評価結果

（環境動態）

検索期間：2010 年 1 月 1 日～2019 年 12 月 31 日

評価対象：適合性区分 a に該当する文献

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1. Information on the study

Data point:	KCA 7.1.3.1.1
Report author	Albers, Ch., et al.
Report year	2018
Report title	Soil Domain and Liquid Manure Affect Pesticide Sorption in Macroporous Clay Till
Document No	Journal of Environmental Quality
Guidelines followed in study	OECD 106
Deviations from current test guideline	1 mM $CaCl_2$ solution (standard: 10 mM solution), at 10°C (standard: 20 – 25°C); 4 concentrations (standard: 5), no explicit measurements of concentrations in the solid phase, i.e. no real mass balances available
GLP/Officially recognised testing facilities	No, not conducted under GLP/Officially recognised testing facilities (literature publication)
Acceptability/Reliability:	Reliable with restrictions (the study does not meet the validity criteria as required according the OECD 106 guideline)

2. Full summary of the study according to OECD format

In this study, it was observed that sorption of strongly sorbing pesticide, glyphosate, varied by more than an order of magnitude across soil domains in 5-m-deep clay till profiles with biopores and fractures. Eight soil domains were identified in each of the profiles: five matrix soils and three in the macropores. Glyphosate showed high variation in sorption between fractures and matrix soil from the same depths. The domain-specific sorption of both tebuconazole and glyphosate was, however, overruled by dilute liquid manure. Liquid manure unexpectedly had a greater effect on glyphosate sorption, which was strongly decreased by dissolved organic matter and phosphate in the manure. The variation in sorption across domains, as well as the effects of liquid manure, should be taken into account when assessing leaching risks.

Materials and methods

Soil sampling

Soil was sampled at two locations, Gjorslev (55°20.988′N, 12°23.672′E) and Lund (55°14.698′N, 12°17.418′E) in the Stevns area of southeastern Denmark. At both sites, soil profiles were excavated to a depth of ~5 m. We sampled composite soil samples from eight domains that were clearly separated on the basis of different soil horizons and the presence or absence of biopores and fractures (Fig. 1). The surface of wormholes (Domain 3) was sampled by scraping off the outer 1 to 2 mm of the pore walls. Deeper soil pores surrounding decayed roots were dissected out, and the outer Fe oxides were scraped off with a knife to sample only the 5- to 10-mm wide, inner, greyish part (Domain 5). The surface of even deeper larger fractures with Mn and Fe oxide coatings was sampled by scraping off the outer 1 to 2 mm (Domain 7). At least 240 g soil was sampled from each fracture domain to have sufficient material for sorption experiments and analysis of sediment parameters. The matrix soil samples (bulk soil in the case of the plow layer, Domain 1) were also compositely sampled, comprising ~1 kg from 20 to 50 subsamples. All soil samples were sieved twice through a 2-mm sieve and stored at 2°C. Soil samples from the reduced zone were packed in airtight aluminum tape on location and sieved in a glove box under a reducing N_2/H_2 atmosphere. The fraction <2 mm was stored in anoxic jars at 2°C.

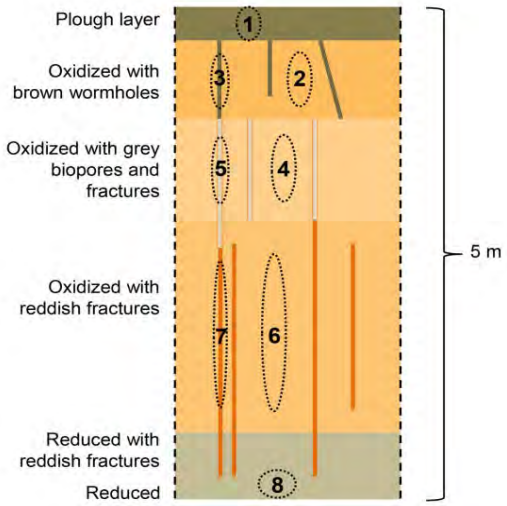


Fig. 1. A schematic representation of the soil profiles in the Gjorslev and Lund sites and their associated soil domains (Domains 1-8). The approximate depth of the lower boundary of each matrix soil domain was (Gjorslev/Lund): Domain 1 (35/35 cm), Domain 2 (105/130 cm), Domain 4 (200/260 cm), and Domain 6 (390/420 cm).

Characterization of the Soil Domains

Soil texture was determined by sieving (0.063-2 mm) and by laser diffraction (<0.063 mm, Mastersizer 3000, Malvern). Water content was determined by drying at 105°C for 24 h. Total carbon and total organic carbon (TOC) were determined on an elemental analyzer (Leco CS-200) on dried (50°C) and crushed samples as they were (total C) or after acid treatment to remove carbonates (TOC). Total inorganic C was calculated as the difference between total C and TOC. The pH was determined in a 1:2.5 soil/liquid slurry with Milli-Q water or 10 mM $CaCl_2$. The $pH_{sorption}$ (i.e., the pH measured at conditions similar to those during the sorption experiments) was also determined with $CaCl_2$, pesticide, and NaN_3 concentrations similar to those used in the sorption experiments. Soil-specific surface area was measured using a Coulter SA 3100 BET analyzer (Coulter Corporation) and calculated using the Brunauer-Emmett-Teller equation. Total Fe and ferrous Fe^{2+} were measured as described by Komadel and Stucki (1988). Iron and manganese oxides were extracted using the citrate-bicarbonate-dithionite (CBD) method and quantified by atomic absorption spectroscopy (PerkinElmer AANALYST 400). Amorphous Fe and Al oxides were extracted using ammonium oxalate solution. Cation exchange capacity was determined by standard method (Chapman, 1965). All analyses of soil parameters were single measurements.

Characterization of Liquid Pig Manure and Soil Extract

Liquid pig manure was sampled from a conventional farm that raised sows and offspring (weaner production) and was stored at 2°C for 4 wk. Topsoil extract was obtained by horizontal rolling of plow layer soil and Milli-Q water (1:1) for 24 h at 22°C. The liquid manure and the topsoil suspension were centrifuged (15 min, 3500 g), and the extracts were stored as frozen subsamples to be used in the subsequent sorption experiments. After thawing, the extracts were sonicated for 30 min before use in sorption experiments. Total organic C in soil and manure extracts and in the aqueous phase of selected sorption experiments was analysed on a TOC analyzer (TOC-Vcph, Shimadzu) after filtration (5 µm polyvinyl difluoride [PVDF], Millipore). Conductivity was determined using a conductivity probe (LE703, Mettler Toledo). The concentrations of major inorganic cations and anions were determined by ion chromatography (Metrohm 819 with a Metrosep A 150/4.0 column). Total Cu, Zn, Al, Ba, Fe, Mn, and S contents were measured on an inductively coupled plasma mass spectrometer (Elan 6100DRC, PerkinElmer) using a multielement scanning method (TotalQuant, PerkinElmer).

Chemicals

(P-methylene-^{14}C)-glyphosate (radiochemical purity = 99 %, specific activity = 122 MBq/mmol) were purchased from Izotop. Glyphosate (purity 97 %) was purchased from Dr. Ehrenstorfer, Germany.

Sorption of Glyphosate

Sorption experiments were performed using a batch-equilibrium method inspired by the Organization for Economic Cooperation and Development (OECD) guideline (OECD, 2000). Eleven-milliliter Pyrex glass vials with 15-mL polypropylene centrifuge vials were used for glyphosate. The final soil/liquid ratio was in all vials 1:10, which in general resulted in between 20 and 95 % sorption of the added pesticide. In each vial, 1 g of soil (wet weight) was mixed with 1 mM $CaCl_2$ solution (8.0 - 9.7 mL) and NaN_3 (20 µL of a 100 g/L solution) was added to repress biodegradation of the pesticides during incubation. One millimolar $CaCl_2$ was used, since it better represented the concentrations in the local soil water than the 10 mM $CaCl_2$ suggested in the OECD guideline. The soil-liquid slurries were then equilibrated at 10°C for 24 h by vertical rotation (7 revolutions/min) before addition of ^{14}C-labeled pesticide and, for the two highest pesticide concentrations, nonradioactive pesticide (both dissolved in 1 mM $CaCl_2$). Initial total concentrations of glyphosate were 30, 120, 1200 (thereof 120 µg/L radioactive glyphosate) and 12,000 µg/L (thereof 120 µg/L radioactive glyphosate). After addition of the pesticides, the vials were rotated at 10°C for another 24 h. The vials were then centrifuged at 1250 g (glass vials) or 3000 g (plastic vials) for 15 min. The pesticide concentration of the aqueous phase was determined by liquid scintillation counting (Tri-Carb 2810 TR, PerkinElmer) of the ^{14}C activity in duplicate 1-mL samples. The ^{14}C activity was counted for 30 min or until 1 % uncertainty (2S, 95 % confidence limit). Sorption to the vials was tested by including reagent blanks without soil, but no such sorption was found. The pesticide concentration in the solid phase (soil) was calculated based on pesticide missing in the aqueous phase.

The sorption experiments were all performed in duplicate. The difference in distribution coefficients between duplicates was <15%, and in most cases, it was <5% . All sorption experiments with soil from reduced zones wer prepared under a N_2 /H_2 atmosphere in a glove box with solutions that had been flushed with N_2.

Freundlich Sorption Models

Glyphosate sorption was described by an extended Freundlich equation, as suggested by de Jonge et al. (2001):

$$C_s = K_{Fex} C_w^{n_{ex}} C_w^{-D}$$

where K_{Fex} is the extended Freundlich coefficient, n_{ex} is the extended Freundlich exponent, and D is a parameter that adds extra curvature to the line in a double-logarithmic plot (i.e., increases the concentration sensitivity compared with the simple Freundlich model). The extended Freundlich model was fitted to the experimental data by nonlinear optimization.

Results

Soil Domains

Both soil profiles had a characteristic depth zonation with eight visually different domains based on different layers and the presence or absence of macropores (Fig. 1). At the Gjorslev site, the upper 35 cm was a relatively homogenous dark brown (10YR 3/2) plow layer (Ap horizon, Domain 1) rich in organic matter (Table 1). The plow layer was followed by an oxidized layer of variable color with a predominantly yellow-brown (10YR 4/4) matrix (Domain 2) perforated by brown (10YR 4/3), vertical wormholes where the soil was enriched in organic matter (Domain 3). Many of the wormholes were present within fractures (geological and desiccation) and extended to a depth of ~ 110 cm. The following layer (105–200 cm) was oxidized with a light brown (10YR 5/3) matrix (Domain 4) and numerous small biopores from decayed plant roots with a diameter of ~ 1 mm. The pores were surrounded by gray (10YR 8/1) pore soil with a diameter of 5 to 10 mm (Domain 5) and a thin, outer layer of Fe oxides. This layer also had gray fractures that extended into the next layer where they changed to reddish. The next layer (200–390 cm) was also oxidized with a light brown (10YR 5/3) matrix (Domain 6) and many parallel fractures (Domain 7). The surface of the larger fractures was coated with Fe and Mn oxides of variable reddish to almost black colors (10YR 4/6). This domain was devoid of visible biopores. The matrix was

reduced at the bottom of the profile (Domain 8), as visible by its gray color (5Y 5/1). The oxidized reddish fracture surfaces extended ~ 50 cm into the reduced zone. Similar horizons and domains were present in the Lund profile, although at slightly different depths (Table 1). Soil parameters for both profiles are available in Table 1 and Table 2.

Table 1. **Main soil parameters from the soil profiles.**

Domain	Sample depth		TOC†		$pH_{Sorption}$‡		Fe_{CBD}§		Mn_{CBD}¶		Surface area	
	Gjorslev	Lund	Gjorslev	Lund	Gjorslev	Lund	Gjorslev	Lund	Gjorslev	Lund	Gjorslev	Lund
	m		%				g kg^{-1}		mg kg^{-1}		m^2 g^{-1}	
1. Plough layer	0.1–0.3	0.2–0.3	0.89	0.67	7.33	7.38	5.6	4.2	286	204	4.1	4.2
2. Matrix	0.6–0.8	0.7–1.2	0.14	0.12	7.30	7.16	9.0	6.4	333	261	16.7	18.5
3. Wormholes	0.4–0.8	0.7–1.2	0.40	0.37	7.88	7.30	7.6	5.9	269	202	12.1	11.3
4. Matrix	1.5–1.6	2.0–2.6	0.05	0.05	8.20	8.26	4.7	3.9	170	153	12.4	13.0
5. Gray macropores	1.1–2.0	2.0–2.6	0.05	0.06	8.19	8.29	2.1	1.1	24	31	11.0	11.6
6. Matrix	2.5–3.5	3.0–3.5	0.05	0.05	8.16	8.31	4.5	2.9	97	97	11.8	12.2
7. Reddish fractures	2.5–3.5	3.0–3.6	0.05	0.06	8.09	8.28	18.8	12.6	798	910	16.0	21.2
8. Reduced zone	4.3–4.5	4.5–4.8	0.18	0.17	8.24	8.53	3.6	0.74	88	44	8.4	11.9

† TOC, total organic C.

‡ $pH_{Sorption}$, the pH measured at conditions similar to those during the sorption experiments.

§ Fe_{CBD}, total Fe oxides (extractable with citrate–bicarbonate–dithionite).

¶ Mn_{CBD}, total Mn oxides (extractable with citrate–bicarbonate–dithionite).

Table 2. **Major soil paramters**

Domain no.	Description	Sample depth (m) Gjorslev / Lund	$pH_{Sorption}$ Gjorslev / Lund	pH_{CaCl2} Gjorslev / Lund	pH_{H2O} Gjorslev / Lund
1	Plough layer	0.1-0.3 / 0.2-0.3	7.33 / 7.38	6.49 / 6.86	7.64 / 7.77
2	Matrix	0.6-0.8 / 0.7-1.2	7.30 / 7.16	6.81 / 6.86	8.09 / 7.92
3	Wormholes	0.4-0.8 / 0.7-1.2	7.88 / 7.30	7.21 / 6.80	8.35 / 7.80
4	Matrix	1.5-1.6 / 2.0-2.6	8.20 / 8.26	7.67 / 7.65	8.67 / 8.63
5	Grey macropores	1.1-2.0 / 2.0-2.6	8.19 / 8.29	7.64 / 7.79	8.77 / 8.77
6	Matrix	2.5-3.5 / 3.0-3.5	8.16 / 8.31	7.62 / 7.47	8.73 / 8.68
7	Reddish fractures	2.5-3.5 / 3.0-3.6	8.09 / 8.28	7.58 / 7.56	8.52 / 8.59
8	Reduced zone	4.3-4.5 / 4.5-4.8	8.24 / 8.53	7.54 / 7.59	N.D. / 8.27

Domain no.	Description	TOC (%) Gjorslev / Lund	TIC (%) Gjorslev / Lund	Surface (m^2/g) Gjorslev / Lund	CEC (cmol/kg) Gjorslev / Lund
1	Plough layer	0.89 / 0.67	0.32 / 0.20	4.1 / 4.2	11.0 / 9.25
2	Matrix	0.14 / 0.12	0.08 / 0.11	16.7 / 18.5	12.5 / 12.2
3	Wormholes	0.40 / 0.37	0.19 / 0.17	12.1 / 11.3	12.0 / 11.9
4	Matrix	0.05 / 0.05	1.95 / 2.41	12.4 / 13.0	8.77 / 7.52
5	Grey macropores	0.05 / 0.06	2.58 / 3.17	11.0 / 11.6	8.80 / 7.75
6	Matrix	0.05 / 0.05	1.88 / 2.98	11.8 / 12.2	8.53 / 7.15
7	Reddish fractures	0.05 / 0.06	1.83 / 2.67	16.0 / 21.2	9.46 / 8.26
8	Reduced zone	0.18 / 0.17	2.21 / 2.85	8.4 / 11.9	7.05 / 4.79

Domain no.	Description	Clay (%) Gjorslev / Lund	Silt (%) Gjorslev / Lund	Fine sand (%) Gjorslev / Lund	Med. sand (%) Gjorslev / Lund	Coarse sand (%) Gjorslev / Lund
1	Plough layer	6.5 / 6.3	41.1 / 39.4	35.9 / 35.7	10.6 / 12.3	6.0 / 6.3
2	Matrix	8.5 / 7.8	46.1 / 43.2	30.0 / 32.3	9.2 / 11.2	6.2 / 5.5
3	Wormholes	8.6 / nd	42.4 / nd	33.2 / nd	9.1 / nd	6.6 / nd
4	Matrix	13.1 / 13.4	42.7 / 43.2	28.8 / 28.5	8.7 / 8.9	6.7 / 6.0
5	Grey macropores	12.9 / 12.5	43.1 / 41.0	27.6/ 25.5	8.8 / 8.8	7.5 / 12.2
6	Matrix	12.9 / 13.6	45.0 / 43.5	27.1 / 26.4	9.0 / 7.4	6.0 / 9.1
7	Reddish fractures	10.6 / 14.1	40.8 / 45.5	24.8 / 26.0	8.8 / 9.9	15.1 / 12.0
8	Reduced zone	10.7 / 14.3	51.3 / 45.5	24.7 / 28.3	8.0 / 7.6	5.3 / 4.3

Sorption Is Domain Specific

Glyphosate sorption followed the Freundlich model with a high concentration dependence ($0.87 < n < 1.32$, Table 3).

Table 3. Extended Freundlich parameters (glyphosate) for sorption to eight soil domains in the Gjorslev and Lund profiles.

Domain	Glyphosate‡					
	K_{Fex}		*n*		*D*	
	Gjorslev	Lund	Gjorslev	Lund	Gjorslev	Lund
1	130	72.5	1.06	1.06	0.040	0.036
2	443	947	1.04	0.98	0.041	0.043
3	239	496	1.07	0.97	0.045	0.040
4	536	125	0.88	1.12	0.039	0.042
5	3849	353	1.01	1.01	0.053	0.053
6	424	359	0.87	0.91	0.037	0.037
7	124	58.1	0.92	0.93	0.011	0.000
8	217	251	1.08	1.32	0.049	0.071

‡ K_{Fex}, the extended Freundlich coefficient; *D*, a parameter that adds extra curvature to the line in a double-logarithmic plot. K_{Fex} equals the predicted distribution coefficient at 1 μg L^{-1} glyphosate.

The extended Freundlich model fitted the sorption data of glyphosate very well, though with a tendency to slightly underestimate sorption at the lowest concentration (Fig. 2). Glyphosate sorption was very concentration dependent (Fig. 2), which is why the extended Freundlich model fitted the sorption data better.

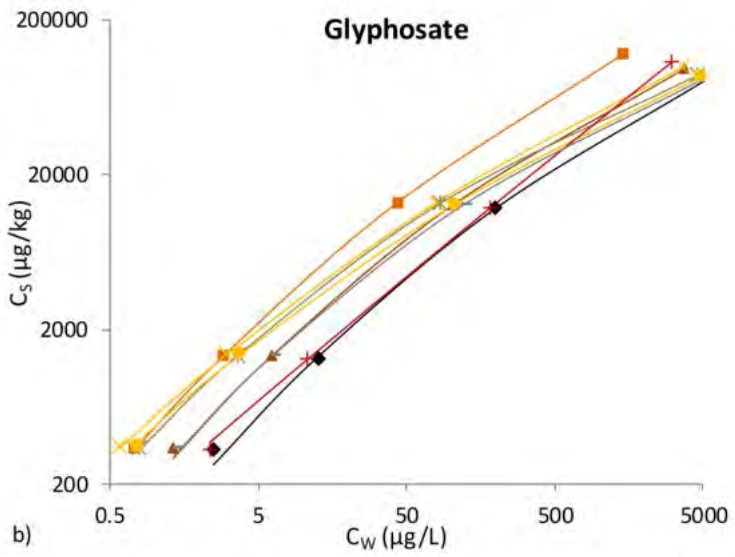


Fig. 2. Sorption isotherms for glyphosate (extended Freundlich model) in the eight soil domains from the Gjorslev profile. Note: Cs is the pesticide concentration in the soil phase, and Cw is the pesticide concentration in the aqueous phase.

The concentration dependence can be exemplified by Domain 6 (matrix soil), where the Kd at the lowest glyphosate equilibrium concentrations (0.8–0.9 mg/L) was 377 for Lund and 453 for Gjorslev, whereas at the highest equilibrium concentrations (4.2–4.8 mg/L), the Kd was only 18 for Gjorslev and 22 for Lund. Domain 7 (reddish macropores from same depth as Domain 6) was an exception with low sorption and little concentration dependence, with a Kd of 55 (Lund) to 137 (Gjorslev) at the lowest concentration and 33 to 34 at the highest. Hence, two very different sorption strengths and concentration dependencies were observed from the same soil depth. Also, at the 0.4- to 1.2-m depth, sorption of glyphosate varied in the two domains at both study sites, being twice as high in matrix soil (Domain 2) than in soil from the wormholes (Domain 3). This fits well with the much lower sorption of glyphosate to the plow layer, which shows some similarities with the wormholes.

There was no correlation between Fe oxide content (expressed either as total Fe oxides or amorphous Fe oxides) and glyphosate sorption (expressed as K_{Fex}) (Fig. 3). There was also no correlation when K_{Fex} was plotted against any other measured soil parameter.

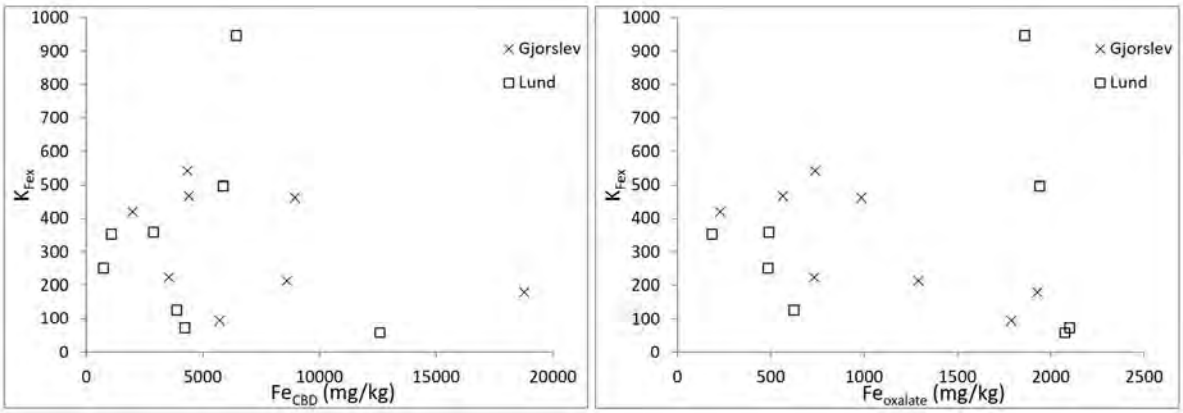


Fig. 3. Relationship between total iron oxide concentration (Fe_{CBD}) or amorphous iron oxides ($Fe_{oxalate}$) and sorption of glyphosate (K_{Fex}) in the eight soil domains at the two study sites. K_{Fex} was determined at a µg/L basis and therefore denotes the calculated partitioning coefficient at 1 µg/L.

It may be important to consider differences in pesticide sorption between soil domains from the same depth when modeling the risk of pesticide leaching. In clayey tills, most water transport takes place in the macropores; sorption studies, on the other hand, would normally be conducted on bulk soil samples, resembling the matrix samples in the present study. Most sorption studies are furthermore performed only with soil from the plow layer, and leaching in the actual fields may therefore be different from the leaching calculated from such sorption studies. For glyphosate, the leaching would most likely be higher than expected, since sorption to the soil of the upper biopores and especially to the surfaces of the metal oxide coated fractures is lower than in their corresponding matrix domains.

Topsoil Extract and Liquid Manure Extract Reduce Pesticide Sorption

The addition of topsoil extract had an effect on glyphosate sorption, decreasing sorption (K_{Fex}) by 3 to 37 % depending on the domain (Table 4), and the addition of liquid manure had an even larger effect. Ten percent liquid pig manure changed sorption (K_{Fex}) dramatically, with a decrease of 83 to 95 % in the Gjorslev Domains 1 to 6 and 8, and 76 to 83 % in the corresponding Lund domains (Fig. 4). Manure additionally changed the other extended Freundlich parameters (n and D), as the sorption of glyphosate was less concentration dependent when manure was present.

Table 4. Sorption parameters for glyphosate with different liquid treatments. Control is without any additions.

	Control			Topsoil extract (50%)			Manure (0.1%)			Manure (1%)			Manure (10%)		
Domain	K_{Fex}	n_{ex}	D	K_{Fex}	n_{ex}	D	K_{Fex}	n_{ex}	D	K_{Fex}	n_{ex}	D	K_{Fex}	n_{ex}	D
Gjor-1	130	1.06	0.040	86	1.16	0.044	96	1.17	0.046	76	1.09	0.037	16	1.14	0.028
Gjor -2	443	1.04	0.041	338	0.98	0.032	454	0.99	0.036	358	1.07	0.045	41	1.28	0.038
Gjor -3	239	1.07	0.045	193	1.07	0.042	240	1.09	0.048	239	1.08	0.045	37	1.13	0.028
Gjor -4	536	0.88	0.039	354	0.97	0.042	543	0.89	0.039	369	1.03	0.049	29	1.33	0.042
Gjor -5	384	1.01	0.053	295	1.02	0.049	408	0.98	0.049	249	1.14	0.057	28	1.28	0.039
Gjor -6	424	0.87	0.037	283	1.00	0.046	386	0.93	0.042	249	1.08	0.049	27	1.31	0.042
Gjor -7	124	0.92	0.011	142	0.99	0.023	201	0.86	0.012	262	0.98	0.030	67	1.14	0.032
Gjor -8	217	1.08	0.049	169	1.12	0.050	223	1.09	0.051	160	1.17	0.052	19	1.36	0.043
Lund-1	72	1.06	0.036	-	-	-	72	1.01	0.030	35	1.11	0.033	12	1.02	0.019
Lund-2	947	0.98	0.043	-	-	-	1026	0.96	0.044	657	1.00	0.043	47	1.20	0.035
Lund-3	496	0.97	0.040	-	-	-	472	0.96	0.038	262	1.07	0.045	23	1.25	0.036
Lund-4	125	1.12	0.042	-	-	-	244	0.98	0.039	253	1.09	0.050	30	1.27	0.040
Lund-5	353	1.01	0.053	-	-	-	350	1.04	0.055	258	1.09	0.053	18	1.39	0.044
Lund-6	359	0.91	0.037	-	-	-	354	0.97	0.045	241	1.07	0.047	30	1.27	0.040
Lund-7	58	0.93	0.000	-	-	-	116	0.85	0.000	259	0.88	0.016	88	1.12	0.031
Lund-8	251	1.32	0.071	-	-	-	264	1.33	0.073	265	1.33	0.073	14	1.29	0.035

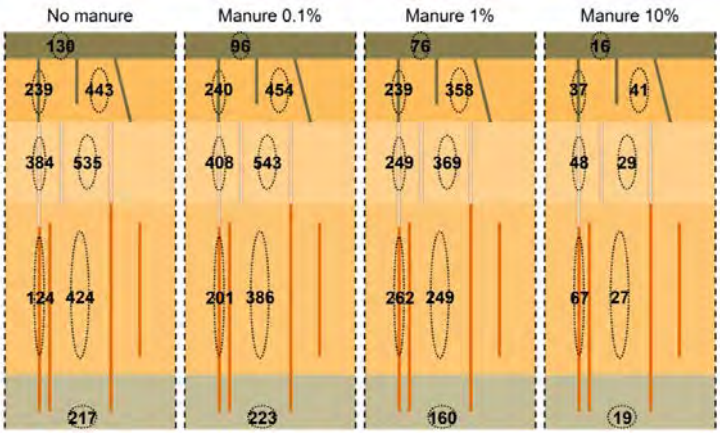


Fig. 4. Effect of liquid pig manure extract (%, v/v) on the sorption (K_{Fex}) of glyphosate in the Gjorslev soil domains. The K_{Fex} equals the predicted distribution coefficient at a glyphosate concentration of 1 µg L^{-1}

Why Do Topsoil Extract and Manure Reduce Glyphosate Sorption

Several soil water parameters have been suggested to influence glyphosate sorption. These include pH, phosphate, divalent metal ions like Cu^{2+} and Zn^{2+} and dissolved organic matter.

Change in pH cannot explain the general decrease in sorption when topsoil extract or pig manure was added.

The manure had a high conductivity (21,900 µS/cm, Table 5). In parallel experiments, it was observed an increase in sorption at increased ionic strengths (data not shown), which has also been reported previously in the literature. The high ionic strength in the manure therefore cannot explain the decreased sorption.

Table 5: Major analyzed parameters for the liquid manure and topsoil extracts. ND = not determined.

Parameter	Unit	Liquid pig manure	Topsoil extract
DOC	mg/L	2648	32
Dry matter	%	1.5	ND
Conductivity	µS/cm	21900	214
PO_4^{3-}	mg/L	182	<0.1
Cu	mg/L	4.9	0.03
Zn	mg/L	7.5	0.12
Fe	mg/L	10.2	22.7
Al	mg/L	0.28	32.0
Na^+	mg/L	623	0.35
K^+	mg/L	2500	0.57
Ca^{2+}	mg/L	<13	3.8
Mg^{2+}	mg/L	<8	0.15
F^-	mg/L	<0.5	1.2
Cl^-	mg/L	1481	4.5
Br^-	mg/L	30.7	0.86
NO_3^-	mg/L	0.64	33.1
SO_4^{2-}	mg/L	10.4	10.3
Ba	mg/L	0.2	0.16
Mn	mg/L	0.4	0.18
S	mg/L	668	11.2
Density	-	1.02	ND

Both the humic and fulvic acid fractions of soil organic matter decreased glyphosate sorption, when added to soil (Fig. 5).

Divalent metal ions and phosphate would be relevant only with manure addition. Divalent metal ions (Cu^{2+} and Zn^{2+}) at concentrations corresponding to 1 and 10% pig manure had no effect on sorption in Domain 1 and increased sorption in Domain 6 (Fig. 5). The Cu^{2+} and Zn^{2+} ions are therefore not likely to have caused the manure effect. Phosphate, on the other hand, reduced glyphosate sorption at concentrations corresponding to those in the pig manure (Fig.5). Both phosphate and dissolved organic matter are therefore likely candidates to explain the manure effect on glyphosate sorption.

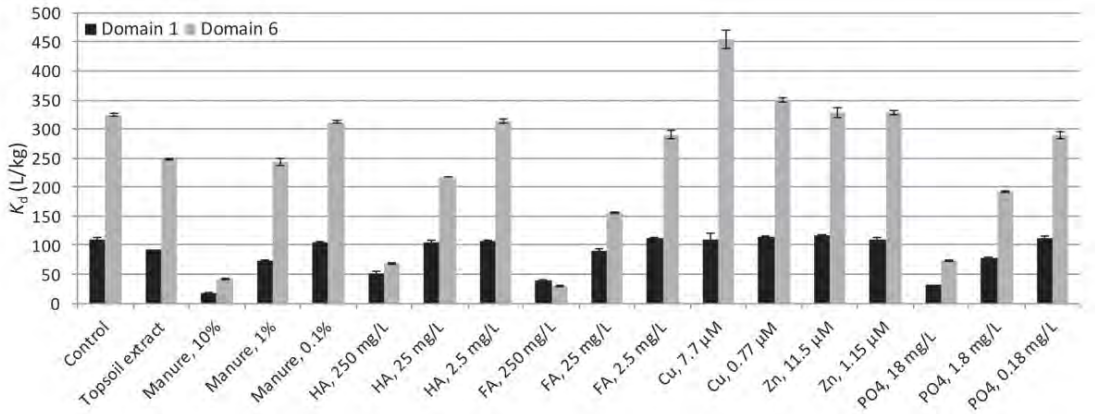


Fig. 5. Effect of topsoil extract, liquid pig manure extract, organic matter (humic acids [HA] and fulvic acids [FA]), divalent metals (Cu and Zn), and phosphate on glyphosate sorption (expressed as the distribution coefficient, K_d) in Domains 1 and 6 from the Gjorslev site. Concentrations correspond to the tested manure concentrations. Error bars are minimums and maximums of duplicate samples. Results from experiments with topsoil extract and liquid manure are included for comparison. Controls are without any additions.

Conclusion

The study has demonstrated that the sorption of glyphosate varies by an order of magnitude across eight identified soil domains in macroporous clayey till. It was expected that glyphosate would show the strongest sorption in domains with high Fe oxide content. This turned out to be wrong, since there was no correlation between glyphosate sorption and any measured soil parameter, including extractable Fe oxides. The domain-specific sorption of glyphosate was by far overruled by addition of liquid manure that strongly decreased glyphosate sorption due to its content of dissolved organic matter and phosphate. The variation across domains and the effects of solutes like the liquid fraction of manure should be taken into account when using sorption data in assessment of leaching risks. Our results suggest that hydrological modeling should focus more on sorption to fracture surfaces and pay less attention to traditional bulk sorption data when predicting pesticide transport through clay macropores. How much sorption influences leaching will, after all, also depend on general hydrological parameters such as pore size, connectivity, and climatic conditions.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The study describes the sorption behavior of glyphosate to different soil domains from two agricultural soils in Denmark. The set-up of the experiment was based on the OECD 106 guideline but with some deviations: The study was conducted with 1 mM $CaCl_2$ solution (standard: 10 mM solution), at 10°C (standard: 20 – 25°C); with 4 concentrations (standard: 5), no concentrations in the solid phase were explicitly measured, i.e. no real mass balances available.
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	Alexa, E. et al.
Report year	2010
Report title	Studies on the biodegradation capacity ^{14}C–labelled glyphosate in vine plantation soils
Document No	Journal of Food, Agriculture & Environment (2010) Vol.8 (3&4): 1193–1
Guidelines followed in study	None
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 (Experimental conditions not sufficiently described to assess validity, relevant endpoint not reported (DT_{50}))

2. Full summary of the study according to OECD format

Glyphosate is among the most widely used broad spectrum herbicides in the world because they are highly efficacious, cost effective, practically non–toxic and degrade readily in the environment. The herbicide is inactivated and biodegraded by soil microbes, degradation rate depends on soil microbial activity and factors that affect this activity. Glyphosate degradation rates vary considerably across a wide variety of soil types and microflora population types. The aim of this paper was to study the biodegradation capacity of glyphosate in soil samples collected from vine plantation from Timis county, Romania, belonging to Banat's University of Agricultural Science, Timisoara, in presence of organic and inorganic supplement, at different concentration levels. After addition of glyphosate–phosphonomethyl–^{14}C–labeled, the accumulated $^{14}CO_2$ (as % of total ^{14}C) was monitored during 44 days. Investigated soil shows a high degradation capacity of over 85 % of total radioactivity after 44 days from the treatment application. Addition of inorganic supplement causes a decrease of glyphosate biodegradation capacity to 10.77–12.87 % of total radioactivity, while in presence of straw the accumulated $^{14}CO_2$ (as % of total ^{14}C) during the 44 days ranged between 59.97 and 87.58 %. The amount of $^{14}CO_2$ released reached the highest level in the first 4 days after herbicide application, both in control and experimental variants with organic and inorganic supplement (from 2.61 to 30.27 % of total radioactivity). By glyphosate addition the growth and multiplication of soil microorganisms, whose biomass is digested in the range of 9–12 days of treatment, according to the daily mineralization rate (DMR) values, is stimulated. Our results on the activity of microorganisms showed that glyphosate degradation in soil is mainly performed by micromyces.

Materials and methods

Chemicals and soil samples

Glyphosate-phosphonomethyl–^{14}C– labeled (Sigma) lot number 012K9428/29, specific activity 2.2 mCi $mmol^{-1}$ and commercially formulated glyphosate of isopropylamine ammonium salt (Roundup) were purchased from Monsanto, Romania. Liquid Scintillation Cocktail (Quicksafe A cocktail) was used in Triathler Liquid Scintillation Counting. All other reagents were of analytical reagent grade.

The soil characterized as cambic moderately gleyed chernozem were sampled in March 2010 from the vine plantation (Burgundy grape variety) of Banat's University of Agricultural Science in Timisoara

(Western part of Romania). Sampling depth was between 0 and 10 cm. The glyphosate treatments and both organic and inorganic fertilizers are usually applied in grape–vine plantation. The soil samples were dried at room temperature for 48 h and crushed to pass a 2 mm sieve.

The basic physico–chemical soil characteristics and chemical composition of added inorganic and organic supplements were as follows:

- soil: clay 42.1 %; sand 29.2 %; silt 28.7 %; pH in H_2O 7.93; organic matter 3.95 %; N total 0.266 %; P 30 ppm; Fe 20,340 ppm; Cu 10 ppm; Mn 300 ppm; Zn 8 ppm

- organic supplement: pH 6.5; N_{total} 14 %; organic matter 7.5 %; P 30 ppm; Zn 35.89 ppm; Cr 42.60 ppm; Ni 25.61 ppm; Cu: 31.51 ppm; Cd 2.01 ppm; Fe 487.7 ppm

- inorganic supplement: N_{total} 15 %; P_2O_5 5 %; K_2O 20 %; CaO 2 %; MgO 1 %; S 9 %; Cu 0.1 %; Fe 0.1 %; Mn 0.5 %; Zn 0.1 %

- wheat straw: cellulose 35 %; lignin 18 %; ash 8 %; hemicellulose 35 %

^{14}C–labelled glyphosate biodegradation radio-assay

Evaluation of ^{14}C–labelled glyphosate biodegradation was done according to Getenga et al. using liquid scintillation counter Triathler (Finland) for radio–assay. In the incubation experiment, 25 g soil samples in duplicates were placed in biometer flasks. The soil was conditioned by being moistened to 85 % of the field water capacity. The biometer flask content is a plastic vial with soil treated with glyphosate, a vial containing 10 ml distillated water, which assures atmosphere saturation with water vapor and a plastic vial filled with 10 ml 0.2 M NaOH , to trap the $^{14}CO_2$ released during mineralization by soil microorganisms. Non–labelled glyphosate solution in distilled water in concentration of 1.5 ppm was added to each soil sample and the initial radioactivity was done by glyphosate–phosphonomethyl–^{14}C–labeled with specific activity 0.5 mCi. The soils were incubated at 20°C, in the dark for 44 days. In order to evaluate the biodegradation of ^{14}C–labeled glyphosate during the incubation period, samples were taken every 4 days. The NaOH solution was mixed with 5 ml of Quicksafe A cocktail in a 20 ml scintillation vial before it was radio–assayed. After every sampling the vial was refilled with fresh 0.2 M NaOH. The amount of $^{14}CO_2$ released during mineralization was quantified on the base of ^{14}C disintegration number.

By adding the percentages at each sampling, the total amount of mineralized glyphosate depending on time is obtained. The mineralization curves of $^{14}CO_2$ accumulated were compared during 44 days.

The experimental treatments were: Control – soil with glyphosate in concentration of 1.5 ppm; OSI – soil with glyphosate and addition of organic supplement 3.2 %; OSII - soil with glyphosate and addition of organic supplement 6.4 %; ISI – soil with glyphosate and addition of inorganic supplement 8 %; ISII – soil with glyphosate and addition of inorganic supplement 16 %; WSI – soil with glyphosate and addition of wheat straw 1 %; WSII – soil with glyphosate and addition of wheat straw 2 %.

Evaluation of microbial response parameters

Microbial communities in soils treated with glyphosate were evaluated using the method described by Seeley *et al*. 20. A soil sample (about 20 g) was treated with 1.5 ppm glyphosate unlabeled solution (Roundup) and incubated at 22 ± 3°C in an Erlenmeyer flask. Daily humidity was corrected so that it does not to fall below 75–80 % of the wet field capacity. After 3 and 10 days we determined the number of culturable microorganisms using the count plate method. For the quantitative determination of eubacterias we used Topping medium: yeast extract 0.25 %, peptone powder 0.25 %, agar 1.8 % and distillated water, pH 7.6. To quantify the number of actinomycetes we used Gause medium: KNO_3 0.1 %, K_2HPO_4 0.05 %, $MgSO_4$ 0.05 %, NaCl 0.05 %, $FeSO_4$ 1 %, corn starch 2 %, agar 2 %, distillated water, pH 7, and for estimation of the micromycetes number we used Czapek Dox medium: $NaNO_3$ 0.3 %, K_2HPO_4 0.1 %, $MgSO_4$ 0.05 %, KCl 0.05 %, $FeSO_4$ 0.001 %, sucrose 0.3 %, agar 1.5 %, pH 5.5. To secure a microbial count the samples were diluted (in 0.1 % sodium pyrophosphate) and plated, and after incubation the colonies that develop were counted. The microbial count of the original samples

was then determined by multiplying the average number of colonies that develop by the degree of dilution (dilution factor of the samples in the plate). Dilutions, expressed as negative exponents, were 10^{-5} for micromycetes and 10^{-7} for eubacterias and actinomycetes determinations. The results were expressed in colony forming units (CFU) per g soil (dry matter).

Results

^{14}C–glyphosate calibration

^{14}C–glyphosate calibration was done on the basis of quench curve method. The curve establishes the relationship between a quench parameter (QP) and the counting efficiency. Quench parameter indicates a relative light production from the sample. In the Triathler the quench parameter (QP) is, in mathematical terms, the center of spectrum gravity in the counting window. The collective effect of quench is a reduction in the number of photons produced and, therefore, detected CPM (counts per minute). The Triathler uses parabolic regression to form the curve. First the quench curve was made by counting a set of standard samples with the same activity but variable quench (Table 1). The Triathler prints the quench parameters and the corresponding efficiencies of the standards. When unknown samples are counted, the quench parameter is measured for each sample. Corresponding efficiency for the measured quench parameter is obtained from the curve and the DPM (desintegrations per minute or absolute radioactivity) corresponding value is calculated ($DPM = CPM \cdot Eff^{-1}$). The efficiency taken from the curve and an error percentage (err %), which is the difference of efficiencies (difference between measured eff. and the one taken from the quench curve, are indicated in Table 2.

Table 1. The data recorded for Triathler calibration.

Sample	Time (s)	Counts	CPM	QP
1	300	1,094,190	218,838	44.144
2	300	550,900	110,180	42.995
3	300	489,535	97,907	34.861
4	300	108,650	21,730	28.142
5	300	27,095	5,419	23.558

Std DPM: 220,000.

Eff.= - 0.0006·qp2 + 0.0743·qp - 1.2895.

Table 2. The efficiencies obtained on the basis of data analysis.

Eff	QP	Eff	err (%)
0.99472	44.14	0.99472	0.00
0.50082	43.00	0.50082	0.00
0.44503	34.86	0.44503	0.00
0.09877	28.14	0.09877	0.00
0.02463	23.56	0.02463	0.00

Results regarding ^{14}C–glyphosate biodegradation

Experimental results regarding the amount of $^{14}CO_2$ (%) released reported to total initial radioactivity, in accordance with prelevation chart are represented in Table 3. The biodegradation degree of glyphosate in soil was estimated as ratio between the number of ^{14}C–glyphosate disintegration in the sample and the number of disintegration in the standard. From these data it can be observed that in both control and experimental variants with organic and inorganic supplement addition, the amount of $^{14}CO_2$ released recorded the maximum value in the first 4 days after herbicide application, ranging from 2.61 % to 30.27 % of total radioactivity. The biodegraded glyphosate amount decreases for all analyzed samples, being less than 1 % after 44 days. The experimental results are in accordance with previous data obtained, which show that the glyphosate biodegradation has only two phases, the initial rapid phase for about

20 days due to microorganisms action on free glyphosate in soil followed by a slow final phase when the microorganisms act on glyphosate adsorbed on the soil compounds. From Table 3 it can be observed that, for control, $^{14}CO_2$ resulting from the glyphosate decomposition reached maximum value after 4 days (30.27 %) and decreases with time advancing: 20.27 % after 8 days, 11.86 % after 12 days, 10.94 % after 16 days and only 3.94 after 20 days reaching 0.35 mg $^{14}CO_2$ after 44 days. In Figure 1a–c the glyphosate mineralization curves expressed as accumulated $^{14}CO_2$ as % from total radioactivity are represented. Accumulated $^{14}CO_2$ in the case of control sample, without fertilizers, increased from 30.24 % after 4 days, to 50.54 % after 8 days from herbicide application, respectively, 80 % after first 24 days and slow growing to 85.96 % of total radioactivity after 44 days (Figure 1a). The soil characteristics influence the degradation capacity of glyphosate in the presence of microorganisms. In the literature there are several papers describing that the adsorption of glyphosate by soils depends on cationic exchange capacity, clay content, pH and organic matter. Studies regarding the effect of pH on the adsorption of glyphosate by soils and clays agreed that an increase of pH decreased the adsorption of glyphosate. It was due to an increase in negative charge of glyphosate and mineral surface with an increase in pH value resulting in a decrease in the adsorption. The analyzed soil has a high content of clay (42.1 %), iron and pH in H_2O (7.93). The experimental results show a high glyphosate biodegradation capacity in control sample (85.96 % after 44 days) and availability of glyphosate to microorganisms, due to low level of glyphosate adsorption on soil particles, according to other studies.

Table 3. Impact of added supplement on $^{14}CO_2$ release (% of total radioactivity).

Variant	Accumulated $^{14}CO_2$ (% of total ^{14}C) in the prelevation time (A-K)										
	A	B	C	D	E	F	G	H	I	J	K
Control	30.27	20.27	11.86	10.94	3.9	2.82	2.27	1.47	1.12	0.69	0.35
OSI	30.23	19.13	12.76	9.54	3.86	2.8	2.27	1.47	1.12	0.91	0.49
OSII	33.66	16.38	13.24	4.13	3.9	2.53	1.9	1.62	0.7	0.7	0.49
ISI	2.61	1.5	0.69	0.62	0.54	0.48	0.46	0.36	0.3	0.26	0.25
ISII	2.73	1.94	1.63	1.49	1.28	1.1	0.91	0.76	0.41	0.42	0.2
WSI	25.51	15.27	4.86	4.08	2.78	2.62	1.94	1.58	1.13	0.81	0.73
WSII	21.96	13.71	7.13	6.58	2.64	2.17	1.65	1.24	1.19	0.96	0.74

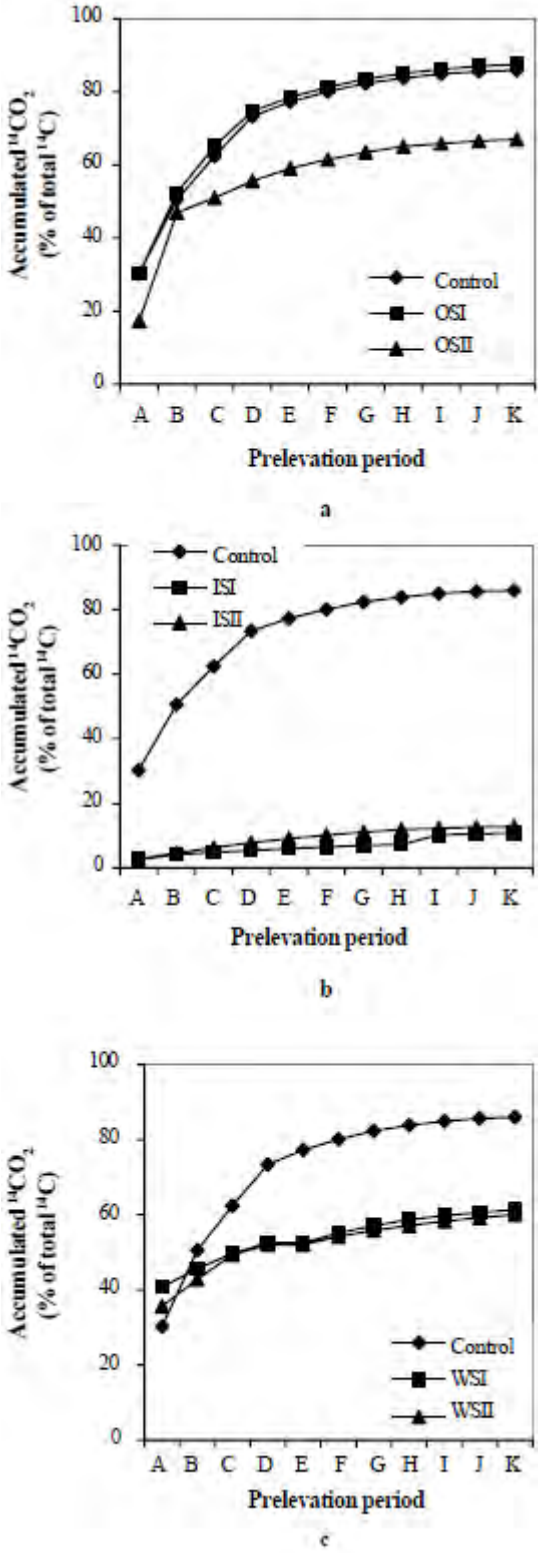
In Figure 1a–c the glyphosate mineralization curves expressed as accumulated $^{14}CO_2$ as % from total radioactivity, in the case of organic and inorganic supplement addition are represented. The experimental results obtained show significant differences between the amount of biodegradable glyphosate according to the type and amount of organic or inorganic fertilizer added. Addition of organic fertilizer at a rate of 3.2 % does not lead to significant changes in curve shape of glyphosate mineralization (Figure 1b). Increasing the amount of organic fertilizer to 6.4 % leads to decrease in the amount of released $^{14}CO_2$. Total accumulated $^{14}CO_2$ after 44 days from the glyphosate application was 87.58 % for organic substrate addition OSI, respectively, 67 % in case of organic substrate addition of OSII. Our results are in accordance with those of Getenga and Kengara, showing that compost addition does not stimulate intense mineralization of glyphosate by microbes.

Mineralization curves in Figure 1b show the reduced availability of glyphosate to biodegradation in the presence of inorganic fertilizers. In case of mineral fertilizers addition in a proportion of 8 % of inorganic supplement, the amount of $^{14}CO_2$ 4 days after the herbicide administration was 2.61 % of the total radioactivity and decreased slowly reaching 0.25 % between 40 and 44 days. Biodegradation capacity of glyphosate in the presence of mineral fertilizers was much reduced compared to the control sample (Table 3, Figure1b). The total amount of $^{14}CO_2$ released after 44 days was only 10.77 % in case of ISI and 12.87 % in case of ISII.

From Table 3 it can be observed that the biodegraded glyphosate percentage was between 2.61 to 2.73 % after the first 4 days and decreased to 0.2 % after 44 days of experimentation. Glyphosate contains functional groups of amine, carboxylate and phosphonate that can form strong coordination bonds with metal ions to give bidentate and tridentate complexes. Addition of inorganic fertilizers rich in metal ions leads to decrease in glyphosate biodegradation ability and reduces the amount of $^{14}CO_2$ released. Cruz

et al. studied the competitive adsorption between glyphosate and phosphate in different Brazilian soils. The results showed that on the clays glyphosate was not easily displaced by phosphate even in the ratio of 10.0 of phosphate/glyphosate. Our results are in accordance with those because in analyzed soil with high content of clays (42.1 %) the glyphosate is not displaced by phosphate ions. On the other hand, the addition of inorganic fertilizer rich in phosphate and nitrate led to micro–organisms orientation on nitrogen and phosphate source easily accessible, respectively, reduced availability of glyphosate for biodegradation. Thus, the amount of $^{14}CO_2$ released is 10 times lower in variants fertilized with inorganic supplement (ISI, ISII). The increased content of mineral fertilizer, in the case of ISII, did not lead to significant changes regarding the release of $^{14}CO_2$ from the glyphosate biodegradation. In WSI and WSII where wheat straw at a rate of 1 % and 2 % was added, there was a noticeable decrease in the amount of $^{14}CO_2$ pursued as a result of glyphosate biodegradation compared with the control. Thus, after 4 days the percentage of released $^{14}CO_2$ was 25.51 % in WSI and 21.96 % in WSII (Table 3). After 8 days from the glyphosate application, the biodegradation capacity decreased to 4.86 and 7.13 %. $^{14}CO_2$ total amount accumulated as a result of glyphosate biodegradation was 61.31 %, in the case of 1 % straw addition and 59.97 % to 2 % straw addition (Figure 1c).

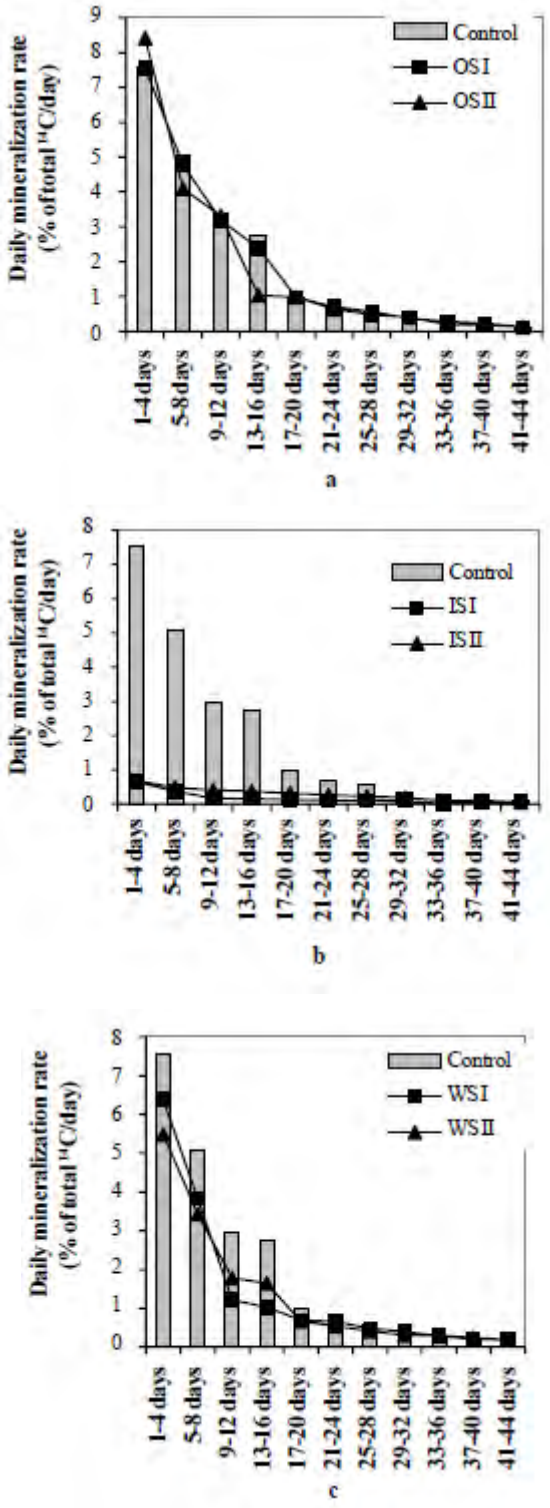
Figure 1. Mineralization of ^{14}C–glyphosate in soil with different supplements (a– control versus OS, b– control versus IS, c– control versus WS).



The daily mineralization rate (DMR) of glyphosate (Figure 2a–c) in the case of different supplement addition was highest for all variants in the first 4 days of experiment, decreasing during incubation. If mineral fertilizers were added (ISI, ISII), the DMR value was much lower than in other cases. The explication is due to existing mineral compounds intake in inorganic fertilizers, compounds with which

glyphosate forms complexes hard accessible for microbial metabolism but also, due to lack of energy substrate supporting the respiratory activity of microorganisms.

Figure 2. Daily mineralization rate of glyphosate (a– control versus OS, b– control versus IS, c– control versus WS).



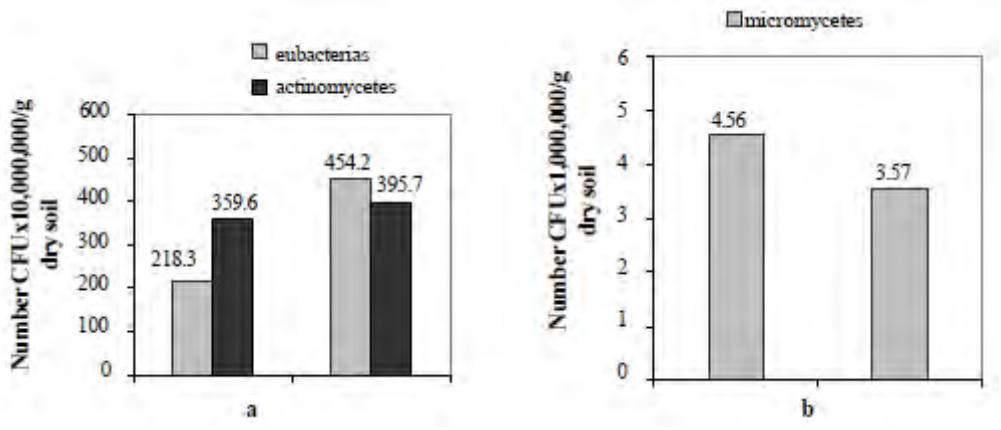
Besides, in the organic material addition, OSI, OSII, WSI and WSII, DMR value was highest in the first 4 days of experimentation. The highest values of $^{14}CO_2$, corresponding to DMR, were determined in OS even exceeding the control (7.567 mg $^{14}CO_2$), DMR value was higher than control value also for range

of 9–12 days. This could be due to labelled carbon release from the microbial protoplasm which assimilates the labelled glyphosate, respectively of fungal biomass.

Results regarding microorganism activity in soil

Glyphosate remains unchanged in the soil for varying lengths of time, depending on soil texture and organic matter content. Soil microorganisms break down glyphosate and many can use glyphosate as a sole source of phosphorus. On the base of results regarding the number of culturable microorganisms existing in the soil with glyphosate (Figure 3a, b) it can be observed that at 10 days after the treatment application, the eubacteria number increases from $218.3x10^5$ to $454.2x10^5$ CFU g^{-1} dry soil.

Figure 3. The variations of microorganisms number after 3 and 10 days since glyphosate addition in control a) eubacterias and actinomycetes, b) micromycetes.



Conclusion

The soil characteristics influence the degradation capacity of glyphosate in the presence of microorganisms. The soil sampled from the vine plantation (Burgundy grape variety) of Banat's University of Agricultural Science in Timisoara shows a high degradation capacity, over 85 % of total radioactivity after 44 days from the treatment application. Addition of inorganic substratum causes a decrease in glyphosate biodegradation capacity to 10.77 – 12.87 % of total radioactivity, while in presence of straw the accumulated $^{14}CO_2$ (as % of total ^{14}C) during the 44 days ranged between 59.97 – 87.58 %. The amount of $^{14}CO_2$ released reached the highest level in the first 4 days after herbicide application both in the control and experimental variants with organic and inorganic substratum (from 2.61 to 30.27 % of total radioactivity). The growth and multiplication of soil microorganisms whose biomass is digested in the range of 9 – 12 days of treatment, according to the daily mineralization rate (DMR) values is stimulated by glyphosate addition. Our results on the activity of microorganisms have shown that glyphosate degradation in soil is mainly performed by micromyces.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The study describes a degradation experiment with glyphosate on an European agricultural soil in the laboratory. Only the mineralization of the substance is reported. Further data like mass balances, residues in soil and a half–life are not reported .The validity of the study cannot be evaluated due to missing information.

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	Al–Rajab, A., Schiavon, M.
Report year	2010
Report title	Degradation of ^{14}C–glyphosate and aminomethylphosphonic acid (AMPA) in three agricultural soils
Document No	Journal of environmental sciences (China), (2010) Vol. 22, No. 9, pp. 1374-80
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Reliable with restrictions (some deficiencies in the test design, not all information reported to check validity of the study)

2. Full summary of the study according to OECD format

Glyphosate (N–phosphonomethyl glycine) is the most used herbicide worldwide. The degradation of ^{14}C–labeled glyphosate was studied under controlled laboratory conditions in three different agricultural soils: a silt clay loam, a clay loam and a sandy loam soil. The kinetic and intensity of glyphosate degradation varied considerably over time within the same soil and among different types of soil. Our results demonstrated that the mineralization rate of glyphosate was high at the beginning of incubation and then decreased with time until the end of the experiment. The same kinetic was observed for the water extractable residues. The degradation of glyphosate was rapid in the soil with low adsorption capacity (clay loam soil) with a short half–life of 4 days. However, the persistence of glyphosate in high adsorption capacity soils increased, with half-live of 19 days for silt clay loam soil and 14.5 days for sandy loam soil. HPLC analyses showed that the main metabolite of glyphosate, aminomethylphosphonic acid (AMPA) was detected after three days of incubation in the extracts of all three soils. Our results suggested that the possibility of contamination of groundwater by glyphosate was high on a long–term period in soils with high adsorption capacity and low degrading activities and/or acid similar to sandy loam soil. This risk might be faster but less sustainable in soil with low adsorption capacity and high degrading activity like the clay loam soil. However, the release of non–extractable residues may increase the risk of contamination of groundwater regardless of the type of soil.

Materials and methods

Chemicals

[Phosphonomethyl–^{14}C]–glyphosate was obtained from ARC–ISOBIO (Belgium) diluted in water. Its specific radioactivity was 385 GBq/mmol and its radiochemical purity 99 %. Non–radioactive glyphosate (purity 98.5 %) was obtained from CIL Cluzeau (France). AMPA, 10 ng/µL in water, was obtained from Dr. Ehrenstorfer GmbH (Germany). Sarcosine (N–methylglycine) $C_3H_7NO_2$, purity 99 %, was obtained from Fluka (Germany). FMOC–chloride (purity 99 %), sodium tetraborate decahydrate (purity 99.5 %), potassium hydroxyde (purity 86 %), potassium dihydrogen phosphate (purity 99.5 %) were also obtained from Fluka (Germany). Acetonitrile was obtained from (SDS, France). All solvents were of high performance liquid chromatography (HPLC) grade.

Selected soils and treatments

Three cultivated soils from the Lorraine region in eastern France were selected on the basis of their texture and pH (Table 1). None of these soils had ever been exposed to glyphosate. Soil types were classified as rendzic leptosol, fluvic cambisol, and stagnic luvisol, hereafter referred to as: clay loam soil, sandy loam soil and silt clay loam soil, respectively. The surface layers (0–25 cm) of all three soils were sampled on the same day.

Soils were air dried and sieved to 2 mm maximum particle size. Soil samples (25 g) were placed in glass jars of 60 mm diameter by 40 mm high. Samples were prepared in triplicates for each soil and each sampling time. An aqueous solution of 0.51 mg glyphosate and 45.1 kBq (equivalent to 1800 g/ha) was added to each soil sample. The volume of aqueous solution was calculated for each soil to obtain samples with moisture content of 80 % of soil retention capacity.

Table 1 Principal characteristics of the soils (surface layers, 0-25 cm) used in this study

Soil	Clay (%)	pH (water)	OC[a] (%)	K_f[b]	Fe oxides[c] (g/kg)	Fe amorphous[d] (g/kg)	Total Cu[e] (mg/kg)	Total P_2O_5 (g/kg)
Sandy loam	10.5	5.1	0.82	34.5	9.73	2.89	7.89	1.24
Silt clay loam	30.6	6.3	1.45	33.6	40.05	8.52	29.80	3.24
Clay loam	34.9	7.9	1.91	16.6	33.16	2.51	14.11	2.74

[a] Organic carbon content; [b] K_f values obtained from Al-Rajab et al., 2008; [c] subtraction of extracted iron by sodium dithionite-citrate and by acid ammonium oxalate; [d] extracted iron by acid ammonium oxalate in darkness; [e] dissolved by HF.

Laboratory degradation studies

Each soil sample was placed in an individual airtight jar (1.5 L). A scintillation vial containing 10 mL water was placed in each jar to maintain a humid atmosphere and prevent desiccation of the soil. A second scintillation vial with 10 mL of 0.5 mol/L NaOH solution was also placed into each jar to trap any CO_2, which evolved from the soil due to mineralization of organic matter and $^{14}CO_2$. The jars were incubated in the dark at 20°C for 80 days. Analyses were performed in triplicates and one control of unspiked soil per type of soil was considered.

Evaluation of soil micro-organism activity

The total CO_2 fixed by the NaOH was evaluated by titrating an aliquot (8 mL) with 0.2 mol/L HCl, in the presence of 3 mL of 20 % $BaCl_2$ and thymolphtalein at 4 % in ethanol, on day 0, 1, 2, 3, 5, 8, 12, 17, 22, 30, 40, 65, and 80. On each sampling date, the replacement of the CO_2 trapping solution by fresh solution allowed air renewal in the jars.

Estimation of mineralization of glyphosate

The amount of $^{14}CO_2$ trapped by NaOH as a result of the mineralization of ^{14}C–glyphosate was determined by liquid scintillation counting. NaOH (1 mL, in duplicates) of each sample received 10 mL Ultima Gold scintillation cocktail (LSC–cocktail) from Packard (USA) in a plastic scintillation vial. Radioactivity was measured during 10 min using a Packard Tri–Carb 1900 CA liquid scintillation counter (Packard, USA).

Residues in soil

Extractable residues of glyphosate were evaluated and analysis as follow. Soils samples in triplicates were removed from incubation for each soil on day 0, 1, 2, 3, 5, 8, 12, 17, 22, 30, 40, 65 and 80 after treatment. The soil of each sample (25 g) was transferred into a 250–mL PPCO (Nalgene, VWR, USA) centrifuge flask. The soil was extracted thrice with 100 mL distilled water (easily available residues) then 3 times with 100 mL of 0.1 mol/L KH_2PO_4. The samples were rotary shaken at (20 ± 2)°C for 2 hr, and then centrifuged at 5000 ×g for 20 min. The supernatants were combined, the volumes adjusted and radioactivity was determined using liquid scintillation as described above. The supernatants of each sample were filtered through Whatman 40 filter papers, and transferred into a round bottom glass bottle (1000 mL), and then frozen at –30°C for 48 hr before being freeze dried (Edwards–Modulyo–RUA). The freeze–dried extracts were dissolved in 7 mL distilled water and filtered through 0.2 µm using Minisart RC–25 filters (Sartorius, France), then the extracts were stored in freezer at –30°C till derivatization and analysis by HPLC.

Analysis

Derivatization of residues

This analysis was carried out only on the aqueous soil extracts. A 0.5 mL of 0.05 mol/L buffer borate was added to 3 mL of the aqueous solution to be analysed, then left to settle for 15 min. Then 3 mL ethyl ether were added and the solution was agitated vigorously for 2 min. The mixture was left to settle. After 15 min, 1.5 mL of the aqueous phase was removed and 0.25 mL acetonitrile added, followed by 0.25 mL of a solution of FMOC–Chloride in acetonitrile (1 g/L). The mixture was left to react for 60 min at

ambient temperature. Two milliliter of ether ethyl was added and the solution was agitated vigorously for 2 min. The solution was left to settle for 1 hr and then the aqueous phase was recovered in a 2–mL vial for high performance liquid chromatography (HPLC) analysis.

Analysis of residues

The residues were analyzed by HPLC in a Varian chromatograph equipped with a fluorescence detector and a β–radioactivity detector (Flo–one β, Packard, USA) in the following operating conditions: Lichrosorb–NH2 column (5 µm, 4 mm × 250 mm) (CIL–Cluzeau, France) thermostated at 30°C, injection volume 50 µL, analysis time 22 min, flow rate 0.8 mL/min, elution KH_2PO_4 0.05 mol/L, pH 5.7, acetonitrile (70/30) (V/V). Detection was performed in the following conditions: (1) β–radioactivity detector: Scintillator Ultima–Flo, flow rate 1.2 mL/min, counting cell 500 µL, and (2) fluorescence detector: λ excitation 260 nm; λ emission 310 nm. Standards of the glyphosate (purity >98.5 %), AMPA (purity >98.5 %, CIL–Cluzeau, France) and sarcosine (N–methylglycine, purity >99 %, Fluka) were used for calibration (0, 10, 20, 50 and 100 µg/L). The retention time was 4.2 min for sarcosine, 6.6 min for AMPA, and 13.3 min for glyphosate.

Non–extractable radioactivity

After extraction by water and KH_2PO_4, all soil samples were air dried. Remaining non–extractable ^{14}C–radioactivity was determined by combustion. An aliquot of 0.3 g was mixed with 0.15 mg cellulose powder and the sample was burnt at 900°C with a 307 Packard Oxidizer (Packard, USA). The released $^{14}CO_2$ was trapped with 10 mL Carbosorb (Packard, USA) and the radioactivity was counted after the addition of 10 mL of Permafluor (Packard, USA).

Statistics

Statistical analyses were performed using Stat Box computer software (Grimmer Software version 6.4). Comparison of the means was done using the Newman–Keuls test at levels of 0.05, 0.01 and 0.001. Curves were plotted using SigmaPlot (Version 10, Systat Software Inc., USA). Data in figures represent the mean and standard deviation of triplicate samples.

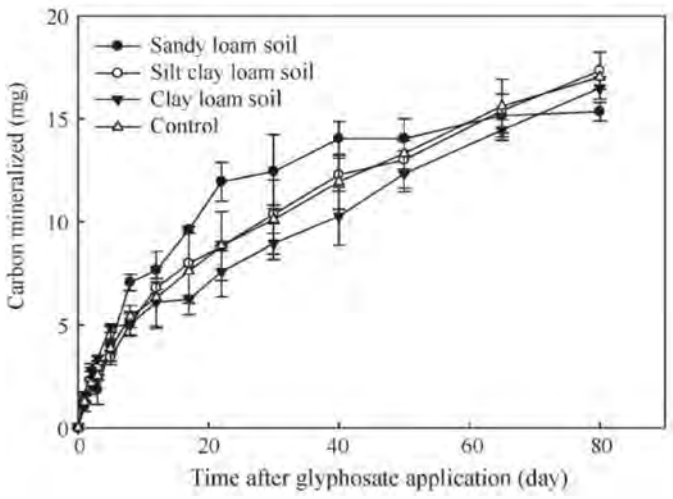
Results

Microbial activity

Total carbon mineralization of treated or untreated soils during the incubation was used as an indicator of the total microbial activity in the soils (Figure 1). Endogenous carbon was steadily mineralized in each soil during incubation and the intensity of mineralization differed slightly among soils between day 5 and day 50. During this period, mineralization was slightly faster in the sandy loam soil (14.4 mg carbon) than in the other two soils (13.73 mg for silt clay loam soil and 11.8 mg for clay loam soil). After 50 days, the slowdown in mineralization activity was more rapid for sandy loam soil than for the other two soils.

At the end of experiment (after 80 days of incubation), the total amount of carbon mineralized was similar for all three soils indicating that each soil presented significant microbial activity and that glyphosate had no toxic effect on soil micro–organisms.

Figure 1 Mineralization activity of microflora of three soils (clay loam, sandy loam and silt clay loam soils). The control is the average of mineralization activity for the three untreated soils.



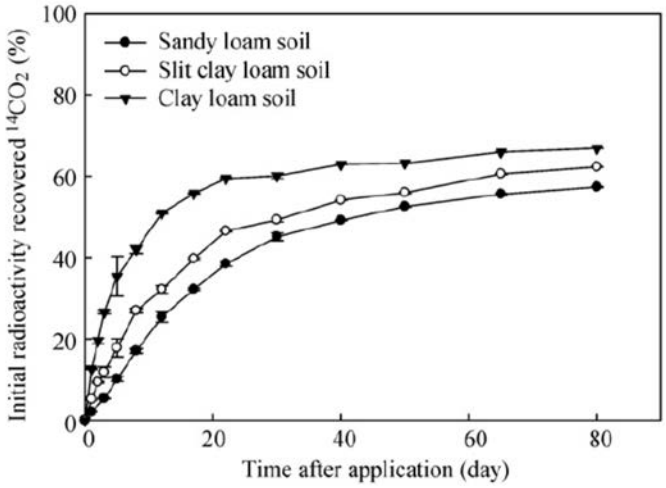
Mineralization of glyphosate

We observed an immediate and high rate of glyphosate degradation after its application on soil (Figure 2). The absence of lag phase indicates that the microflora of soil already had an enzymatic system capable of degrading glyphosate and as such did not need an adaptation period.

Mineralization of glyphosate after 17 days of incubation reached 32.2 % to 39.7 % of the initial amount applied to the two soils (sandy loam (pH 5.1) or silt clay loam (pH 6.3)). However, the mineralization rate was more rapid and intense for the clay loam soil (pH 7.9) with 48.4 % reached by 12 days of incubation. Thereafter, the mineralization of glyphosate declined gradually for all three soils. The endogenous activity of mineralization was comparable for the three investigated soils. The fast mineralization of glyphosate in clay loam soil appears due exclusively to a bioavailability more important than in other two soils.

We have previously shown that the adsorption of glyphosate in clay loam soil (K_f = 17) is lower than the other two soils (K_f = 34) (Al–Rajab et al., 2008). The half–lives of glyphosate derived from the mineralization rates were significantly different for the three soils, and were 42, 31, and 12 days for sandy loam, silt clay loam, and clay loam soils respectively. These results show that the degradation of glyphosate in biologically active agricultural soils could be influenced by the adsorption of glyphosate. Otherwise, the effect of organic matter content in the soil on mineralization of glyphosate was not clear under the conditions of this study.

Figure 2 Mineralization of ^{14}C–glyphosate to $^{14}CO_2$ in three soils incubated at 20°C.



Glyphosate degradation products – Extractable residues

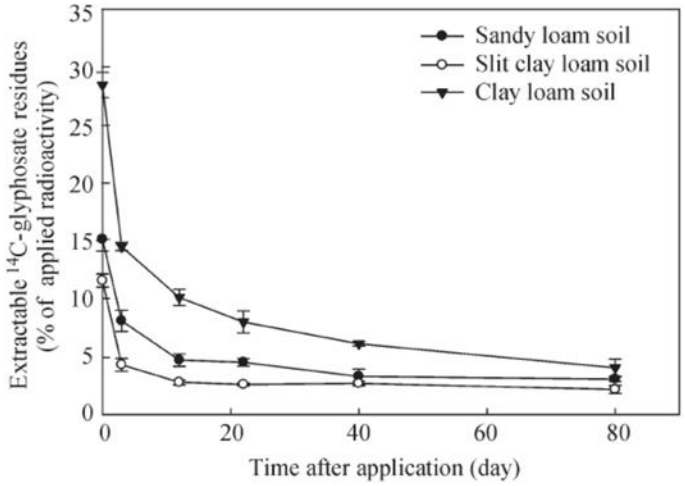
The soil was extracted separately three times with distilled water, then three times with 0.1 mol/L KH_2PO_4. The extraction rate of glyphosate residues with H_2O is influenced by: (1) the degradation,

which produces new products (metabolites) that differ in their water solubility and their reactivity with soil constituents; (2) by the process of adsorption–desorption, and (3) the formation of non–extractable residues over time; these sequestered residues are not available to be extracted by H_2O.

The extraction rate of glyphosate with water is an indication of the accessibility of the residues for microbial degradation and/or their transfer to groundwater under natural conditions. The extraction of glyphosate residues with water is directly related to the K_f measured for these soils (Figure 3). The observed difference of glyphosate extractable residues with water between the sandy loam soil and silt clay loam soil (which have the same K_f value) is certainly related to their texture. For the sandy loam soil, the sandy texture and unstable structure results in a better accessibility to the extraction solution, which in turn leads to a greater extraction efficiency when compared to clay loam soil.

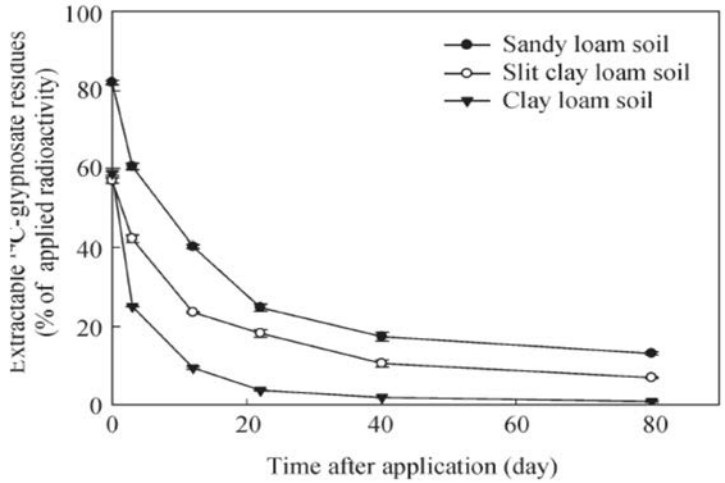
The extraction curves are opposite to those of the mineralization, with the same ranking of soils. These results indicate that the degrading activity of the microflora of soil is linked to the rate of glyphosate available for passage in the aqueous phase.

Figure 3 Evolution of extractable ^{14}C–glyphosate residues with H_2O from the three soils during incubation at 20°C.



On the other hand, the extraction of glyphosate from soil with 0.1 mol/L KH_2PO_4 was more efficient than extraction with H_2O. It did not seem affected by the level of bonds energy between the soil and residues of herbicide (Figure 4). In fact, in the sandy loam soil of Kf = 34, the percentage of glyphosate ^{14}C–phosphonomethyl extracted at T0, immediately after treatment, was (81.9 ± 0.55) % of the initial amount applied (Figure 4). Thereafter, this value decreased slowly to reach (13.0 ± 0.41) % of the initial amount applied at the end of incubation. In contrast, in the silt clay loam soil, with similar value of Kf = 34, the percentage of extracted residues at day 0 was only (56.9 ± 0.7) %, which is similar to that obtained for the clay loam soil which has a different Kf value of 17. This difference may be due to the high clay content in these two soils (silt clay loam and clay loam) and their structures, which reduces the performance of extraction of KH_2PO_4. We can assume that the treatment in a dry soil may cause an entry of glyphosate into the microporisity of aggregates during the capillary invasion by the aqueous solution of treatment (Guimont et al., 2005). The size of this compartment would be defined at the time of treatment and may depend on the physicochemical and physical properties (size of microporal compartment), and the moisture rate of soil at application time. This availability to extraction decreased overtime, more quickly in the sandy loam soil than in the other two brown soils, and at the end of experiment it reached 13.0 %, 6.9 %, and 0.8 % of the initial amount for sandy loam, silt clay loam, and clay loam soils, respectively. The evolution of extraction rate with KH_2PO_4 over time in the three soils is related to the mineralization of residues and the rate that non–extractable residues become available for mineralization and extraction.

Figure 4 Evolution of extractable ^{14}C–glyphosate residues with KH_2PO_4 from the three soils during incubation at 20°C.



Glyphosate degradation products – Degradation products

The analysis of water extracts by HPLC showed the appearance of two degradation products of glyphosate AMPA and/or sarcosine. However, this analysis of glyphosate residues by HPLC did not allow us to measure the sarcosine because its retention time was too short and equal that of co–eluted and unlabelled organic compounds. This analysis showed only the very rapid onset of AMPA in the extracts and its predominance compared to glyphosate as of the day 12 of application for the clay loam soil.

The appearance of AMPA during incubation varied significantly depending on the speed of mineralization of glyphosate in each soil (Table 2). In sandy loam soil, there was only 12.7 % of AMPA present on day 3 after treatment, whereas 87.3 % of the initial radioactive glyphosate was present on the same day. Thereafter, the percentage of AMPA increased gradually overtime, reaching 58.9 % of residues after 22 days of incubation, and 91.1 % at the end of the experiment.

Table 2 Mass balance of ^{14}C–glyphosate residues during incubation over 80 days as percentage of initial radioactivity (%)

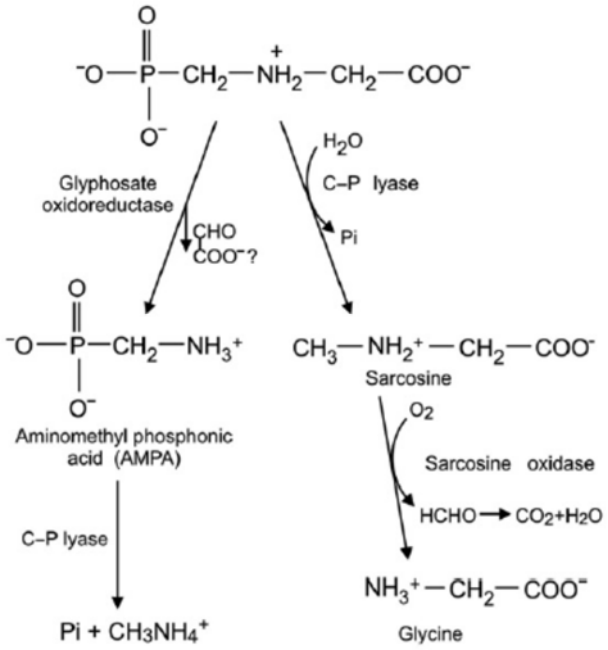
Incubation time (day)	Sandy loam soil		Silt clay loam soil		Clay loam soil	
	Glyphosate	AMPA	Glyphosate	AMPA	Glyphosate	AMPA
0	100	nd	100	nd	100	nd
3	87.3	12.7	79.7	20.3	51.5	48.5
12	71.0	29.0	58.5	41.5	40.2	59.8
22	41.1	58.9	25.6	74.4	12.0	88.0
40	22.3	77.7	22.5	77.5	5.6	94.4
80	8.9	91.1	14.9	85.1	0.9	99.1

AMPA: aminomethylphosphonic acid. nd: not detected.

The extractable residues of glyphosate with water are easily available to the degradation or transfer by water in soil. The half–life of glyphosate extractable with water was estimated and was found to vary depending on the biological activity of soil. It was 19 days for the sandy loam soil, 14.5 days for the silt clay loam soil and 4 days for the clay loam soil.

Together, our results suggest that the rupture of the $–CH_2–NH–$ bond giving rise to AMPA is easier than breaking the $–CH_2–PO_3H_2$ bond that results in either sarcosine and phosphorus, or methylamine and phosphorus (Figure 5). The break of the $–CH_2–NH–$ bond may depend on the overall activity of the microflora and the retention of glyphosate by the soil; while the rupture of the $–CH_2–PO_3H_2$ bond could be related to a more specific bacterial population. This difference in the rupture speed of these two links leads to some accumulation of AMPA in the soil (Figure 5).

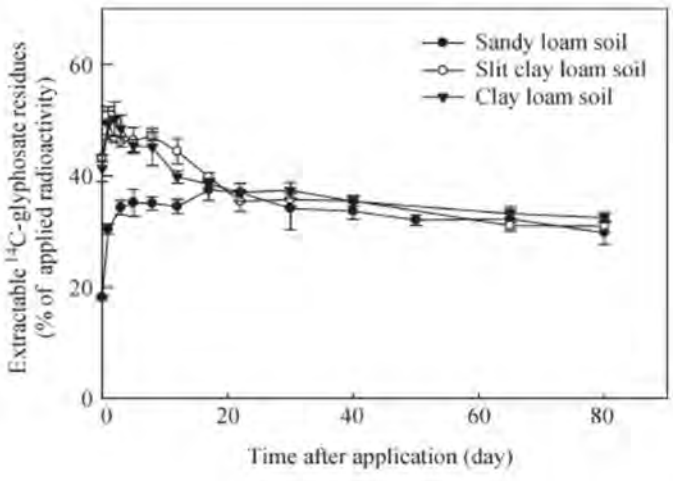
Figure 5 Microbial degradation of glyphosate in soil through sarcosine or AMPA (Liu et al., 1991)



Non–extractable glyphosate residues

The non–extractable residues represent the fraction, which cannot be extracted from the soil by the series of KH_2PO_4 extractions (exhaustive extraction) (Figure 6). Upon application of glyphosate on a sandy loam soil, we observed the formation of non–extractable residues at 18.1 % of the initial applied amount of herbicide. Subsequently, it progressed during 3 days to 35 %, staying stable until day 22, and then decreased very gradually over time until 30 % of initial applied amount of glyphosate was present at the end of experiment. In contrast, the formation of non–extractable residues for the clay loam and the silt clay loam soils was more intense and rapid than in the sandy loam soil. It reached 41.3 % and 43 % of the initial applied amount for the clay loam and silt clay loam soils respectively at day 0, and 49.4 % for both soils at day 1. For both soils, the rate stayed stable after day 2 until which decreased to 32.4 % and 30.9 %, respectively by the end of experiment. The rates of non–extractable residues seems specific for each soil, but are defined by day 3 after treatment.

Figure 6 Evolution of non–extractable residues in three soils during incubation time at 20°C



The rate of non–extractable residues is probably dependent on the physico–chemical properties and physical aspects of the soils including the size of the microporal compartment. This rapid formation of non–extractable residues immediately after treatment with a maximum reached within 2 to 8 days after application is very specific for glyphosate and could probably be due to: (1) the high solubility of glyphosate in water (10.5 g/L) (Agri–tox, 2009), (2) the physico–chemical properties that allow glyphosate to immediately establish high energy bonds with the constituents of soil, (3) the physico–

chemical properties of soils (texture, meso and microporisity), and/or (4) the treatment conditions.

The treatment of herbicide on a dry soil promotes the capillary invasion and the rapid transport of the solution of treatment in the microporisity intra aggregate (Guimont et al., 2005) subsequently making the glyphosate inaccessible to KH_2PO_4. Furthermore, the clayey texture promotes the importance of the microporosity. This explains the similar behaviour of clay loam and silt clay loam soils in the formation of non–extractable residues of glyphosate. In fact, these two soils have very different K_f values (17 and 34 respectively) but they have the same texture. These two soils, particularly the silt clay loam soil, differs strongly from the sandy loam soil which forms relatively a low rate of non–extractable residues and whose texture is sandy although having the same K_f (34) as the silt clay loam soil. We also noted that the initiation of the degradation of glyphosate did not affect the evolution of extractable residues rate. This implies that AMPA was not playing different role comparing to glyphosate. The very slow decrease of non–extractable residues showed that these residues can return by diffusion, and under the effect of a concentration gradient, to areas accessible to microorganisms to subsequently undergo mineralization. We note that from day 22 until the end of incubation the rates of non–extractable residues of glyphosate were similar for the three soils. The mineralization of glyphosate in three soils affects only the extractable fractions with water and KH_2PO_4 influenced by the forces adsorption defined by K_f.

The ^{14}C mass balance for each sample revealed a deficit (loss) that fluctuated from (4 ± 2) % at day 0 (application of glyphosate) to (6.0 ± 3.4) % after 80 days of incubation independent of soil type and different sampling dates over time. These losses were probably partially caused by the handling of samples during analyses (extraction and concentration). Because of these low losses, results were corrected and returned to 100 % by distributing the deficit on the various compartments assessed in proportion to their respective importance.

Conclusion

We simultaneously monitored in controlled conditions the principal processes involved in ^{14}C–glyphosate dissipation and their interactions in three agricultural soils over a period of 80 days. The results of this experiment showed that for agricultural soils with a significant and comparable biological activity, the fate of glyphosate and its potential in polluting water is closely related to the adsorption and the formation of non–extractable residues, which are dependent on soil texture and its moisture condition at the time of treatment. Our results showed that for a clay soil at basic pH, the glyphosate could be available to reach the groundwater in few days after treatment if the conditions are favourable for precipitation. Conversely, in the case of an acid sandy soil, the potential pollution of groundwater by glyphosate is greatly reduced by the strong adsorption of its residues in the soil. In case of rain following treatment, the risk of groundwater pollution by glyphosate will be low but may continue to be present for long time since the mineralization is slow. In this system, the silt clay loam soil is apparently less favourable for water pollution since it showed a strong adsorption of glyphosate and the formation of large amount of non–extractable residues. In the three investigated soils, a low level of water pollution (background) could be occurred over a long time by the sequestered residues of glyphosate, which are either gradually released into the soil solution, or circulated by the water through the soil.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The study describes a soil degradation experiment with glyphosate in three agricultural soils from the EU. The study conditions are sufficiently described. However some deficiencies were identified: the test vessels were air–tight and did not allow for air exchange; no information whether the applied test solution was mixed with the soil; $^{14}CO_2$ was passively (and potentially not quantitatively) collected; soil moisture was too high (80 % of soil retention capacity); for analytical method no LoD/LoQ provided; no radioactive material balance can be established; identification of glyphosate and AMPA was done only in aqueous extracts. Only few quantitative results are reported, mainly graphical plots; calculation method of DT_{50} not reported.

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

1. Information on the study

Data point:	KCA 7.1.2.1.1 and KCA 7.1.4.1.1
Report author	Al-Rajab, A. et al.
Report year	2014
Report title	Behavior of the non-selective herbicide glyphosate in agricultural soil
Document No	American Journal of Environmental Science 10 (2): 94-101, 2014
Guidelines followed in study	None
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 (some deviations from study guidelines, not all necessary data reported to derive comprehensive DT_{50} values, preferential flow in the soil column)

2. Full summary of the study according to OECD format

Glyphosate [*N*-phosphonomethyl]glycine is a systematic, non-selective, organophosphorus herbicide used worldwide in agriculture and industrial zones. Following its application, residues of glyphosate can threaten soil or aquatic organisms in adjacent water. In this study, we followed the degradation, stabilization, remobilization and leaching of ^{14}C-glyphosate in three agricultural soils in laboratory incubations and in lysimeters under field conditions. Glyphosate degradation was relatively rapid with a half-life of 14.5 days in the silt clay loam soil incubated at 20°C. Glyphosate's degradation product, Aminomethylphosphonic Acid (AMPA), represented more than 85 % of residues after 80 days of laboratory incubation. Leaching of glyphosate in lysimeters of three different investigated soils under outdoor conditions was very slow, less than 1 % of the initial applied amount has been detected in the leachates after 100 days of experimentation. Glyphosate rapidly formed non-extractable residues after treatment. In summary, glyphosate was removed from soil very rapidly and its leaching seems to be very slow regardless the type of treated soil. On the other hand, the contamination risk of groundwater with its metabolite AMPA at long term is probably due to the release of the non-extractable residues.

Materials & Methods

Chemicals

[Phosphonomethyl-^{14}C]-glyphosate diluted, purity 99 % was purchased from ARC-ISOBIO (Belgium). Glyphosate [*N*-phosphonomethyl]glycine, purity 99 % was purchased from Cluzeau (CIL, Paris). Aminomethylphosphonic Acid (AMPA), 10 ng µ/L in water, was purchased from Dr. Ehrenstorfer GmbH (Germany). Sarcosine [*N*-methylglycine], purity 99 % was purchased from Fluka (Germany). H_2PO_4, FMOC-chloride, Potassium hydroxide and Sodium tetraborate decahydrate were purchased from Fluka (Germany). Methanol and acetonitrile (HPLC grade) were purchased from SDS (France).

Sampling

Soils used in this study were obtained from three different agricultural lands in Lorraine region (France). Therefore, based on information provided by the landowners, these soils were never exposed to direct agricultural application of glyphosate and their properties were as following: Sandy loam soil (Sand:Silt:Clay (59:30:11), pH 5.1; % organic matter 0.82); silt clay loam soil (Sand:Silt:Clay (16:53:31), pH 6.3; % organic matter 1.45); and clay loam soil (Sand:Silt:Clay (35:30:35), pH 7.9; % organic matter 1.91).

In the laboratory studies, soils were air dried then sieved at 2 mm and stored in fridge at 4°C until treatment. Otherwise, in the outdoor leaching study, lysimeters were prepared in site using an undisturbed soil for each type of soil separately, a total of 7 columns of each soil were used in this study.

Lysimeters were polyvinyl chloride pipes of 10 cm wide and 35 cm long. Therefore, the 21 lysimeters of the three selected soils were placed in the experimental field of ENSAIA (54500 Vandoeuvre-lès-Nancy, France) for 100 days.

Extraction of Glyphosate

The efficacy of different solvents for extraction of glyphosate from soil was evaluated as follows. A 5 g portion of each soil (in triplicate) was treated with a 0.5 mL solution of H_2O (concentration of 19.4 Bq/g) of [Phosphonomethyl-^{14}C]-glyphosate and 0.1 µg/g of unlabelled glyphosate. Treated soil was placed into a 250 mL PPCO (Nalgene®, VWR, USA) centrifuge bottle and 25 mL of selected solvent were added. Five different solvents were tested separately for the glyphosate extraction efficacy: Ammonium oxalate monohydrate 0.1 M; potassium dihydrogen phosphate (KH_2PO_4) 0.1 M; a mixture of (NH_4OH 0.5 M + KH_2PO_4 0.1 M + H_3PO_4 0.5 %); $CaCl_2$ 0.1 M and distilled water. Bottles were rotary shaken for 2 h, then centrifuged at 5000 g for 20 min, the supernatant of each sample was recovered. Extraction of each sample has been repeated twice, the supernatants of the same sample were combined and a portion of 1 mL counted by Liquid Scintillation Counter (LSC). Thereafter, extraction of glyphosate from soil samples was effectuated with (KH_2PO_4) 0.1 M.

Laboratory Degradation Study

About 25-g soil samples were placed in glass jars (60 mm diameter, 40 mm high). Samples of silt clay loam soil were prepared in triplicates for each sampling time. Each sample was amended by 0.51 mg of glyphosate and 45.1 kBq in water. Final soil moisture was 80 % of the soil retention capacity. After treatment, each sample was added to a Mason jars (1.5 L). At the same time, a plastic vial of 10 mL H_2O was added to each jar in order to maintain the humidity of soil (Al-Rajab *et al*., 2009). Another plastic scintillation vial with 10 mL of 0.5 N NaOH was placed into each jar for trapping $^{14}CO_2$. Jars were incubated at 20°C in the dark for 80 days. The radioactivity trapped in NaOH was counted at each sampling time using a Liquid Scintillation Counter LSC Packard Tri-Carb 1900 CA (Packard, USA). 1 mL of NaOH was added to 10 mL of scintillation cocktail in a plastic scintillation vial to measure the radioactivity in the LSC during 10 min. At each sampling date, the 25-g soil samples were extracted separately using KH_2PO_4 as described previously. Then, after the 3rd and last extraction, soil samples were air-dried at the lab ambient temperature for 3 days. The remaining ^{14}C-radioactivity in the samples after extraction was referred as (non-extractable residues) which was determined by combustion at 900°C using a 307 Packard Oxidiser (Packard, USA).

Leaching Study

Lysimeters were prepared and placed in the experimental field of Lorraine University (France) 3 months before the treatment. During the experimentation of 100 days, the average temperature was 10°C; total precipitation was 235 mm; in total 8 leachates samples were collected. Leached radioactivity from each lysimeter was determined directly after collection, Therefore, water samples were stored at -18°C until analysis.

Analytical Methods

^{14}C-Radioactivity has been determined using a Liquid Scintillation Counter LSC. Glyphosate residues were determined using a Varian HPLC (USA) equipped with two detectors: A fluorescence detector and a β-radioactivity detector. A Lichrosorb (NH_2) column (4×250 mm, 5 µm) purchased from (CIL-Cluzeau, France) was used and thermostated at 30°C. Fluorescence detector was set at (λ 260 and 310 nm), while the flow rate of 1.2 mL/min was adopted in the β-radioactivity detector with a counting cell of 500 µL. The mobile phase was a mixture of (KH_2PO_4 0.05 mol^{-1}, pH 5.7)/acetonitrile (70/30: V/V) at flow rate of 0.8 mL/min. The injected volume was 50 µL. Within these conditions, the retention times were 4.2, 6.6 and 13.3 for sarcosine, AMPA and glyphosate respectively. Determination of the non-extractable residues in soil has been effectuated by combustion of 0.5 g portions at 900°C using an oxidizer (Packard, USA). Statistical analyses were conducted using Stat Box(Version 6.4, Grimmer Software, France).

Results

Extraction of Glyphosate

Extraction recovery of glyphosate varied from 4 to 74 % of the initial applied amount (Table 1). $CaCl_2$ (0.1 M) and water were the less effective solvents in glyphosate extraction in the three investigated soils. However, ammonium oxalate (0.1 M) was the most efficient solvent with a recovery rate ranged from 60 to 74 %. The only issue with the extraction with ammonium oxalate was that the extracts were very dark and need an intensive clean up. On the other hand, potassium dihydrogen phosphate (KH_2PO_4 0.1 M) was adopted as a suitable solvent since the extracts were clear and it showed an acceptable recovery rate varied from 45 to 49 % in investigated soils (Table 1). Recovery rate with citric acid (20 %) was not high enough (less than 37 %) for the three investigated soils.

Dissipation of Glyphosate

Results showed an immediate and high degradation rate of glyphosate after its application on the soil (Figure 1). Mineralization of glyphosate after 17 days of incubation reached 39.7 % of the initial amount applied. Thereafter, the mineralization of glyphosate declined gradually. The half-life of glyphosate derived from the mineralization rates was 31 days for silt clay loam soil. However, the extraction curves are opposite to those of the mineralization (Figure 2). The percentage of extracted residues from the silt clay loam soil at T0 was only 56.9±0.7 %. This availability to extraction decreased overtime, it reached 6.9 % of the initial amount for silt clay loam soil. HPLC analysis showed the appearance of two degradation products of glyphosate AMPA and sarcosine. However, this analysis of glyphosate residues by HPLC did not allow us to measure the sarcosine because its retention time was too short and equal that of co-eluted and unlabeled organic compounds. The half-life of glyphosate extractable was 14.5.

Table 1. Extraction efficiency of glyphosate from the selected soils using different solvents

Extraction efficiency (%: Mean±standard deviation: n = 3)			
Solvent	Sandy loam	Silt clay loam	Clay loam
KH_2PO_4 (0.1M)	44.9 (±0.3)	48.8 (±0.7)	48 (±0.5)
Ammonium oxalate (0.1 M)	59.9 (±0.7)	73.5 (±0.2)	61.1 (±0.1)
Citric acid (20%)	34.2 (±0.1)	36.4 (±0.2)	28.9 (±0.2)
$CaCl_2$ (0.1 M)	5.7 (±0.5)	3.6 (±0.9)	10.3 (±0.6)
H_2O	14.3 (±0.2)	23.5 (±0.1)	31.7 (±0.1)

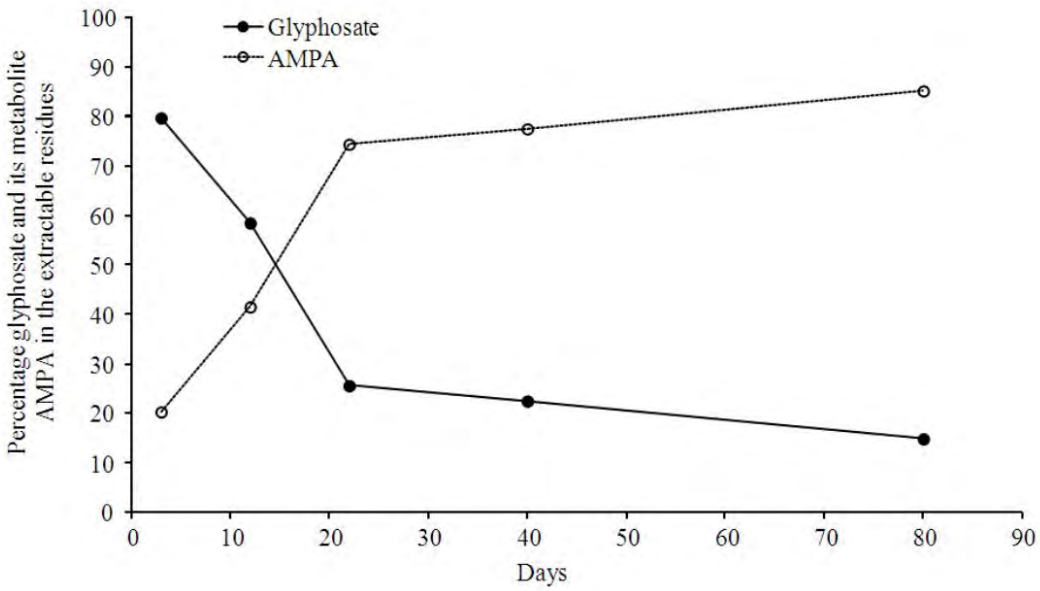


Figure 1. Residues evolution of glyphosate and AMPA in the extractable residues in silt clay loam soil during incubation at 20°C

Discussion

Extraction of Glyphosate

Extraction and determination of glyphosate in agricultural soil is problematic due to its high solubility and its physic-chemical properties (Botero-Coy *et al*., 2013). In the present study, extraction recovery of glyphosate varied from 4 to 74 % of the initial applied amount (Table 1). $CaCl_2$ (0.1 M) and water were the less effective solvents in glyphosate extraction in the three investigated soils. However, ammonium oxalate (0.1 M) was the most efficient solvent with a recovery rate ranged from 60 to 74 %. The only issue with the extraction with ammonium oxalate was that the extracts were very dark and need an intensive clean up.

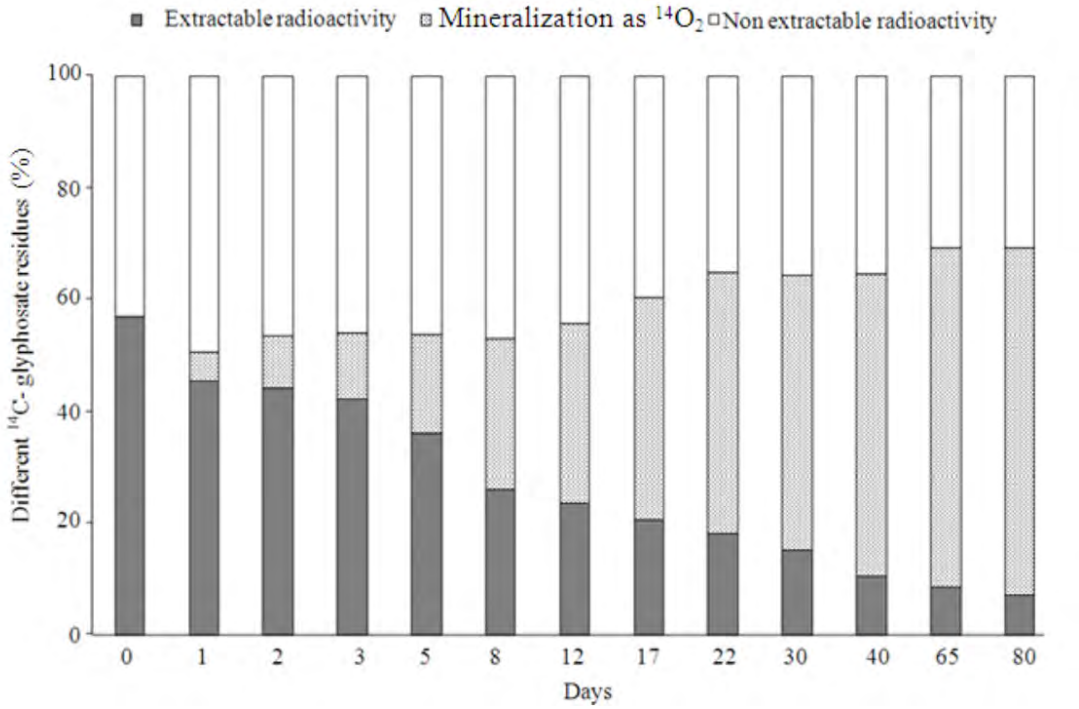


Figure 2. Evolution of different portions of ^{14}C-glyphosate residues (extractable, mineralization as $^{14}CO_2$ and Non-extractable) in silt clay loam soil during incubation at 20°C

On the other hand, potassium dihydrogen phosphate (KH_2PO_4 0.1 M) was adopted as a suitable solvent since the extracts were clear and it showed an acceptable recovery rate varied from 45 to 49 % in investigated soils (Table 1), this rate was similar to that one reported by other studies (Cheah and Lum, 1998; Landry *et al*., 2005). Recovery rate with citric acid (20 %) was not high enough (less than 37 %) for the three investigated soils. Non-extractable residues of glyphosate in soil increase with the time; consequently, glyphosate will be less available for extraction or degradation.

Dissipation of Glyphosate

Monitoring of mineralization of glyphosate labelled on the phosphonomethyl group allows assessing both the loss of glyphosate and AMPA. We observed an immediate and high degradation rate of glyphosate after its application on the soil (Figure 1). The absence of lag phase indicates that the microflora of soil already had an enzymatic system capable of degrading glyphosate and as such did not need an adaptation period. Mineralization of glyphosate after 17 days of incubation reached 39.7 % of the initial amount applied. Thereafter, the mineralization of glyphosate declined gradually. The fast mineralization of glyphosate in the soil appears due to its bioavailability. The half-life of glyphosate derived from the mineralization rates was 31 days for silt clay loam soil. On the other hand, the effect of organic matter content in the soil on mineralization of glyphosate was not clear under the conditions of this study. The extraction rate of glyphosate is an indication of the accessibility of the residues for microbial degradation and/or their transfer to groundwater under natural conditions. The extraction

curves are opposite to those of the mineralization (Figure 2). The percentage of extracted residues from the silt clay loam soil at T0 was only 56.9±0.7 %. We can assume that the treatment in a dry soil may cause an entry of glyphosate into the microporisity of aggregates during the capillary invasion by the aqueous solution of treatment (Guimont et al., 2005; Al-Rajab et al., 2010b). The size of this compartment would be defined at the time of treatment and may depend on the physicochemical and physical properties and the moisture rate of soil at the application moment. This availability to extraction decreased overtime, it reached 6.9 % of the initial amount for silt clay loam soil. The evolution of extraction rate with KH_2PO_4 over time in the soil is related to the mineralization of residues and the availability of non-extractable residues for mineralization or extraction. A similar behaviour of extractable residues of glyphosate over time was reported by (Getenga, 2004; Miles, 1998). HPLC analysis showed the appearance of two degradation products of glyphosate AMPA and sarcosine. However, this analysis of glyphosate residues by HPLC did not allow us to measure the sarcosine because its retention time was too short and equal that of co-eluted and unlabelled organic compounds.

The appearance of AMPA during the first days of incubation is due the fast mineralization of glyphosate in soil, reaching about 85.1 % of residues after 80 days of treatment (Figure 1). Our results are consistent, to some extent, with those obtained by (Cheah and Lum, 1998) who reported the rate of AMPA in the extracts of a sandy loam soil increased gradually over incubation time and reached 50 % of residues after 45 days of treatment. The half-life of glyphosate extractable was 14.5, this value is in accord with the half-lives of 6 to 9 days reported in other study for glyphosate in four agricultural soils incubated at 25°C (Eberbach, 1998) as well as the 19.2 days half-life observed in a sandy loam soil by (Cheah and Lum, 1998). However, much longer half-lives have also been reported by (Getenga, 2004) whereby the half-life of glyphosate was 85.6 days in a clay soil. The fraction of non-extractable residues represent the residues which cannot be extracted from the soil by the series of KH_2PO_4 extractions (exhaustive extraction) (Figure 2). The formation of the non-extractable residues NER in the silt clay loam soil reached 43 % of the initial applied amount at T0 and 49.4 % at T1. The rate stayed stable until T2 after which it decreased to 30.9 % by the end of experiment. The rate of non-extractable residues decreased over time unlike other pesticides such as atrazine where the rate of non-extractable residues increases gradually over dozens of days (Winkelmann, 1991). The rate of non-extractable residues is probably dependent on the properties and physical aspects of the soils including the size of the microporal compartment. This rapid formation of non-extractable residues immediately after treatment is very specific for glyphosate. The treatment of herbicide on a dry soil promotes the capillary invasion and the rapid transport of the solution of treatment in the microporisity intra aggregate, subsequently making the herbicide inaccessible for extraction (Guimont *et al*., 2005). We also reported that the initiation of the degradation of glyphosate did not affect the evolution of extractable residues rate. The very slow decrease of non-extractable residues showed that these residues can return by diffusion and under the effect of a concentration gradient, to areas accessible to microorganisms to subsequently undergo mineralization.

Leaching of Glyphosate

This study showed that water circulation in the soil might has an important role in contamination of groundwater with glyphosate. The diminution of soil macroporosity on the surface layer (where most residues usually present) with the time slows the water infiltration and might encourage the desorption of glyphosate residues. The circulation of glyphosate residues in soil could be due to a preferential water flow regarding the presence of its residues in the 1st collected leachates (Figure 3). In disaccording with results reported by (Dousset et al., 2004), our study showed that the residues of glyphosate were detected in the first leachates samples of three soils, the cumulated precipitation was 85 mm. Detection of glyphosate residues in the 1st leachates was due to the preferential flow (Laitinen et al., 2006). In the case of silt clay loam soil, the maximum residues concentration of 9.5±7 µg/L has been reached after 2 months of application. However, (De Jonge and Jacobsen, 2000) have reported residues concentration of glyphosate much higher than what was obtained from the current study. Concentration of leached residues decreased dramatically after 2 months until the end of experiment (Figure 3). Our findings were in accord with results reported by (De Jonge and Jacobsen, 2000; Landry et al., 2005) who detected the glyphosate residues in the soil leachates after 3 months of application. Overall, the total residues

(extractable and non-extractable) of glyphosate in the soil should be considered to evaluate its persistence in the soil, not only the extractable residues.

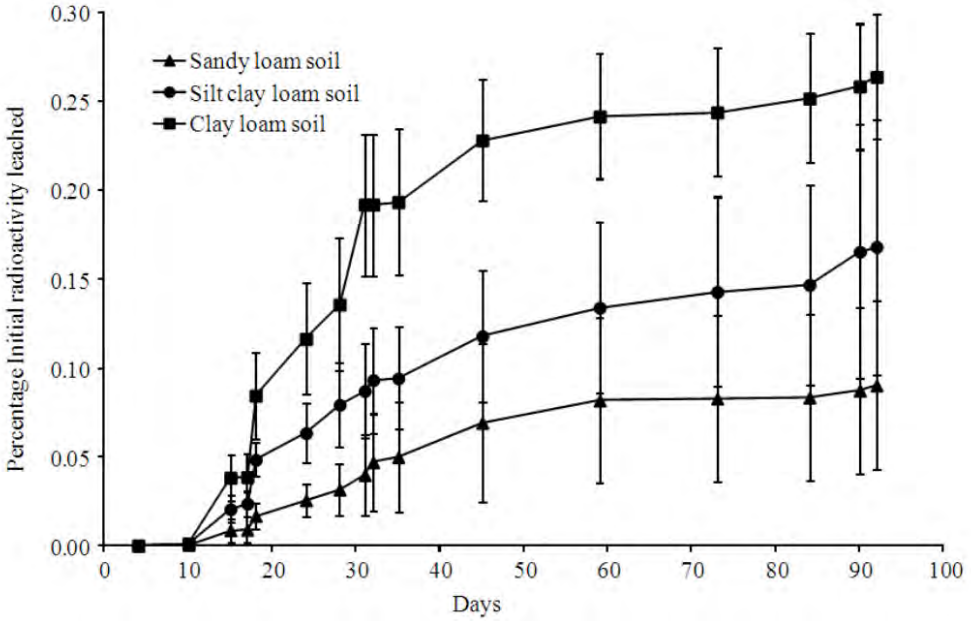


Fig. 3. Radioactivity leached from lysimeters of the investigated soils treated with ^{14}C-glyphosate under outdoor conditions

Conclusion

The present study monitored the residue dynamics of glyphosate in agricultural soil in controlled and outdoor conditions. Results obtained for the fate study suggest that the water pollution with this herbicide is closely related to the adsorption and the formation of non-extractable residues, which are themselves dependent on soil texture and its moisture condition at the time of treatment. In case of rain following treatment, the risk of groundwater pollution by glyphosate will be low but may continue to be present for long time since the mineralization is slow. The silt clay loam soil could be less favourable for water pollution since it showed a formation of large amount of non-extractable residues. In the semi-field lysimeters study, leaching of glyphosate was limited, but its metabolite AMPA seems to be the main potential pollutant of the groundwater. The water circulation mode in the soil was preferential flow which facilitate a fast leaching of residues to reach the groundwater.

In summary, these results suggest that the organophosphorus herbicide glyphosate is rapidly degradable in the agricultural soil. Leaching of glyphosate seems to be very slow regardless the type of the soil. Release of the non-extractable residues of glyphosate probably increases the risk of groundwater pollution with its metabolite AMPA at long term. More investigations are requested for a better understanding of the effect of soil content of organic carbon and soil microflora on environmental behavior of glyphosate.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The article describes the dissipation and leaching behavior of glyphosate and its metabolite AMPA in three French soils. The soil dissipation experiments were conducted with ^{14}C-labelled glyphosate and show few deviations from the relevant OECD guideline. However, no detailed values on the measurements per time point are reported for all soils.

The outdoor leaching experiments were conducted with 35-cm long undisturbed columns from the three different soils. ^{14}C-labelled glyphosate was applied. Preferential flow was identified as the main reason for the fast detection of glyphosate in the leachate.

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

E-Fate: Reliability criteria for the detailed assessment of full-text documents

Data requirements (indicated by the corresponding EU data point)	Criteria for "Reliable" articles	Criteria met? Yes / No / Uncertain
General criteria for reliability considered for all data requirements indicated by the corresponding EU data points as specified in EC Regulation (EU) No 283/2013	1. For guideline-compliant studies (GLP studies): OECD, OPPTS, ISO, and others. The validity/quality criteria listed in the corresponding guidelines met.	No
	2. Previous exposure to other chemicals is documented (where relevant).	Yes
	3. The test substance is dissolved in water or non-toxic solvent	Yes
	4. Glyphosate, when the test substance, is sufficiently documented - identity of the test material reported (i.e. purity, source, content, storage conditions)	Yes
	5. Only glyphosate is the tested substance (excluding mixture), and information on application of glyphosate is described	Yes
	6. The endpoint measured can be considered a consequence of glyphosate (or a glyphosate metabolite)	Yes
	7. Study design / test system is well described, including when relevant: concentration in exposure media (dose rates, volume applied, etc.), dilution/mixture of test item (solvent, vehicle) where relevant.	Yes
	8. Analytical verifications performed in test media (concentration)/ collected samples, stability of glyphosate in test media documented	No
	9. An endpoint can be derived. Findings do deliver a regulatory endpoint, and/or is useful as supporting information	Yes
	10. Assessment of the statistical power of the assay is possible with reported data.	Yes
	11. If statistical methodology was applied for findings reported, then the data analysis applied is clearly reported (e.g., checking the plots and confidence intervals)	Yes
	12. Field locations relevant/comparable to European conditions. Soils not completely matching the OECD criteria but from Europe or to some extent representative for the European Agriculture.	Yes
	13. Characterization of soil: texture (sandy loam, silty loam, loam, loamy sand), pH (5.5-8.0), cation exchange capacity, organic carbon (0.5-2-5 %), bulk density, water retention, microbial biomass (~1 % of organic carbon)	Yes
	14. Other soils where information on characterization by the parameters: pH, texture, CEC, organic carbon, bulk density, water holding capacity, microbial biomass	Yes
	15. For tests including agricultural soils, they should not have been treated with test substance or similar substances for a minimum of 1 year	Yes
	16. For soil samples, sampling from A-horizon, top 20 cm layers; soils freshly from field preferred (storage max	No

E-Fate: Reliability criteria for the detailed assessment of full-text documents

Data requirements (indicated by the corresponding EU data point)	Criteria for "Reliable" articles	Criteria met? Yes / No / Uncertain
	3 months at 4 +/- 2°C).	
	17. Data on precipitation is recorded	Yes
	18. The temperature was in the range between 20-25°C and the moisture was reported	Yes
	19. The presence of glyphosate identified in samples collected from groundwater, soil, surface waters, sediments or air from European areas	Yes
	20. Analytical results present residues measurements which can be correlated with the existing residues definition of glyphosate	Yes
	21. Analytical methods clearly described and adequate Statement of specificity and sensitivity of the analytical methods is included	No
	22. Radiolabel characterization: purity, specific activity, location of label	Yes
	23. If degradation kinetics are included: expect to see data tables provided, model description. Statistical parameters for kinetic fit.	No
	24. Glyphosate monitoring data: description of matrix analysed, and analytical methods fully described as above.	No
	25. For environmental fate studies: clear description of application rate and relevance to approved uses.	Yes

1. Information on the study

Data point:	KCA 7.1.4.3
Report author	Aronsson, H. et al.
Report year	2010
Report title	Leaching of N, P and glyphosate from two soils after herbicide treatment and incorporation of a ryegrass catch crop
Document No	Soil use and management (2011), Volume 27, Number 1, pp. 54–68
Guidelines followed in study	None
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 (experiment not sufficiently described to evaluate the validity of the results)

2. Full summary of the study according to OECD format

During 2005–2007, studies were carried out in two field experiments in southwest Sweden with separately tile–drained plots on a sandy soil (three replicates) and on a clay soil (two replicates). The overall aim was to determine the effects of different cropping systems with catch crops on losses of N, P and glyphosate. Different times of glyphosate treatment of undersown ryegrass catch crops were examined in combination with soil tillage in November or spring. Drainage water was sampled continuously in proportion to water flow and analysed for N, P and glyphosate. Catch crops were sampled in late autumn and spring and soil was analysed for mineral N content. The yields of following cereal crops were determined. The importance of keeping the catch crop growing as long as possible in the autumn is demonstrated to decrease the risk of N leaching. During a year with high drainage on the sandy soil, annual N leaching was 26 kg⁄ha higher for plots with a catch crop killed with glyphosate in late September than for plots with a catch crop, while the difference was very small during 1 yr with less drainage. Having the catch crop in place during October was the most important factor, whereas the time of incorporation of a dead catch crop did not influence N leaching from either of the two soils. However, incorporation of a growing catch crop in spring resulted in decreased crop yields, especially on the clay soil. Soil type affected glyphosate leaching to a larger extent than the experimental treatments. Glyphosate was not leached from the sand at all, while it was found at average concentrations of 0.25 µg⁄L in drainage water from the clay soil on all sampling occasions. Phosphorus leaching also varied (on average 0.2 and 0.5 kg⁄(ha yr) from the sand and clay, respectively), but was not significantly affected by the different catch crop treatments.

Materials and methods

Experimental fields

The study was conducted over 2 years (2005–2007) in field experiments with a similar treatment design, but located at different sites, Lanna and Lilla Böslid in southwest Sweden. In both experiments, leaching was measured in separately tile–drained experimental plots where drainage flow was measured continuously and water was sampled in proportion to flow. Precipitation and air temperature were recorded at both sites.

Lanna site, clay soil.

Lanna research station (58°20′N, 13°07′E) is situated in a region which has a mean annual temperature of 6.1°C and mean annual precipitation of 558 mm (Lanna, 1961–1990). The experimental field, which was established in 2001, consists of 10 plots (790 m^2). Each plot was separately tile–drained at ca. 1 m depth and the drains were backfilled with 10–cm gravel at the bottom and then with the soil. The soil at Lanna consists of 47 % clay (<2 µm) in the topsoil (0–0.3 m depth) and 55–60 % clay in the subsoil

(0.3–0.9 m depth). During the study, the topsoil had an organic matter content of 4.4 % and a mean pH of 6.6. The mean amount of ammonium lactate soluble P was 3.4 mg⁄100 g dry soil which is considered as low P status. The soil contains numerous cracks and macropores in the upper 1.0 m of the profile. More details on this soil are given by Bergström *et al*. (1994). At Lanna, the same plots were used during the two experimental years, with the same treatment being applied on each plot during the two consecutive years (with two replicates).

Lilla Böslid site, sandy soil.
Lilla Böslid experimental farm (56°35′N, 12°56′E) is located ca. 240 km south of Lanna. The mean annual temperature is 7.2°C and the mean annual precipitation is 803 mm (Halmstad, 1961–1990). The sandy soils in this region are commonly drained as the groundwater levels are often high because of a clay layer under the sand deposits. This experimental field was constructed in 2002, and consists of 36 separately tile–drained plots, each 320 m^2. The tile drains are at 0.9–m depth. The soil is an unstructured sand with 9 % clay in the topsoil (0–0.3 m depth) and 1–2 %in the subsoil (0.3–0.9 m depth). At the time of study, the topsoil had a mean organic matter content of 4.9 % and a pH value of 6.1. The mean amount of ammonium lactate soluble P was 12.8 mg⁄100 g dry soil. This value indicates that this soil is rich in P and that reduced P application rates are recommended for spring cereals. At Lilla Böslid, the experimental lay–out allowed two experimental years on different plots by dividing the field into two sections and using one section each year (with three replicates).

Experimental design and management practices
During the year before the experiment started, a spring cereal was grown at Lilla Böslid and winter wheat at Lanna. The experiments started in 2005 by undersowing a catch crop of ryegrass (*Lolium perenne L.*) in a cereal crop in three of five treatments at Lanna and in all treatments at Lilla Böslid (Table 1). At Lanna, glyphosate was applied in four treatments at the beginning of October, and in one in spring. Glyphosate treatment in October was combined with tillage in November (mouldboard ploughing, 25–cm depth) or in April (stubble cultivation, 6–cm depth). At Lilla Böslid, different times of glyphosate treatment in autumn were tested in combination with mouldboard ploughing (25–cm depth) in November or April. There was also one treatment without use of herbicide and spring ploughing, which was considered as a control treatment representing the best scenario for low N leaching. At Lilla Böslid, the soil was tine–cultivated to ca. 10 cm depth just before ploughing. Dates of tillage and glyphosate treatment are shown in Table 1. At Lanna, glyphosate was applied as Glyphomax Bio at a dose of 3.5 or 4.0 L⁄ha and at Lilla Böslid as Round–up Bio, 3.5 L⁄ha. The crop following incorporation of the catch crop was a spring cereal (oats or barley). It was fertilized with 100–110 kg N⁄ha at Lanna and with 90 kg N⁄ha at Lilla Böslid. A dose of 10 kg⁄ha of mineral P was applied at Lilla Böslid in 2006 and the same amount at Lanna in 2007.

Sampling and analyses of water, soil and crops
Drainage water from the plots at both sites was led to an underground monitoring station with temperatures never >15°C and <10°C during the main drainage periods when discharge rates were recorded using tipping buckets connected to a data logger which stored accumulated daily drainage volumes from each plot. Flow–proportional water samples of 15 mL were taken using a peristaltic pump after every 0.2 mm discharge. The samples for each plot were collected in individual polyethylene bottles which were emptied every 2 weeks during drainage periods for analysis of total–N, NO_3–N, total–P and PO_4–P. During sampling, the bottles were prepared with sulphuric acid for conservation of glyphosate. Glyphosate and the degradation product of aminomethylphosphonic acid (AMPA) were analysed for the same samples on 5–6 occasions during each of the two drainage seasons. At Lanna, glyphosate was analysed in samples from treatments A–D and at Lilla Böslid, in treatments F–J (Table 1). These events were primarily chosen to represent periods when drainage started in autumn with high flow periods. The first samples were taken before glyphosate treatment to ensure that any leaching detected originated from the experimental treatments. During the first year, the samples from replicates were pooled for analyses of glyphosate because of the high cost of analyses, but during the second year, all samples were analysed individually. Prior to analysis, the water samples were pretreated with a C18 ion exchange column for removal of non–polar substances, which also caused some filtration of particles (unknown size). Then glyphosate was derived with trifluoroacetic acid⁄trifluorethanol before combined gas chromatograph⁄mass spectrometer (GC⁄MS) analyses. The partitioning between particle–bound and

dissolved glyphosate was not examined and some particles were also filtered before analysis. Thus, the analysis mainly covered the amount of dissolved glyphosate, but it is also likely that some particle–bound glyphosate was included as water samples were acidified during storage, which may have resulted in some dissolution of particle–bound glyphosate.

Table 1. The different experimental treatments at the two sites during the 2 years, with planned and actual time of glyphosate treatment and catch crop incorporation

		Time of glyphosate treatment			Time of incorporation		
	Catch crop	Plan	Act yr 1	Act yr 2	Plan	Act yr 1	Act yr 2
Lanna, clay soil							
A	Per. ryegr.	1 Oct	4 Oct 2005	4 Oct 2006	10 Nov	11 Nov 2005	10 Nov 2006
B	Per. ryegr.	1 Oct	4 Oct 2005	4 Oct 2006	1 Apr	28 Apr 2006	12 Apr 2007
C	Per. ryegr.	1 Mar	14 Apr 2006	19 Mar 2007	1 Apr	28 Apr 2006	12 Apr 2007
D	–	1 Oct	4 Oct 2005	4 Oct 2006	10 Nov	11 Nov 2005	10 Nov 2006
E	–	1 Oct	4 Oct 2005	4 Oct 2006	1 Apr	28 Apr 2006	12 Apr 2007
Lilla Böslid, sandy soil							
F	Per. ryegr.	20 Sep	26 Sep 2005	26 Sep 2006	10 Nov	24 Nov 2005	24 Nov 2006
G	Per. ryegr.	20 Sep	26 Sep 2005	26 Sep 2006	1 Apr	12 Apr 2006	2 Apr 2007
H	Per. ryegr.	5 Oct	4 Oct 2005	10 Oct 2006	10 Nov	24 Nov 2005	24 Nov 2006
I	Per. ryegr.	5 Oct	4 Oct 2005	10 Oct 2006	1 Apr	12 Apr 2006	2 Apr 2007
J	Per. ryegr.	20 Oct	31 Oct 2005	22 Nov 2006	1 Apr	12 Apr 2006	2 Apr 2007
K	Per. ryegr.	–	–	–	1 Apr	12 Apr 2006	2 Apr 2007

Per. ryegr., perennial ryegrass.

Calculations and statistical analysis

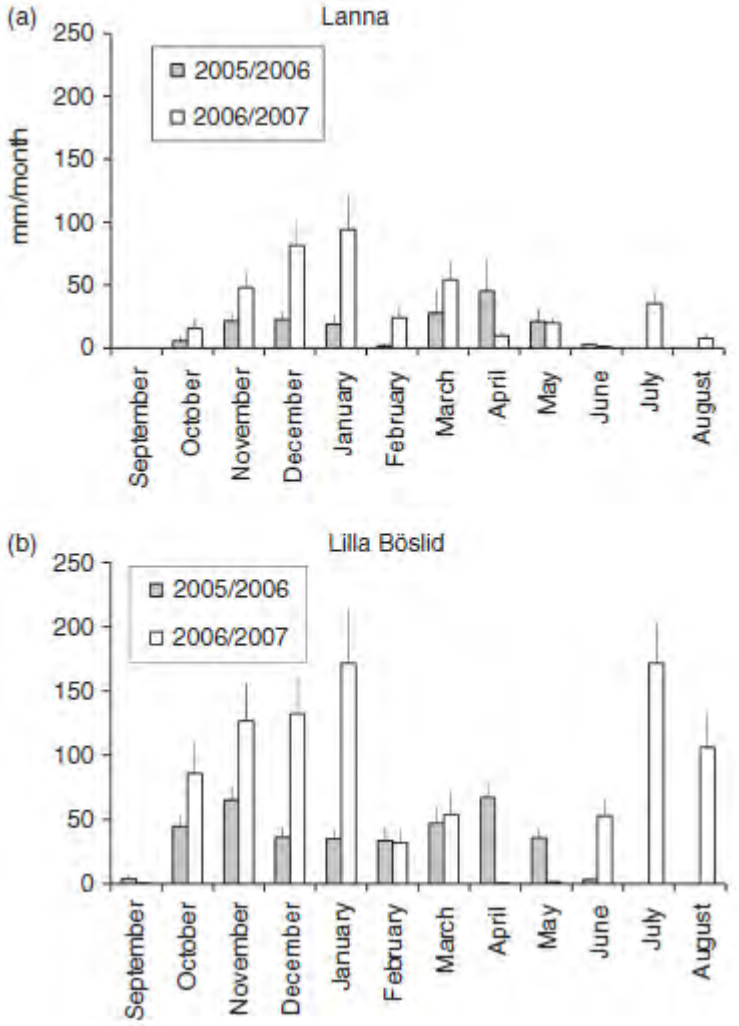
Analysis of variance was carried out by the Mixed procedure in SAS 9.1 (SAS Institute Inc. 2003: SAS/Stat 9.1 Users' Guide. Cary, NC, USA) for the statistical analysis of differences in yields, catch crop biomass and N and P contents, soil mineral N, leaching of N and P and concentrations of glyphosate between treatments. The t–test at P = 0.05 was used for pairwise comparisons by the PDIFF statement. Block was used as the random variable in analysis of a single year. For the Lanna site, the average for the 2 years was analysed by calculating an average per plot and by using block as random variable. For the Lilla Böslid site, where the experiment was carried out in separate plots during the 2 years, year was used as random variable when analysing the average for the 2 years.

Results

Drainage and climate conditions

The two experimental years represented varying climate and drainage conditions, 1 yr with a cold winter with relatively small drainage amounts, and one mild winter with high drainage. At Lanna, the mean temperature during December 2005–March 2006 was –3.5°C, while it was +2.1°C during the same period 2006–2007. For Lilla Böslid, corresponding values were –2.6°C and +4.3°C. At Lanna, the measured precipitation was 480 mm during 2005–2006 and 759 mm during 2006–2007 (1 September–31 August). At Lilla Böslid, the corresponding figures were 542 mm and 950 mm, respectively. During the first year, the precipitation was considerably lower than the long–term mean value for both sites, but higher during the second year. Different precipitation and temperature conditions during winter clearly affected drainage and the N and P leaching during the two experimental years. The high rainfall resulted in much drainage during the autumn and winter of 2006–2007. During summer 2007, southwest Sweden was exposed to several large low pressure cells, which resulted in extremely rainy conditions and major drainage events. There was some variation in drainage water totals between individual plots as shown in Figure 1 where standard deviations for all plots are included. These differences could not be attributed to different experimental treatments except for 2005–2006 at Lilla Böslid, when treatment K had higher drainage than most of the other treatments (P = 0.03).

Figure 1. Mean monthly drainage (mm) from all plots during the two experimental years at the two sites Lanna (a) and Lilla Böslid (b). Standard deviations are shown with narrow bars.



Management practices, catch crop growth and crop yields

The planned time of glyphosate treatment in September and beginning of October corresponded quite well to the actual time at both sites (Table 1). From field observations, the catch crop was still intact 1 week after treatment, but after 3 weeks, it was totally killed in both years. However, glyphosate treatment in late October at Lilla Böslid was delayed by up to 4 weeks because of bad weather conditions, especially in 2006 (Table 1) when the catch crop was treated in late November. This resulted in a poor effect of the glyphosate, and only 50 % of the catch crop was killed 3 weeks after treatment. Glyphosate treatment in spring (treatment C at Lanna) resulted in problems with the timing. Obtaining an effect of the herbicide, while simultaneously being able to cultivate this heavy clay soil, was a challenge. In spring 2007, when there was a very dense catch crop, it was particularly difficult to incorporate the catch crop material in this treatment, and about 20–40 % of the catch crop was estimated to be still growing at harvest of the following crop. Shallow cultivation in spring worked much better after glyphosate treatment in autumn (treatments B and E) with respect to incorporation of plant material, although this tillage practice is not common for this type of soil.

Leaching of glyphosate

At the sandy soil at Lilla Böslid, drainage water was analysed for glyphosate on eight occasions during the experimental period (November 2005, December 2005, April 2006, October 2006, November 2006, December 2006, January 2007 and March 2007). Glyphosate was only detected twice and occurred at trace levels, that is at concentrations above the detection limit (ca. 0.01 µg/L), but under the limit for determination of the concentration (ca. 0.05 µg/L). These occasions were in treatments F and I at sampling on 20 December 2006 and in treatment J on 8 January 2007.

AMPA was not found at all. As a result of bad weather conditions during October–November 2006, glyphosate application in treatment J was not possible until 22 November. If there had been a risk of glyphosate transport, it would probably have arisen during conditions like these, but the risk seemed to be very small for this soil. The adsorption of glyphosate in the sandy soil was probably very efficient, probably because of Al/Fe–oxides, the same as for P. At the Lanna clay soil, glyphosate was found at concentrations above the determination limit in all samples except two during the experimental period (Table 2). Thus, application of glyphosate both in autumn and in spring resulted in some transport to drainage water, but with this experimental design, it was possible that application of glyphosate during 2005–2006 also affected to some extent the results from 2006 to 2007. Even at sampling in spring 2005, before the start of the experiment at Lanna, traces of glyphosate were found in drainage water. This probably originated from autumn 2004 when glyphosate was applied to borders between the experimental plots. Concentrations were low, on average 0.25 µg/L, and only exceeded 1 µg/L on one occasion (January 2007 in treatment D). The concentrations of glyphosate measured at Lanna were similar to those found in monitoring of streams in agricultural catchments in southern Sweden (Adielsson *et al.*, 2007).

Table 2. Measured concentrations of glyphosate and its metabolite AMPA

	A		B		C		D	
	Glyphosate	AMPA	Glyphosate	AMPA	Glyphosate	AMPA	Glyphosate	AMPA
Glyphosate treatment	4 Oct 2005 4 Oct 2006		4 Oct 2005 4 Oct 2006		14 Apr 2005 20 Mar 2006		4 Oct 2005 4 Oct 2006	
24 Apr 2005	Trace*	nd*	Trace*		–	–	Trace*	nd*
15 Nov 2005	0.39	nd	0.86	Trace	–		0.85	0.40
27 Nov 2005	nd	nd	0.19	nd	–		0.01	ed
5 Apr 2006	0.23	nd	0.48	Trace	Trace*	Trace*	0.16	Trace
2 May 2006	–	–	–	–	0.29	Trace	–	–
1 Jun 2006	Trace	nd	0.08 (0.05)	nd	–	–	0.04 (0.01)	nd
1 Nov 2006	0.53[a] (0.09)	Trace	0.66[a] (0.48)	Trace	0.19[a] (0.04)	Trace	1.04[a] (0.37)	Trace
15 Nov 2006	0.21[a] (0.11)	Trace	0.44[b] (0.02)	0.10	0.10[a] (0.06)	Trace	0.51[a] (0.07)	0.10
8 Jan 2006	0.18[a] (0.01)	Trace	0.66[b] (0.12)	Trace	0.12[a] (0.06)	Trace	0.18[a] (0.06)	Trace
15 Jan 2006	0.13[a] (0.01)	Trace	0.50[b] (0.01)	Trace	0.10[a] (0.02)	Trace	0.20[a] (0.09)	Trace
13 Mar 2007	0.14[a] (0.04)	Trace	0.31[b] (0.02)	Trace	0.07[a] (0.02)	nd	0.13[a] (0.03)	Trace
21 May 2007	Trace	Trace	0.06[a] (0.04)	Trace	0.14[a] (0.07)	Trace	0.05[a] (0.03)	Trace

Standard deviations are shown in brackets. Different superscript letters indicate significant differences between treatments ($P = 0.01$). *Samples taken before treatment with glyphosate. nd, not detected. Trace, between detection and determination limit, ca. between 0.02 and 0.05 µg/L.

Discussion

Results from this study indicate that soil texture was the dominant factor in influencing both P and glyphosate losses, whereas different treatments had small or no effects. For glyphosate, this was not surprising, as soil structure and transport pathways have been shown to be of major importance for glyphosate leaching (Vereecken, 2005; Borggaard & Gimsing, 2008). The immediate detection of glyphosate in drainage water from the clay soil at Lanna clearly shows that there are rapid pathways for water and solutes in this soil, as reported previously by Larsson & Jarvis (1999). The glyphosate analyses did not distinguish between dissolved and particle–bound glyphosate; however, as 70–80 % of the P losses were in particle–bound form, this might also be an important transport form for glyphosate. In studies on two soils in Denmark, the contribution of colloid–facilitated transport was up to 27 % and 52 % for a sandy loam and a sandy soil, respectively (de Jonge *et al.*, 2000). It is probable that total leaching of glyphosate, especially from the clay soil, was underestimated in this study as it is uncertain of the extent to which particle–bound glyphosate was included in the analyses. Soil tillage practices affect transport pathways through the soil. For example, conservation tillage has been shown to increase the amount of macropores and related preferential flow paths (Shipitalo *et al.*, 2000), but time of ploughing may also affect the partitioning between different types of losses. Spring ploughing instead of autumn ploughing protects the surface against destruction of soil aggregates over winter and is highly relevant in minimizing particle–bound P losses by erosion (Kronvang *et al.*, 2005), especially in combination with a catch crop (Ule´ n, 1997). In contrast, losses of dissolved compounds may increase

when the soil is not cultivated in autumn. This was reported in studies of glyphosate losses in Norway (Stenrød *et al.*, 2007) and Denmark (Lærke Baun *et al.*, 2007) where tillage in autumn increased the leaching of particulate–bound glyphosate, while there was increased leaching of dissolved glyphosate when the soil was not tilled in autumn. These findings are supported by the results from Lanna, where there are indications of higher losses of total–P after ploughing in autumn, but differences in concentrations or yearly transport are ns. Spring tillage at Lanna (treatment B) gave significantly higher concentrations of glyphosate in drainage water than the other treatments on four occasions ($P = 0.01$) in 2006–2007, which may indicate that spring tillage conserved transport pathways through the topsoil during winter. However, it is not possible to draw conclusions about the partitioning between dissolved and particle–bound glyphosate. Another study on the Lanna soil in lysimeters shows that losses of particle–bound glyphosate were negligible and that almost all leached glyphosate was in dissolved form (Bergström *et al.*, 2010). There were no indications of increased transport of dissolved P in spring–ploughed plots, with or without a catch crop over winter. However, catch crop plant material may constitute a risk of dissolved P leaching if exposed to freezing, as shown by Bechmann *et al.* (2005).

In the sandy soil at Lilla Böslid, glyphosate was efficiently sorbed, which was also true for P. The high P status of this soil did not seem to increase the risk of P losses, although studies have shown a relationship between high P content of the soil and P leaching (Heckrath *et al.*, 1995). The larger proportion of dissolved P at Lilla Böslid, compared with Lanna, could be an indication of enhanced P desorption because of high soil P content, but this is probably not the case as P concentrations in drainage water were consistently low and stable. There is also considered to be an increased risk of glyphosate transport in soils with high P content, as PO_4–P and glyphosate may compete for the same surface binding sites on soil mineral particles (Gimsing & Borggaard, 2002). However, the P and glyphosate sorption capacity of the subsoil and the degree of saturation of sorption sites have a large impact on actual P losses, and there was no indication of saturated conditions in the sandy soil at Lilla Böslid.

The results from the sandy soil at Lilla Böslid show that the time available for catch crop growth and N uptake during autumn significantly affected the accumulation of N in the soil and the risk of N leaching during the following winter, although it is somewhat surprising that there is no clear correlation between soil mineral N in autumn and N leaching. The results also show that glyphosate treatment in September or early October resulted in fast release of N available for leaching. This confirms the findings by Snapp & Borden (2005) that N mineralization increases when the catch crop is treated with glyphosate 8 days before incorporation, compared with no treatment before incorporation. The time of catch crop incorporation after chemical kill–off in autumn seems to be of minor importance according to the results from both sites. This is somewhat surprising for the sandy soil, as several studies have shown that time of tillage in autumn clearly influences N mineralization and N leaching from this type of soil (e.g. Wallgren & Lindén, 1994; Djurhuus & Olsen, 1997; Stenberg *et al.*, 1999). In the present study, glyphosate treatment obviously had a similar effect to incorporation on N release in the soil, at least during the second year. For the clay soil at Lanna, the results are similar to those found in a study in an adjacent field (Aronsson & Stenberg, 2010), where time of tillage in autumn or spring did not affect N leaching to any large extent.

Growing a catch crop may affect the yield of the main crop because of inter–plant competition, although this effect is often small or negligible (Ohlander *et al.*, 1996). The catch crop may also affect the following crop after incorporation.

This effect can be positive as a result of the fast remineralization of catch crop N (Lyngstad & Borresen, 1996). It may also be negative as a result of the immobilization of catch crop N or depletion of soil mineral N content in spring as a result of N uptake by the catch crop. This pre–emptive effect (Thorup–Kristensen, 1993) probably contributed to decreased yields at Lanna together with regrowth of the catch crop and at Lilla Böslid. Incorporation of a living catch crop in November–December or in February–March instead of April would probably have been more suitable for remineralization of catch crop N, as suggested by Torstensson & Aronsson (2000). To improve synchronization with the N requirements of the following crop, the N mineralization dynamics must be considered rather than increasing N fertilization rates after incorporation of catch crops.

Conclusion
To develop recommendations to achieve decreased nutrient leaching and pesticide contamination of water, the leaching and contamination risks need to be considered in relation to each other, and crop production aspects also need to be considered. It is clear from this study that N leaching was considerably lower from the clay soil (2–22 kg N/ha/yr) than from the sandy soil (15–53 kg N/ha/yr). It was also clear that spring incorporation of a catch crop could not be recommended on the clay soil as it negatively affected crop yields. Glyphosate treatment causes some risk of contamination of percolating water on the clay soil, irrespective of time of application, while time of incorporation does not affect leaching of N and P on either clay soil or sandy soil. This suggests that clay soil should not be given special priority for the use of catch crops with chemical treatment or for the use of excluded tillage in autumn. The reasons are that the overall risk of N leaching is relatively low and that the beneficial effects on N leaching may be counteracted by some risk of glyphosate leaching. Moreover, there is no reduction in P losses.

For the sandy soil, N leaching was much higher than from the clay soil and keeping the soil covered with a catch crop until November or until spring considerably reduced N leaching during high–flow conditions compared with chemical kill–off in September or mid-October. It was difficult to achieve a good herbicide effect with delayed chemical treatment, indicating that there always has to be a compromise between N leaching and successful weed control. Despite the low risk of glyphosate and P leaching, the results suggest that for a sandy soil, glyphosate treatment should be excluded during autumn when growing catch crops to maximize the reduction in N leaching. Incorporation of the catch crop as late as possible in autumn or in very early spring probably reduces the risk of decreased crop yields of the following crop as a result of N uptake by the catch crop.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The study describes a long–term leaching experiment in Sweden on agricultural soils with glyphosate. The method is not sufficiently described to evaluate the validity of the results.

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

1. Information on the study

Data point:	KCA 7.3
Report author	Bento, C. P.M. et al.
Report year	2017
Report title	Glyphosate and AMPA distribution in wind-eroded sediment derived from loess soil
Document No	Environmental Pollution 220 (2017) 1079 -1089
Guidelines followed in study	None
Deviations from current test guideline	No
GLP/Officially recognised testing facilities	No, not conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Reliable

2. Full summary of the study according to OECD format

Glyphosate is one of the most used herbicides in agricultural lands worldwide. Wind-eroded sediment and dust, as an environmental transport pathway of glyphosate and of its main metabolite aminomethylphosphonic acid (AMPA), can result in environmental- and human exposure far beyond the agricultural areas where it has been applied. Therefore, special attention is required to the airborne transport of glyphosate and AMPA. In this study, we investigated the behavior of glyphosate and AMPA in wind-eroded sediment by measuring their content in different size fractions (median diameters between 715 and 8 μm) of a loess soil, during a period of 28 days after glyphosate application. Granulometrical extraction was done using a wind tunnel and a Soil Fine Particle Extractor. Extractions were conducted on days 0, 3, 7, 14, 21 and 28 after glyphosate application. Results indicated that glyphosate and AMPA contents were significantly higher in the finest particle fractions (median diameters between 8 and 18 μm), and lowered significantly with the increase in particle size. However, their content remained constant when aggregates were present in the sample. Glyphosate and AMPA contents correlated positively with clay, organic matter, and silt content. The dissipation of glyphosate over time was very low, which was most probably due to the low soil moisture content of the sediment. Consequently, the formation of AMPA was also very low. The low dissipation of glyphosate in our study indicates that the risk of glyphosate transport in dry sediment to off-target areas by wind can be very high. The highest glyphosate and AMPA contents were found in the smallest soil fractions (PM10 and less), which are easily inhaled and, therefore, contribute to human exposure.

Materials & Methods

Soil

We used the topsoil of a silty loam loess soil from Huldenberg, Belgium. The soil was air-dried and then sieved through a 1-mm sieve. It was tested for glyphosate and AMPA residues and found free of glyphosate and AMPA. The main soil properties of the sieved soil are shown in Table 1. Figure 1 shows the grain size distribution of the soil after disintegration of all aggregates.

Table 1. Soil properties of the loess soil used in this study

Parameters	Value
Particle size distribution:	
<2 µm (clay) (%)	10
2–50 µm (silt) (%)	79
>50 µm (sand) (%)	11
pH $CaCl_2$	5.8
Organic matter (OM) (%)	3.2
Particle density (g cm^{-3})	2.5
N total (g kg^{-1})	1.7
P available (mg kg^{-1})	0.4
K available (mg kg^{-1})	209
Mg available (mg kg^{-1})	121
Na available (mg kg^{-1})	10
C/N ratio	9

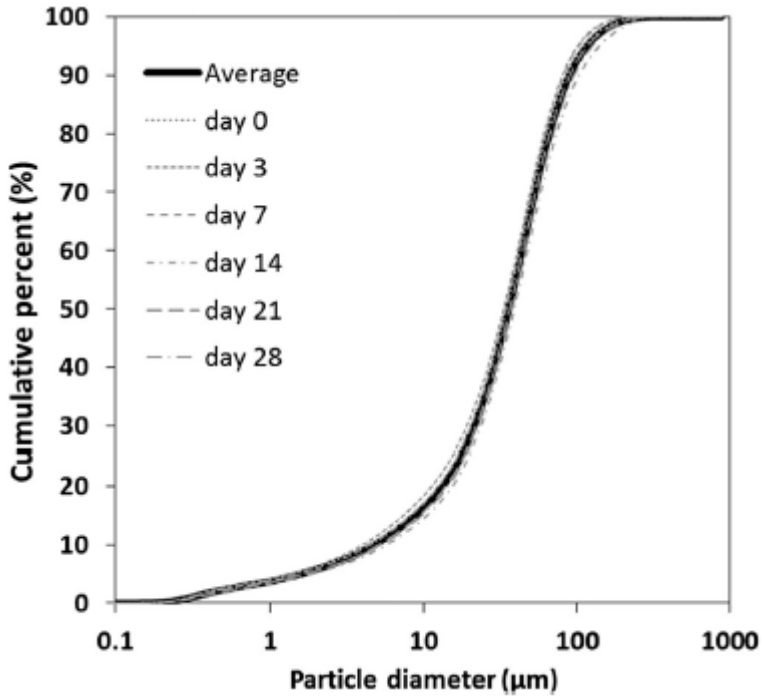


Figure 1. Particle size distribution of the start soil after disintegration of all aggregates.

Glyphosate preparation and application in the soil

Preparation of glyphosate solution

Glyphosate solution was prepared by diluting 980 mL of CLINIC®, a glyphosate-based herbicide that contains 360 g/L of glyphosate, in Millipore water to achieve a final stock solution of 0.42 g/L. A concentration of glyphosate in soil of 8.4 mg/kg was used in this study, which corresponds to an application rate of 1.26 kg a.i./ha (typically applied in agricultural fields), assuming a soil depth of 1 cm and a bulk density of 1.5 g/cm^3.

Application in soil

A plastic sheet was put on the ground and an approximately 5-cm thin layer of the air-dried and sieved soil (42 kg) was spread on it. The soil was then sprayed with the prepared glyphosate solution (see Section 2.2.1). During the application, the soil was thoroughly mixed with a rake. The soil was then stored in a plastic bag at room temperature (22ºC) and dark conditions. A small portion of the soil was collected after glyphosate application and oven-dried (105ºC) for 24 h to determine the initial soil moisture content, which was found to be 5.4 % (w/w).

Facilities and instrumentation

The experiment was carried out in the facilities of the Geography and Tourism Research Group of the Katholieke Universiteit Leuven, Belgium. A closed-return wind tunnel was used. The tunnel has two test sections, both of which were used in this study. The dimensions of the large test section are 760 cm (length) x 120 cm (width) x 60 cm (height), and those of the small test section are 150 cm (length) x 35 cm (width) x 30 cm (height). A detailed description of the wind tunnel can be found in the technical report by Goossens and Offer (1988).

Apart from the wind tunnel, we also used a modified version of the Soil Fine Particle Extractor developed in a previous study by Goossens (2012). This instrument draws up the sediment, previously spread on a table, with a plastic hose attached to a BASE 440 three-engine vacuum cleaner connected to a cyclone dust separator (RIBO, Villanova, Italy). The hose is 300 cm long and 4 cm in diameter; the separator is 70 cm high and 40 cm in diameter. Coarse particles settle in the separator and are thus removed from the sample. Separation is accomplished by the circular motion of the particles and enhanced by selective gravitational settling. Some of the smallest particles remain suspended in the separator. After initial separation in the separator, the dust enters a tube 139 cm long and 16 cm in diameter, which operates as an elutriator. Dust is then accelerated through a small pipe 36 cm long and 7.6 cm in diameter and hits an impactor (diameter: 8.7 cm) installed near the bottom of a settling chamber. Only the finest particles will suspend in the chamber. These particles then enter a 200-cm long plastic tube. Further granulometrical separation is performed in this tube, which operates as a second elutriator. Particles then enter the vacuum cleaner and settle in a 50-L deposition chamber, where they can be collected. Three 1200-W engines that generate a suction rate up to 510 m^3/h and create an under pressure of 2200-mm H_2O power the instrument. For this study, only one engine (170 m^3/h) was used.

Experimental design

To perform each experimental run, a total of 8 kg of pre-treated soil (enough to fill the sediment tray in the wind tunnel) was taken one day before each experimental run. The soil was then oven-dried at 37.5°C for 24 h to ensure a soil moisture 2 % (the highest soil moisture allowed to guarantee wind erosion; see Nourzadeh et al. (2013)). Soil samples (in duplicate) were always taken before and after the drying process to control for any effect on glyphosate decay and AMPA formation/decay. The oven-dried soil was then subjected to wind erosion, which was carried out in the wind tunnel. In the small test section, a tray 150 cm long x 35 cm wide x 2 cm deep was installed. The upwind 75 cm were filled with a piece of wood; the downwind 75 cm were covered with a thin sheet of plastic (to avoid direct contact between the glyphosate-treated soil and the metal of the tray). The oven-dried soil was then put into the tray. Its surface was carefully flattened using a slat. The wind tunnel was then closed and turned on to allow the soil sediment to erode until the entire tray was empty. We used a free-stream wind speed of 10.0 m/s, which was well above the deflation threshold of the sediment used (6.5 m/s according to visual observations made before the test). It took approximately 1 h until the tray was empty. After each run, sediment samples (in triplicate) were collected (≥2 g for most of the samples; and always ≥1 g) at 4 different places in the wind tunnel using a clean brush. The distances from the trailing edge of the tray were as follows: sample 1: 10 cm; sample 2: 480 cm, sample 3: 1290 cm, and sample 4: 1865 cm. Due to aeolian selection, the samples become finer as they are taken further from the source. Because of the restricted length of the wind tunnel, sample 4 was the finest sample that could be obtained with the wind tunnel technique. To collect even finer samples we used the Soil Fine Particle Extractor and 3 more samples were collected. After each wind tunnel run, the tunnel was first thoroughly cleaned with the vacuum cleaner. A sample (sample 7) was then taken from the deposition chamber of the vacuum cleaner, which at this stage was directly connected to the cyclone separator. The sediment in the separator was

then mixed with the remaining dust in the deposition chamber and put on a clean table. After assembling the entire Soil Fine Particle Extractor, the sediment on the table was sucked up and samples 5 and 6 were collected just downwind from the cyclone separator (sample 5) and in the deposition chamber of the vacuum cleaner (sample 6). All experimental runs (wind tunnel + Soil Fine Particle Extractor) and collection of samples were conducted on days 0, 3, 7, 14, 21 and 28 after glyphosate application. All samples were stored in plastic tubes and frozen at -18°C until glyphosate and AMPA analysis.

Particle size distribution and organic matter content

To analyze the particle size distribution of samples 2 to 7 we used a Malvern Mastersizer S laser particle size analyzer (Malvern Ltd, Malvern, UK). Sample 1, which exclusively consisted of large aggregates, was analyzed optically with a microscope. For the latter sample, we collected a subsample from the main sample and measured the nominal diameter of all aggregates. Using these data, the aggregate size distribution of the sample could be determined. To get an idea of the internal particle size distribution of the large aggregates themselves, we also collected several of these aggregates, carefully crushed and dispersed them, and then analyzed them with the Mastersizer instrument. The OM content was estimated by oxidation at 600ºC and detected by close infra-red using a SC-144DR equipment (LECO Corporation, St Joseph, MI, USA). When there was insufficient sample for analysis, the triplicates were mixed together.

Glyphosate and AMPA content

Glyphosate and AMPA contents in the samples were analyzed as described by Bento et al. (2016). Briefly, glyphosate and AMPA were extracted from 1 g of soil or wind-eroded sediment with 5 mL of 0.6 M KOH (potassium hydroxide, p.a. 85 %). After shaking and centrifuging the samples, 1 mL of the supernatant was transferred to a 10-mL plastic tube. Isotopically labelled glyphosate and AMPA were added at this point and then a derivatisation step was carried out with FMOC to improve retention and MS/MS detection as described by Bento et al. (2016). Solvent standards with isotopically labelled internal standards were prepared together with all the samples for each batch of samples, and derivatized the same way. Glyphosate and AMPA contents were then determined by liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) using an XBridge™ Shield RP C18 column 100 mm x 2.1 mm i.d. (Aquity UPLC I-Class coupled to a Micromass Ultima triple-quadrople MS, Waters, The Netherlands). Chemicals used, mobile phases and instrumentation conditions of the HPLC-MS/MS were as described by Yang et al. (2015b) and Bento et al. (2016). With each batch of samples, two blank soil samples of the loess soil used in this study were fortified at 0.5 mg/kg and added as quality control (QC) samples. To ensure the quality of the analysis when processing real samples, the fortified samples were analysed twice, at the beginning and at the end of each batch. The quantification of the sample batch was considered satisfactory when the QC recoveries were between 70 and 120 %. A detailed description of the method validation and quality control can be found in Bento et al. (2016).

Statistical analysis

All statistical analyses were performed in SPSS 22, and the graphs in Figure 4 were produced in SigmaPlot 10.0. A one-way ANOVA to ln-transformed data followed by Dunett T3 post-hoc tests was performed to test for significant ($p < 0.05$) differences in clay, silt or organic matter (OM) content between extracted size fractions of the wind-eroded sediment. Besides, a power function was applied to the non-aggregated samples (sample 3-7) to test the correlation between the clay or OM content and the particle size of the samples. To test for significant differences of glyphosate or AMPA residues between extracted size fractions of the wind-eroded sediment, an analysis of covariance (ANCOVA) to ln-transformed data followed by Bonferroni tests was performed ($p < 0.05$). The assumption of homogeneity of regression slopes was not violated. Moreover, a categorical principal components analysis (non-linear PCA) was performed to determine the relationship between sediment properties (clay, silt, OM) and glyphosate or AMPA content in the wind-eroded sediment. The loading of a given variable was considered meaningful if its absolute value was ≥ 0.40 for a given component. Besides, a Pearson correlation was computed to assess the relationship between glyphosate or AMPA contents and clay, silt or OM. A reconstruction of the distribution of glyphosate in the original soil in the sediment

tray before the start of each wind tunnel experiment was also performed. This was done by considering the glyphosate content for a large number of narrow grain size classes, which could be estimated by applying an exponential regression analysis to the data (only the samples without aggregates, i.e., samples 3-7).

Results & Discussion

Physicochemical composition of the wind-eroded sediment

Particle size distribution

The particle size distribution of the different extracted fractions of the wind-eroded sediment is shown in Figure 2a. Sample 1 was composed of large, macroscopic aggregates only. Sample 2 consisted of individual grains and micro-aggregates, mixed with a few macroscopic aggregates. Samples 3-7 only contained individual grains with some small micro-aggregates (as verified under the microscope) and were mostly composed of particles ≤100 µm in diameter. More than 96 % of the particles of samples 5-7 were ≤50 µm in diameter. The median diameters of the samples were: 715 ± 69 µm (sample 1), 58 ± 2 µm (sample 2), 33 ± 1 µm (sample 3), 29 ± 1 µm (sample 4), 18 ± 1 µm (sample 5), 8 ± 1 µm (sample 6) and 11 ± 3 µm (sample 7). These median diameters are further used as reference codes in the data analysis presented here.

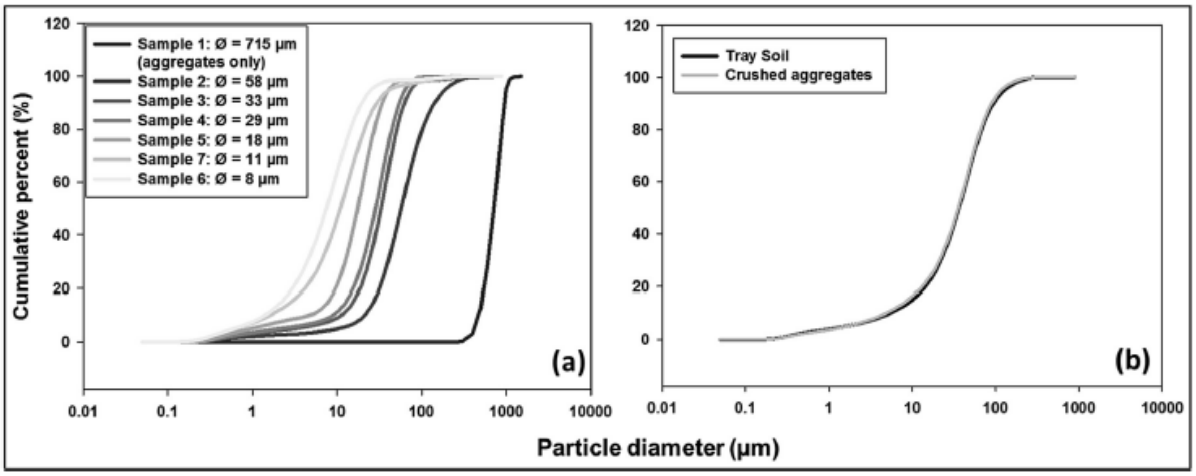


Figure 2. **Particle size distribution of (a) the different extracted fractions of wind-eroded sediment; (b) the crushed aggregates and the original sediment in the sample tray. Ø = median diameter.**

Crushing of the macroscopic aggregates (sample 1) and analyzing their grain size distribution showed that the aggregates are perfect compositions of the original tray sediment (Figure 2b), with a median particle diameter of 36 ± 2 µm for both the aggregates and the original tray soil.

Clay, silt and OM content

The clay (<2 µm), silt (2-50 µm) and OM content of the different extracted fractions of the wind-eroded sediment are shown in Figure 3. The clay content was significantly higher for the finest extracted size fraction (median diameter of 8 µm) and lowered significantly with increasing particle size (Figure 3), except for the samples with a 715-µm median diameter which consisted exclusively of macroscopic aggregates. A strong negative correlation was also observed between the clay content and the particle size of the non-aggregated samples (median diameters between 8 and 33 µm; Clay (%) = 67.7 $MDES^{-0.78}$, R^2 = 0.99; MDES = median diameter of the extracted sample). Likewise, the OM content was highest for the finest extracted fractions (samples with median diameter of 8 and 11 µm) and lowered significantly with increasing particle size (Figure 3). Nevertheless, this decrease in OM was no longer significant after a particle size ≥33 µm. A strong negative correlation was also observed between the

OM content and the particle size of the non-aggregated samples (OM (%) = 13.1 $MDES^{-0.61}$, $R^2 = 0.90$). All samples were mostly composed of silt (Figure 3). The silt content decreased as the samples became coarser, but to a lower extent compared to clay and OM. In the aggregated samples (median diameters of 58 and 715 µm), the silt content was significantly lower than in the non-aggregated samples.

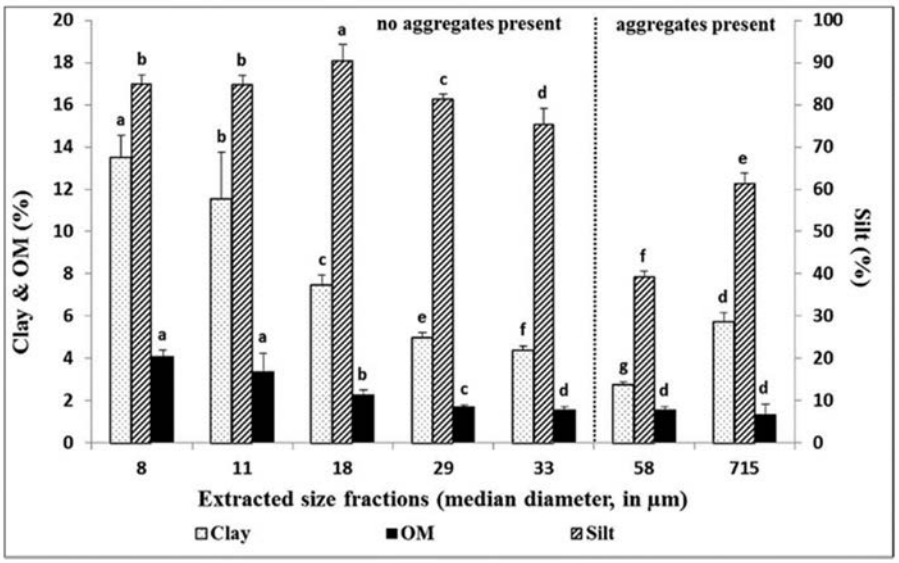


Figure 3. **Clay, silt and organic matter (OM) content of the extracted size fractions. The 715-µm samples consist exclusively of large aggregates. Different lowercase letters within the same type of bars mean significant differences in silt or clay or OM between extracted size fractions ($p < 0.05$).**

Glyphosate and AMPA content in the wind-eroded sediment

Relationship between glyphosate or AMPA and particle size

Glyphosate content (Figure 4a) varied between 5.5 and 16 µg/g, with a significantly higher content in the finest extracted fractions (median diameters from 8 to 18 µm). AMPA content (Figure 4b), on the other hand, was rather low, varying between 0.07 and 0.7 µg/g. Here too, AMPA content was significantly higher in the finest extracted fractions. In Figure 5, the relationship between glyphosate (or AMPA) content and particle size of the wind-eroded sediment is better shown. Here, it is clearly visible that glyphosate and AMPA contents were highest in the finest samples (median diameter: 8 µm) and became lower with increasing particle size until ≈33 µm (Figure 5a and b). Note that this does not necessarily mean that the highest amounts of glyphosate and AMPA in a sample occur in the finest fractions of that sample: the mass of coarse grains is much higher than that of fine grains, so even when the concentration is higher in the fine fractions it is possible that the coarse fractions contain more glyphosate and AMPA in weight. A larger spread was observed for AMPA (Figure 5b) than for glyphosate (Figure 5a). However, this larger spread is not meaningful since it just reflects the increase of AMPA content in the course of time (see Figure 4b). For the individual days, the lower AMPA content with increasing particle size became better visible. It also became stronger over time. The effect of the presence of macroscopic aggregates in a sample was also very prominent (Figure 5). Once macroscopic aggregates were present (samples with median diameters of 58 and 715 µm), glyphosate and AMPA contents remained constant regardless of how numerous or how large the aggregates were. This seems to be related with the fact that the aggregates are perfect compositions of the original soil in the sediment tray (Figure 2b) regardless of their size. Because, in an aggregate, the largest mass is represented by the coarsest grains, glyphosate and AMPA contents will be rather low, approaching the concentration in the coarsest individual grains, albeit a little higher because of the presence of a higher percentage of fine particles in the aggregates. When comparing the glyphosate content in the different sediment fractions with its content in the parent soil, it was, on average, 1.4 times higher in the finest fractions of the wind-eroded sediment (median diameters between 8 and 18 µm) than in the parent soil. In contrast, the

coarsest fractions (median diameters between 29 and 58 µm) had glyphosate contents that were, on average, 1.2 times lower than that in the parent soil. Only the samples entirely composed of macroscopic aggregates (median diameter of 715 µm) matched the glyphosate content of the parent soil, confirming once again that the large aggregates are perfect compositions of the original soil in the sediment tray. Clymo et al. (2005) also reported a much higher concentration of the herbicide pendimethalin in the PM2.5 fraction when compared to their field soil, but not for the herbicide metolachlor. According to these authors, pendimethalin is less volatile than metolachlor and therefore, the former has a higher affinity to the particle phase while the latter has a higher affinity to the gas phase. Glyphosate is also on-volatile and tends to strongly adsorb to soil particles; therefore its preference to the particle phase is also expected.

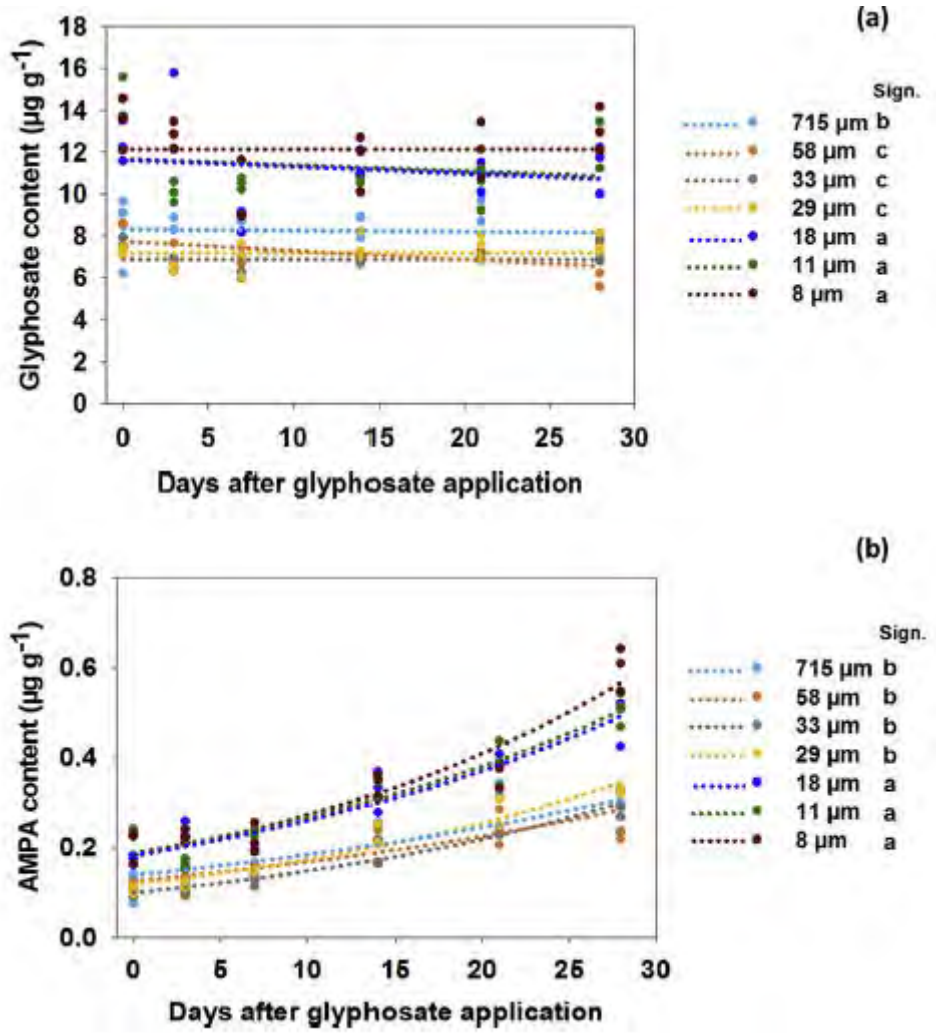


Figure 4. **Glyphosate (a) and AMPA (b) content in the different extracted size fractions of the wind-eroded sediment during the 28 days after glyphosate application, and respective trendlines. Note the different vertical scales between (a) and (b). To the right of the legends, different lowercase letters mean significant differences in glyphosate (a) and AMPA (b) content between extracted size fractions, using an ANCOVA followed by Bonferroni tests ($p < 0.05$).**

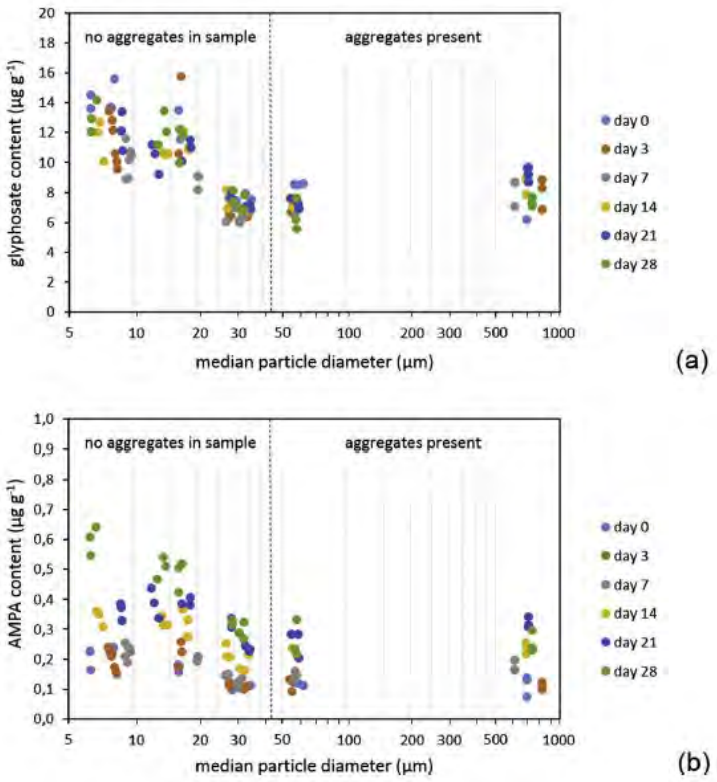


Figure 5. **Relationship between (a) glyphosate content and particle size, (b) AMPA content and particle size.**

Relationship between glyphosate or AMPA and clay, silt and OM

Figure 6 shows the results of the categorical principal components analysis performed to determine the relationship between the studied sediment properties (clay, silt and OM) and glyphosate and AMPA content. The proportion of variance-accounted-for by the first component is 61.1 %, whereas the second component accounts for 28.1 %. Thus, the two components together account for a considerable proportion (89.2 %) of the variance. All sediment properties analyzed in this study loaded in the first component together with glyphosate and AMPA, whereas only the duration of the experiment (days) loaded in the second component together with AMPA (Figure 6). The studied sediment properties do, therefore, play a major role in adsorbing glyphosate and AMPA. The duration of the experiment, on the other hand, was only meaningful for AMPA.

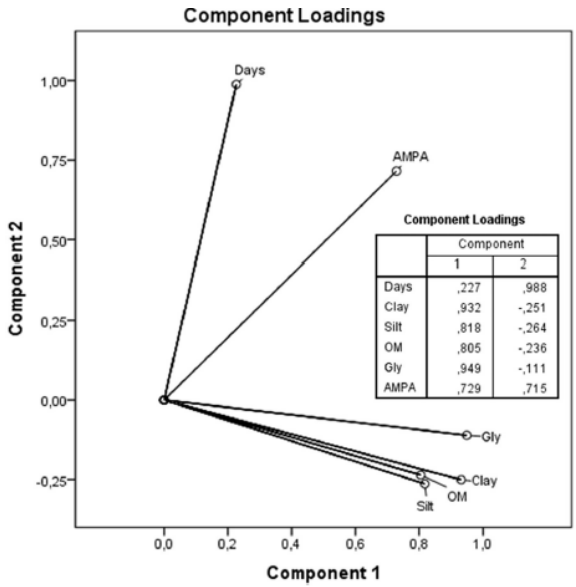


Figure 6. **Categorical principal components analysis (non-linear PCA). Gly - glyphosate; OM - organic matter.**

The order to which glyphosate and AMPA contents in the wind-eroded sediment are influenced by the studied sediment properties is as follows: clay >OM >silt (Figure 6). Glyphosate content correlates significantly and positively to the clay content (R^2 = 0.63, p <0.01). For coarser soil fractions, such as silt, the relationship with glyphosate content is considerably less expressed (R^2 = 0.27) but still significant (p <0.01). Significantly positive correlations were also observed between AMPA content and clay (R^2 = 0.16, p <0.01), and AMPA content and silt (R^2 = 0.10, p <0.01) (Figure 6). Organic matter also appears as a strong factor influencing glyphosate adsorption to wind-eroded sediment (Figure 6): glyphosate content correlates significantly and positively to the OM content (R^2 = 0.49, p <0.01). However, one should realize that a positive correlation between glyphosate content and OM would be observed anyway because both are a function of particle size (both are higher for smaller particles, see Figure 3). Therefore, the effect of OM on glyphosate adsorption cannot be confirmed with certainty. In summary, these results show that the highest concentrations of glyphosate and AMPA in the finest fractions are related to the higher clay and OM content in these same fractions, although the role of silt cannot be ignored. Sprankle et al. (1975) also reported that glyphosate was readily adsorbed to clay and OM, and that less glyphosate was adsorbed by a sandy loam soil than by a clayey loam soil.

Glyphosate and AMPA content through time and consequences for their airborne off-site transport with dust

The fact that glyphosate and AMPA contents are highest in the fine fractions of the soil has important consequences for the airborne off-site transport of these compounds, because particles <20 µm have the capacity of being transported in long-term suspension. This can easily be shown by calculating the aeolian threshold for long-term suspension, which, according to the model of Pye and Tsoar (1990), is $u_\infty/u^* < 0.1$, where u_∞ is the terminal fall velocity and u* the friction velocity. Using this criterion, 20-µm particles are already transported in long-term suspension when u* <0.3 m/s. Assuming a roughness length z_0 of 3-10 cm (typical value for agricultural areas, depending on the type of crop, see Ramli et al. (2009)), this corresponds to a 10-m height wind speed of 3.5-4.4 m/s, which are very typical values for many inland agricultural areas. For 10-µm particles, the critical wind speed is much lower: only 1.2-1.4 m/s (at 10 m height). At these wind speeds, particles are able to travel tens to even several hundreds of km before they settle back to the Earth's surface. During the 4-week experiment, nearly no glyphosate decay took place (Figure 4a). Consequently, the formation of AMPA (Figure 4b) was very slow and remained low during the experimental period. Glyphosate and AMPA decay mostly by microbial activity (Bento et al., 2016; Gimsing et al., 2004; Nomura and Hilton, 1977), and for the latter a minimum soil moisture is required (Bento et al., 2016; Schroll et al., 2006). In our study, the soil

moisture content during storage, after applying glyphosate but before the 24-h drying process prior to each wind tunnel test, was 5.4 %. This soil moisture content revealed to be very low to allow for soil microbial activity and consequent glyphosate decay. According to Bento et al. (2016), the decay of glyphosate and AMPA in a silty loam loess soil is already significantly slower at a soil moisture content of 8 % (w/w; 20 % of water holding capacity). Schroll et al. (2006) also showed that pesticide mineralization (using glyphosate) was nearly inexistent for soil moisture contents ≤2.6 % (w/w; equivalent to the ≤-20 MPa reported by these authors). Very important in this context is that wind erosion of fine, dusty particles only occurs when the topsoil (and, therefore, also the particles themselves) is sufficiently dry. Nourzadeh et al. (2013) tested several types of loamy soils using a field wind tunnel and found that the maximum moisture content to allow wind erosion of these soils was only 2 %, well below the limit for a substantial decay of glyphosate. Besides wind erosion, for many silty soils tillage erosion is a second (and in many cases even more important) mechanism for emission of fine particulates.

For tillage-emitted particles the probability for off-site transport is also highest when the particles are dry. Since the decay of glyphosate in our study occurred already extremely slowly for a soil moisture content of 5.4 %, its decay would be nearly inexistent for such dry wind-eroded sediment. Therefore, if glyphosate is applied during a dry period and emission of fine particles happens thereafter (either by wind erosion if the soil cover is still small, or by tillage activities if there is already some cover), then the potential for airborne glyphosate transport to off-site areas is considerable. In fact, Farenhorst et al. (2015), in a 2-year study where bulk deposition samples were collected and analyzed for several pesticides and some metabolites, showed that glyphosate and AMPA were the compounds detected at the highest concentrations. These authors also reported that glyphosate "accounted for 65 % of the total pesticide deposition over the 2 years" and that its deposition was >5 times larger in the dryer year. Other studies also reported the occurrence of glyphosate and/or AMPA in the atmosphere (Humphries et al., 2005; Messing et al., 2011; Quaghebeur et al., 2004).

Potential contribution of glyphosate and/or AMPA contaminated airborne dust to human exposure

Figure 7 shows the reconstruction of the distribution of glyphosate in the original non-aggregated soil in the sediment tray before the start of each wind tunnel experiment. As expected, the glyphosate distribution was nearly identical for the six experimental runs, and it was predominantly concentrated in the finest fractions. On average for the six experimental runs, 13 % of the glyphosate in the original soil was concentrated in the PM2.5 fraction (particles <2.5 μm), 15 % in the PM4 fraction, and 28 % in the PM10 fraction. It is currently unknown whether the distribution of glyphosate in Figure 7 also applies to the macroscopic aggregates, but because the aggregates are almost perfect compositions of the original soil in the sediment tray (see Figure 2b) the distribution of glyphosate within the aggregates is probably not far off from that shown in Figure 7. For AMPA, 14 % was concentrated in the PM2.5 fraction, 15 % in the PM4 fraction, and 29 % in the PM10 fraction. These results reconfirm that glyphosate and AMPA are considerably susceptible to be transported with airborne dust. After having accomplished their airborne transport trajectory, the glyphosate and/or AMPA containing soil particles will settle to the ground, thereby contaminating the deposition area. When the deposition is induced by rainfall and the particles and the soil become wet, glyphosate and/or AMPA will most probably further decay. When dry deposition occurs and the conditions remain dry for a while, glyphosate may remain in the deposited sediment until the soil becomes wet and the soil microorganisms active. But during their airborne transport the particles may also become inhaled, especially the finest fractions (PM10 and smaller), which can penetrate deeply into the human respiratory system. Some of the smallest particles (PM2.5) may even remain fixed in the lungs.

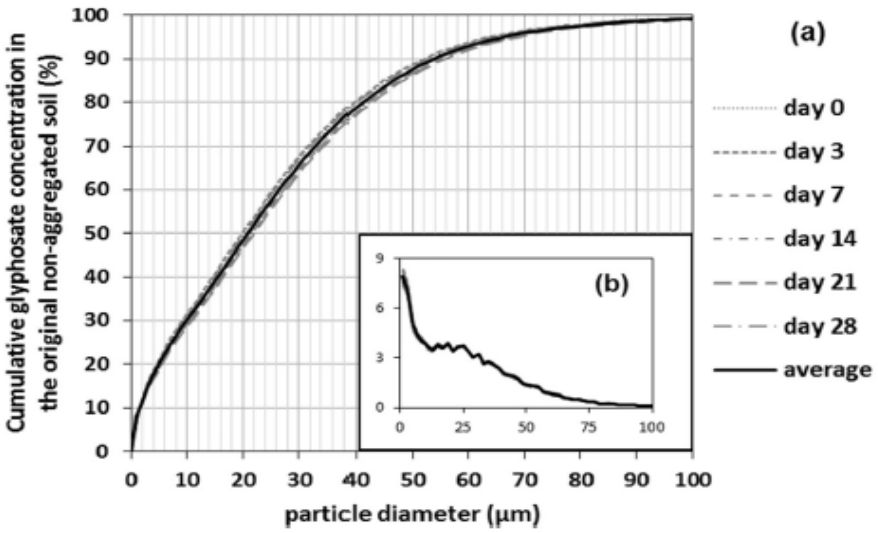


Figure 7. **Calculated cumulative (a) and non-cumulative (b) distribution of glyphosate in the original soil (after destruction of the aggregates) for the six experimental days.**

Inhalation of such fine particulates has already been associated to various health problems and around 2.1 million people world-wide are estimated to die every year from lung and respiratory system diseases caused by fine particulate matter (Kim et al., 2015; Shah et al., 2013). The risks to human health are expected to increase even more when these fine particulates are polluted with adsorbing chemicals. Bartos et al. (2009), who assessed the airborne exposure health risks associated to several toxic compounds, reported that 83-94 % of the cumulative cancer risk was due to particle-bound chemicals. To our knowledge, a human health risk assessment focusing on exposure to glyphosate and AMPA contaminated airborne particulates has not been done so far. As shown by our study, airborne particulates can be contaminated with very high concentrations of glyphosate and AMPA. According to Williams et al. (2000), the oral and dermal absorption of glyphosate and AMPA is low to very low, and they don't bio-accumulate. Any ingested glyphosate or AMPA is, therefore, expected to be eliminated unmetabolized through the urine or feces (Brewster et al., 1991; Williams et al., 2000). Nevertheless, in the review article of Williams et al. (2000), values of absorbed glyphosate in the body system of rats between 15 % and 36 % were reported, and absorbed percentages were dependent on the applied dose levels and on single or repetitive oral exposures. Brewster et al. (1991), who studied the tissue distribution of glyphosate in rats after a single oral dose, reported that significant (>1 %) doses of administered glyphosate were detected in the small intestine, bone, colon and kidney. These authors also reported that >94 % of the body burden was unmetabolized glyphosate and that only 1.2 % of the administered dose of glyphosate was still remaining in some tissues after one week, mostly in the bone. Glyphosate is reported as being harmless to humans or mammals mostly due to the absence of the shikimate pathway in animals (the mode of action of glyphosate in plants is the disruption of this shikimate pathway) (Brewster et al., 1991; Helander et al, 2012). However, studies have shown that glyphosate induced DNA damage (Mañas et al., 2009b), inhibited the activity of human serum enzymes (El-Demerdash et al., 2001), and that glyphosate exposure was related to Parkinson's disease (Barbosa et al., 2001; Gui et al., 2012; Wang et al., 2011). AMPA, on the other hand, has been shown to induce DNA damage and to produce chromosomal aberrations in human lymphocites (Mañas et al., 2009a). According to the IARC (2015), there are few studies on the effects of AMPA, but the existing ones all gave positive results as regards its genotoxicity. The IARC (2015) also reported that there is strong evidence that glyphosate and AMPA can induce oxidative stress. Inhalation of glyphosate is normally disregarded and considered a minor route of exposure in humans due to its low vapor pressure (IARC, 2015). Nevertheless, as shown in our study, very high concentrations of glyphosate may occur in airborne particulate matter that can be inhaled by humans and, thus, exposure through this route might have been underestimated in assessments done so far. The considerations above show that there is sufficient reason to take off-site airborne transport of glyphosate and/or AMPA contaminated soil fractions seriously, especially during sufficiently long periods of draught.

Conclusion

Our study indicates that glyphosate and AMPA contents are highest in sediment particles <10 µm (PM10), and that their content diminishes with increasing particle size. The risk of off-site airborne transport of glyphosate and AMPA with dust is, therefore, very high. Because glyphosate and AMPA hardly decay under dry conditions of the soil, this risk is intensified if glyphosate is applied in arid and semi-arid areas or during long periods of draught. If glyphosate and AMPA contaminated PM10 fractions of soil are emitted to the atmosphere, they may be inhaled by humans and animals. This contributes to the risk of human and animal exposure and, therefore, more attention should be paid to this route of exposure in environmental and human health risk assessment studies. Moreover, glyphosate applications during dry periods in regions susceptible to wind erosion should be avoided.

Assessment and conclusion by applicant:

The article describes the glyphosate and AMPA distribution in wind-eroded sediment derived from a Belgian loess soil. The distribution of the substances as dust via air and their dissipation and formation behavior is evaluated. Correlations to different soil parameters are presented.

The article was seen as reliable (Category 1).

E-Fate: Reliability criteria for the detailed assessment of full-text documents

Data requirements (indicated by the corresponding EU data point)	Criteria for "Reliable" articles	Criteria met? Yes / No / Uncertain
General criteria for reliability considered for all data requirements indicated by the corresponding EU data points as specified in EC Regulation (EU) No 283/2013	1. For guideline-compliant studies (GLP studies): OECD, OPPTS, ISO, and others. The validity/quality criteria listed in the corresponding guidelines met.	No
	2. Previous exposure to other chemicals is documented (where relevant).	No
	3. The test substance is dissolved in water or non-toxic solvent	Yes
	4. Glyphosate, when the test substance, is sufficiently documented - identity of the test material reported (i.e. purity, source, content, storage conditions)	Yes
	5. Only glyphosate is the tested substance (excluding mixture), and information on application of glyphosate is described	Yes
	6. The endpoint measured can be considered a consequence of glyphosate (or a glyphosate metabolite)	Yes
	7. Study design / test system is well described, including when relevant: concentration in exposure media (dose rates, volume applied, etc.), dilution/mixture of test item (solvent, vehicle) where relevant.	Yes
	8. Analytical verifications performed in test media (concentration)/ collected samples, stability of glyphosate in test media documented	Yes
	9. An endpoint can be derived. Findings do deliver a regulatory endpoint, and/or is useful as supporting information	Yes
	10. Assessment of the statistical power of the assay is possible with reported data.	Yes
	11. If statistical methodology was applied for findings reported, then the data analysis applied is clearly reported (e.g., checking the plots and confidence intervals)	Yes
	12. Field locations relevant/comparable to European conditions. Soils not completely matching the OECD criteria but from Europe or to some extent representative for the European Agriculture.	Yes

E-Fate: Reliability criteria for the detailed assessment of full-text documents

Data requirements (indicated by the corresponding EU data point)	Criteria for "Reliable" articles	Criteria met? Yes / No / Uncertain
	13. Characterization of soil: texture (sandy loam, silty loam, loam, loamy sand), pH (5.5-8.0), cation exchange capacity, organic carbon (0.5-2-5 %), bulk density, water retention, microbial biomass (~1 % of organic carbon)	Yes
	14. Other soils where information on characterization by the parameters: pH, texture, CEC, organic carbon, bulk density, water holding capacity, microbial biomass	Yes
	15. For tests including agricultural soils, they should not have been treated with test substance or similar substances for a minimum of 1 year	No
	16. For soil samples, sampling from A-horizon, top 20 cm layers; soils freshly from field preferred (storage max 3 months at 4 +/- 2°C).	No
	17. Data on precipitation is recorded	No
	18. The temperature was in the range between 20-25°C and the moisture was reported	No
	19. The presence of glyphosate identified in samples collected from groundwater, soil, surface waters, sediments or air from European areas	Yes
	20. Analytical results present residues measurements which can be correlated with the existing residues definition of glyphosate	Yes
	21. Analytical methods clearly described and adequate Statement of specificity and sensitivity of the analytical methods is included	Yes
	22. Radiolabel characterization: purity, specific activity, location of label	No
	23. If degradation kinetics are included: expect to see data tables provided, model description. Statistical parameters for kinetic fit.	No
	24. Glyphosate monitoring data: description of matrix analysed, and analytical methods fully described as above.	No
	25. For environmental fate studies: clear description of application rate and relevance to approved uses.	Yes

1. Information on the study

Data point:	KCA 7.1.2.1.1, KCA 7.1.2.1.2, KCA 7.1.3.1, KCA 7.1.4.2
Report author	Bergstrom, L. et al.
Report year	2011
Report title	Laboratory and Lysimeter Studies of Glyphosate and Aminomethylphosphonic Acid in a Sand and a Clay Soil
Document No	Journal of environmental quality (2011), Vol 40, No 1, pp. 98–108
Guidelines followed in study	OECD 106 Guideline
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 (Not all validity criteria of the studies were met)

2. Full summary of the study according to OECD format

Due to the increasing concern about the appearance of glyphosate [N– (phosphonomethyl) glycine] and its major metabolite aminomethylphosphonic acid (AMPA) in natural waters, batch laboratory and lysimeter transport studies were performed to assess the potential for leaching of the compounds in two agricultural soils. Unlabelled and ^{14}C–labelled glyphosate were added at a rate corresponding to 1.54 kg a.s./ha on undisturbed sand and clay columns. Leachate was sampled weekly during a period of 748 d for analyses of glyphosate, AMPA, total ^{14}C, and particle–bound residues. Topsoil and subsoil samples were used for determination of glyphosate adsorption, glyphosate degradation, and formation of AMPA and its degradation. The influence of adsorption on glyphosate degradation was confirmed, giving very slow degradation rate in the clay soil (half–life 110–151 d). The kinetics of AMPA residues suggest that although AMPA is always more persistent than glyphosate when formed from glyphosate, its degradation rate can be faster than that of glyphosate. The kinetics also suggest that apart from glyphosate being transformed to AMPA, the sarcosine pathway can be just as significant. The long persistence of glyphosate was also confirmed in the lysimeter study, where glyphosate+AMPA residues constituted 59 % of the initial amount of glyphosate added to the clay soil 748 d after application. Despite large amounts of precipitation in the autumn and winter after application, however, these residues were mainly located in the topsoil, and only 0.009 and 0.019 % of the initial amount of glyphosate added leached during the whole study period in the sand and clay, respectively. No leaching of AMPA occurred in the sand, whereas 0.03 g/ha leached in the clay soil.

Materials and methods

Lysimeter Experiment

Soil Characteristics, Lysimeter Collection, and Management

Three undisturbed soil columns of a sandy soil and four of a clay soil were used. The smaller number of sand columns was based on the fact that sandy soils are usually more homogeneous and therefore show less variability in flow processes (Bergström and Shirmohammadi, 1999). Some physical and chemical properties of the two soils used are listed in Table 1. The soil columns were collected using coring equipment in which a polyvinyl chloride pipe (1.18–m long and 0.295–m inner diam.) is gently pushed into the soil by a steel cylinder with cutting teeth, which rotates around the pipe as it penetrates the soil (Persson and Bergström, 1991). After collection at the two field sites, Lanna in southwest Sweden (58°21′ N, 13°08′ E) and Nåntuna close to Uppsala (59°49′ N, 17°39′ E), the columns were prepared for gravity drainage by removing about 0.07 m of soil at the base, which was replaced by gravel, two stainless steel meshes, and a fiberglass lid, giving a final length of the soil columns of ~1.05 m. The lysimeters were then placed in vertical pipes permanently installed below ground at a lysimeter station located at the Swedish University of Agricultural Sciences in Uppsala, Sweden (Bergström, 1992).

Table 1. Selected soil characteristics of the Lanna clay and the Nåntuna sand. Standard laboratory methods were used throughout (Bergström et al., 1994).

Layer	Soil texture (Gr/Sa/Si/Cl)†	Organic matter	Cation exchange capacity	Bulk density	pH‡	Water content at tensions (cm)		
						0§	100	15,000
cm	%		$cmol_c\ kg^{-1}$	$g\ cm^{-3}$		$m^3\ m^{-3}$		
Clay								
0–30	1.3/6.0/46.2/46.5	4.4	28.4	1.24	7.2	0.524	0.359	0.193
30–60	0.6/2.7/40.6/56.1	0	33.6	1.43	7.4	0.477	–	0.249
60–90	0.2/1.8/37.4/60.6	0	–	1.46	7.4	0.468	–	0.297
Sand								
0–30	0/87.8/4.5/7.7	2.0	4.7	1.43	7.4	0.448	0.180	0.034
30–60	0/95.4/4.6/0	1.0	1.8	1.47	6.4	0.427	0.165	0.019
60–90	–	1.0	1.4	1.46	7.0	0.450	0.065	0.016

† Gr = gravel, >2 mm; Sa = sand, 0.06–2 mm; Si = silt, 0.002–0.06 mm; Cl = clay, <0.002 mm.

‡ Determined in water.

§ Equivalent to porosity.

All management practices performed on the lysimeters were intended to reproduce field conditions as closely as possible. Just before sowing in each year, the soil in each lysimeter was hand–tilled to simulate light harrowing. Spring barley (Hordeum distichum L.) was sown at a rate of 2 g per lysimeter on 21 May 2006, 26 May 2007, and 30 May 2008. On each occasion, mineral fertilizers were applied at rates of 100 kg N/ha, 22 kg P/ha, and 56 kg K/ha. The barley was harvested on 1 Sept. 2006, 28 Sept. 2007, and 16 Sept. 2008 by cutting the aboveground plant parts at ground level.

In addition to natural precipitation, all lysimeters received supplemental irrigation on two occasions during the 2–yr experimental period (in total, 22 mm). On each occasion, water was added with spray bottles over a few hours at rates typical of heavy rain storms, but not exceeding the infiltration capacity of the soil.

Chemical Application

Glyphosate was applied to two lysimeters of the sand soil and to three lysimeters of the clay soil on 18 Sept. 2006 at a rate corresponding to 1.54 kg a.s./ha, which represents a normal dose in Swedish cereal production systems. Radiolabeled [^{14}C] glyphosate (ARC 1313 glyphosate-[phosphonomethyl–^{14}C], 50 mCi/mmol, American Radiolabeled Chemicals, Inc., St. Louis, MO) was used to obtain fast screening of the leachate samples using scintillation counting analysis. The radiolabeled portion (5.32 MBq) was mixed with formulated (Roundup BIO, contains 486 g glyphosate/L as isopropylamin salt, Monsanto Crop Sciences), unlabeled glyphosate (in total 10.5 mg/lysimeter), which was dissolved in 11 mL (0.16 mm) of water. This solution was applied to the lysimeters by dripping it on the soil surface using a syringe. After the solution had been applied, 5 mL (0.07 mm) of water was drawn up into the syringe and also applied to each lysimeter. In addition to glyphosate, KBr at a rate of 0.268 g Br^- per lysimeter (~40 kg Br^-/ha) was applied to provide information on the movement of water through the soil columns. The KBr was dissolved in water (0.4 g KBr in 5 mL), which was applied separately to the lysimeters, also using a syringe.

Soil and Water Sampling

On 17 Oct. 2007, samples of the topsoil (0–30 cm) and subsoil (30–80 cm) of each soil were collected for determination of adsorption and degradation characteristics. These samples were taken from the lysimeter of each soil used as control (i.e., no glyphosate added). Three soil cores from each lysimeter were collected with a tube drill. The individual samples were then mixed by layers into a topsoil and a subsoil sample for each lysimeter. On 5 Oct. 2008, after leaching measurements were terminated, soil samples were collected from the lysimeters to which glyphosate had been applied to determine the residual amounts of glyphosate and AMPA about 2 yr after application. Three cores from each lysimeter were taken with a tube drill and divided into three layers (0–30, 30–60, and 60–90 cm), which were pooled to one sample for each lysimeter and layer. After collection, all soil samples were stored in a freezer (–20°C) until analyzed.

Leachate from the lysimeters was collected and weighed each week during the 2–yr period when

drainage water was available. After collection, all leachate samples were stored in a freezer (–20°C) until analyzed. The amount of ^{14}C was measured in 10 mL of the leachate using a Beckman LS 6000TA liquid scintillation counter (Beckman Coulter Inc, Fullerton, CA) after addition of 10 mL of Insta–Gel Plus (PerkinElmer, Waltham, MA).

Adsorption Study

The adsorption study was performed according to the OECD 106 guideline (OECD, 2001). Adsorption data were obtained at five different concentrations in two replicate samples. Four grams dry weight (DW) of field–moist soil were shaken at 200 rpm on a shaker for pre–equilibration with 39 mL of 0.01 M $CaCl_2$ for 24 h at 20°C in 50–mL plastic tubes. Thereafter, the soil slurry was spiked with 1 mL of a mixture of labeled (1.98 kBq) and unlabeled glyphosate in 0.01 M $CaCl_2$ to give five initial concentrations in the range 0.1 to 10 µg/g dw of soil. After shaking for 24 h, the tubes were centrifuged for 20 min at 4000 rpm and then the radioactivity was measured in 10 mL of the supernatant. Tubes without soil and ^{14}C–labeled glyphosate were included for subtraction of background radiation, and tubes without soil were used to give the initial amount of ^{14}C activity added. No significant adsorption of glyphosate occurred on the plastic tubes. A pre–study showed that adsorption equilibrium was obtained after 24 h of contact time between soil and solution, which also indicates that negligible amounts of AMPA had been formed.

Adsorption data were fitted by nonlinear regression to the Freundlich adsorption isotherm:

[1]

$$c_{soil} = K_f c_{aq}^{1/n}$$

where c_{soil} (µg/g) is the adsorbed amount, c_{aq} (µg/mL) is the concentration in the aqueous phase, K_f [$\mu g^{1-1/n}$ $(mL)^{1/n}$/g] is the Freundlich adsorption coefficient, and $1/n$ (–) the measure of nonlinearity.

Degradation Study

Glyphosate dissolved in water (1.4 mg/mL) was applied dropwise (1.0 mL) to 15 g of fresh soil. The soil was dried and mixed, after which an additional amount of fresh soil (to give 140 g DW in total) was thoroughly mixed into the spiked soil to give an initial concentration of 10 µg glyphosate per g DW of soil. Portions corresponding to 10 g of dry soil were transferred to 50–mL plastic tubes. The water content was adjusted to 60 % of the water–holding capacity. The tubes were sealed with plastic caps that allow gas exchange and incubated at 20°C in the dark. After 2, 4, 8, 16, 32, and 64 d, two tubes were put in the freezer (−20°C) until analysis for residual concentrations of glyphosate and the metabolite AMPA. The weight of the tubes was measured once a week during the incubation, and when necessary, the moisture content was adjusted to 60 % of the water–holding capacity.

Residual values of glyphosate were used for a least squares fitting procedure to determine values of the parameters of the function for first order exponential decay:

[2]

$$c_G(t) = c_{G0} e^{-k\,t}$$

where c_G (mg/kg) is the residual concentration of glyphosate at time t days after application, c_{G0} (mg/kg) is the initial concentration of glyphosate, and k (d^{-1}) is the first–order rate coefficient for degradation.

A branched reaction scheme was applied to describe the degradation of glyphosate to AMPA and sarcosine (Karpouzas and Singh, 2006; Borggaard and Gimsing, 2008) and the degradation of AMPA (Figure 1). According to this scheme and assuming first–order kinetics, the rate of AMPA formation and degradation is then

[3]

$$\frac{dc_A}{dt} = 0.66 k_1 c_G - k_2 c_A$$

where c_A (mg/kg) is the concentration of AMPA at the time t. Because the concentrations of glyphosate and AMPA were expressed in units mg/kg, the value of c_{G0} obtained from Eq. [2] was multiplied by the stoichiometric factor 0.66 (i.e., the ratio of the molecular weights of the dominant species of AMPA and

glyphosate at pH 7) in these calculations. The equation describing the concentration of AMPA was obtained by combining Eq. [2] and [3], and integrating:

[4]

$$c_A = \frac{0.66 k_1 c_{G0}}{k_2 - k}(e^{-kt} - e^{-k_2 t})$$

In this branched pathway, k for glyphosate degradation in Eq. [2] equals the sum of k_1 for AMPA formation and k_3 for sarcosine formation. Then $k_3 = k - k_1$ and the fractions of glyphosate transformed into AMPA and sarcosine are k_1/k and k_3/k, respectively. Since no more than 100 % of the glyphosate can be transformed into AMPA, the upper limit for k_1 is k, in which case $k_3 = 0$. The maximum concentration of AMPA, c_{Amax}, occurs at time t_{Amax} when $dC_A/dt = 0$. Inserting this value into Eq. [3], replacing c_G and c_A in Eq. [3] by their expressions in Eq. [2] and [4], respectively, and rearranging gives the following:

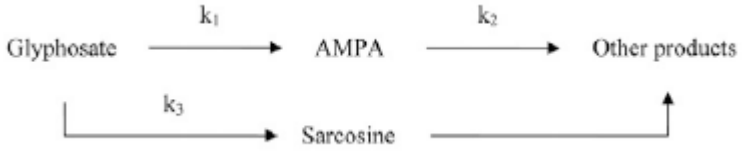
[5]

$$t_{A\,max} = \frac{\ln(k) - \ln(k_2)}{k - k_2}$$

Nonlinear Regression

Least squares fits of data on adsorption and on residual values of glyphosate and AMPA were fitted to their respective equations by nonlinear regression. Residual values of AMPA were fitted using the values of c_{GO} and k for glyphosate degradation as obtained from Eq. [2]. The calculations were performed on a PC with the application SigmaPlot for Windows version 10.0 (Systat Software, Inc., San Jose, CA); the nonlinear regression method is based on the Levenberg and Marquardt method.

Figure 1. Branched reaction scheme with the first-order rate coefficients k_1 and k_3 for the degradation of glyphosate to aminomethylphosphonic acid (AMPA) and sarcosine, respectively, and k_2 for the degradation of AMPA



Glyphosate and AMPA Analyses

Reagents

Analytical standards used for calibration were (trivial names in italics): N−(phosphonomethyl) glycine, *glyphosate*, (Riedel−de−Haën, Sigma−Aldrich, Sweden AB) and (aminomethyl) phosphonic acid, *AMPA*, (Dr. Ehrenstorfer GmbH, Augsburg, Germany). Internal standards were ^{13}C; ^{15}N; ^{2}D−labeled AMPA and ^{13}C; and ^{15}N−labeled glyphosate (LCG Standards AB, Borås, Sweden). Concentrated HCl, ethyl acetate, and NaOH (analytical reagent grade from VWR, Stockholm, Sweden), were used for extraction and solvation. The AG1−X8, 100−200, formate form (Bio Rad Laboratories, Sundbyberg, Sweden) and Isolute C18 EC 200 mg (Sorbent AB, Gothenburg, Sweden) were used for ion exchange and clean−up. Trifl uoroacetic anhydride (TFAA) and trifluoroethanol (TFE), both analytical reagent grade from Sigma Aldrich Sweden AB (Stockholm, Sweden), were used for the derivatization. The 0.22−µm glass fiber filters # GSWP04700 were from Millipore VWR (Stockholm, Sweden).

Calibration

Stock solutions of glyphosate and AMPA were diluted in water to concentrations of 100 µg/mL and stored at +4°C. A solution containing 1 µg/mL of glyphosate and AMPA was prepared daily as a working standard. The labeled glyphosate and AMPA were diluted in deionized water to a concentration of 1 µg/mL and stored at −20°C in 2−mL portions.

Clean−Up and Derivatization: Water Samples

A 50−mL volume of a water sample and 0.1 µg each of glyphosate and AMPA internal standard were adjusted to pH 2 with 6 M HCl in a plastic tube. The sample was left to precipitate for 1 h and centrifuged at 5000 rpm for 10 min. The upper, clear phase was adjusted to pH 7 to 8. Ag1−X8 (2.3 g) was weighed

into an empty 6 mL−plastic column equipped with a piece of cotton at the bottom, and the column was wetted with deionized water. A 3−mL (200 mg) C18 SPE column was activated with 3 mL of methanol and 3 mL of water and connected on top of the AG1−X8 column. An empty 75−mL plastic column was connected on top of the C18 and Ag1−X8 columns, and the sample was applied at a rate of 2 mL/min. The two upper columns were removed and the analytes were eluted with 3×4 mL of 0.6 M HCl at a rate of 1 mL/min and collected in a 100−mL pear−shaped fl ask. The sample was evaporated to approximately 2 mL under vacuum, quantitatively transferred to an 8−mL glass tube and evaporated to dryness under an air stream at 50°C. The derivatization was performed by adding 1 mL of trifluoroethanol and 2 mL of trifluoroacetic anhydride, and the sample was held at 100°C for 1 h. After being cooled to room temperature, the sample was evaporated under nitrogen and redissolved in 1.00 mL of ethyl acetate before analysis.

Clean−Up and Derivatization: Particle−Bound Glyphosate and AMPA in Leachate

Leachate samples from three lysimeters of each soil were analyzed for particle−bound glyphosate and AMPA. These samples comprised two samples from the untreated lysimeters and four samples from the glyphosate−treated lysimeters on sampling occasions when the highest concentrations of glyphosate and AMPA were detected in the leachate. A 300−mL portion of each sample was filtered through a 0.22−µm glass fiber filter. The filter was weighed before and after filtration, dried at 105°C, and the dry weight of the particles was calculated. The dry filter and the particles were analyzed for glyphosate and AMPA by extraction with 7 mL of 0.1 M NaOH following the same procedure as for soil samples (see below).

Clean−Up and Derivatization: Soil Sample

Ten grams of soil were extracted with 40 mL (for the degradation study) or 75 mL (for the lysimeter soil residue analysis) of 0.1 M NaOH by shaking for 30 min at 200 rpm, sonicated for 10 min and centrifuged for 10 min at 5000 rpm. The internal standards (0.1 µg each of glyphosate and AMPA) were added to a portion (40 µL and 4 mL for the degradation and the lysimeter studies, respectively) of the clear upper part of the sample, which was then analyzed according to the procedures described for the water samples. The portion from the degradation study was evaporated and derivatized directly after precipitation of the extract, since no column clean−up was needed due to the high residual concentrations in these samples.

Instrumentation

The gas chomatography−mass spectrometry (GC−MS) analyses were performed with a Hewlett−Packard 6890 GC (Agilent Technologies Sweden AB), equipped with a 30 m by 0.25 mm i.d. (0.25−µm fi lm thickness) fused silica capillary column (HP−5 for GC–MS), a mass spectrometer 5973, a split/splitless injector, and the software Chemstation, all from Agilent Technologies (Kista, Sweden). One microliter of the samples was injected (in the splitless mode at 270°C, oven temperature 70°C). After 2 min, the oven temperature was raised to 170°C at 30°C/min and then from 170 to 250°C at 120°C/min. Helium (N47 grade, 99.997 %) was used as the carrier gas and the flow rate was 1.2 mL/min. The mass spectrometer was operated in the electron impact (EI) mode; the transfer line and manifold temperatures were 270 and 230°C, respectively. Fragment ions were detected by selected ion monitoring (SIM) and used for identification of the AMPA and glyphosate derivates as shown in Table 2.

Table 2. Molecular weights, retention times (RT) and specific selected ions for compound derivatives.

Molecule	Molecular weight	RT (min)	Quantification ion (m/z, % relative abundance)	Qualification ion (m/z, % relative abundance)
AMPA†	371	4.49	126 (100)	302 (23)
AMPA‡	375	4.49	130 (100)	306 (22)
Glyphosate	511	5.35	411 (100)	384 (50)
Glyphosate‡	513	5.35	413 (100)	386 (48)

† AMPA = aminomethylphosphonic acid.

‡ Internal standard.

Verification of compound identification was based on comparison of the areas of the selected ions in the samples with those of the standards. For quantification, the response areas for AMPA and glyphosate target ions were calculated in relation to those of the internal standards. The response was found to be

linear in the practical concentration range (2.5−100 pg) of individual components injected.
The quantification levels for glyphosate and AMPA were 0.1 µg/L in water and 0.01 µg/g in soil. In some samples, however, the quantification level was higher due to the specific background.

Results

Adsorption of Glyphosate

The high correlation coefficients (R^2 ≥0.997; Table 3) obtained when sorption data for both soils and soil layers were fitted to the Freundlich adsorption isotherm show that they could be accurately described by this model. The values of the K_f parameter obtained were considerably higher in the clay soil than in the sand and are similar to values previously reported for glyphosate sorption to soils of similar textures (Vereecken, 2005). In the sand, K_f was higher in the topsoil than in the sub−soil, whereas the opposite was true for the clay. The correlation between K_f and the amount of clay in the different soils was 0.987. Although based on only four soils (topsoil and subsoil in the respective soils), this result supports the generally held view that glyphosate is primarily sorbed to clay particles and their associated iron oxides (Vereecken, 2005). Normalization of the distribution coefficients for glyphosate should therefore also account for the amount of clay and oxides present in soil and not organic carbon only, which is used to calculate K_{oc}. The 1/*n* parameter, which expresses the degree of linear relationship between c_{soil} and c_{aq}, was close to 1 for both layers of the clay soil and the sand topsoil, showing an almost constant distribution coefficient between sorbed and dissolved glyphosate in these soil layers in the range of concentrations studied. In the subsoil of the sand, the parameter 1/*n* was 0.82, indicating that the availability of sites for sorption in this layer becomes limiting at high glyphosate concentrations.

Table 3. Freundlich coefficients (K_f) (± SE, *n* = 10) for adsorption of glyphosate obtained by nonlinear regression according to Eq. [1].

Soil	K_f	1/*n*	R^2
	μg^{1-n} (mL)^n g^{-1}	–	–
Sand topsoil	40.0 ± 2.9	0.92 ± 0.04	0.997
Sand subsoil	28.7 ± 1.2	0.82 ± 0.02	0.998
Clay topsoil	118 ± 4.4	0.95 ± 0.01	1.000
Clay subsoil	165 ± 10.7	1.03 ± 0.02	0.999

Degradation of Glyphosate and AMPA

Best fits of glyphosate and AMPA residue data to Eq. [2] and [4], respectively, are shown in Figure 2 and the parameter values obtained in Table 4 and Table 5, respectively. Initial extraction efficiencies of glyphosate were 112 to 123 % as shown by comparing the initial concentrations obtained (Table 4) with the nominal value of 10 µg/g DW. All parameter values were significantly different from zero (p <0.05, n = 12). The models gave good fits of the data for all soils (R^2 ≥0.90), except for glyphosate in the clay subsoil (R^2 = 0.56). This poor fit could be due to difficulties in getting glyphosate homogeneously distributed in this clay−rich (56.1 %) subsoil with no organic matter (0 %). Another explanation could be that the R^2 values obtained by nonlinear and linear regression are not comparable. In nonlinear regression, R^2 refers to the fraction of the variance explained and is the model efficiency (EF). A disadvantage of EF is its dependency on the slope of the curve, as it is always relatively small for relatively flat decline patterns, or can even be negative for curves describing for instance formation and degradation of metabolites, irrespective of the scatter of measured data around the calculated curve (FOCUS, 2005). Therefore, from visual inspection of the fits to the data (Figure 2) and from the generally small standard errors in the parameters determined (Table 4 and 5), we concluded that the equations provide relevant quantitative information.

Figure 2. Best fits (A) to Eq. [2] of data on glyphosate and (B) to Eq. [4] of data on aminomethylphosphonic acid (AMPA) concentrations for sand topsoil (•), sand subsoil (○), clay topsoil (▼), and clay subsoil (^) (mean ± SE, n = 2). dw = dry weight.

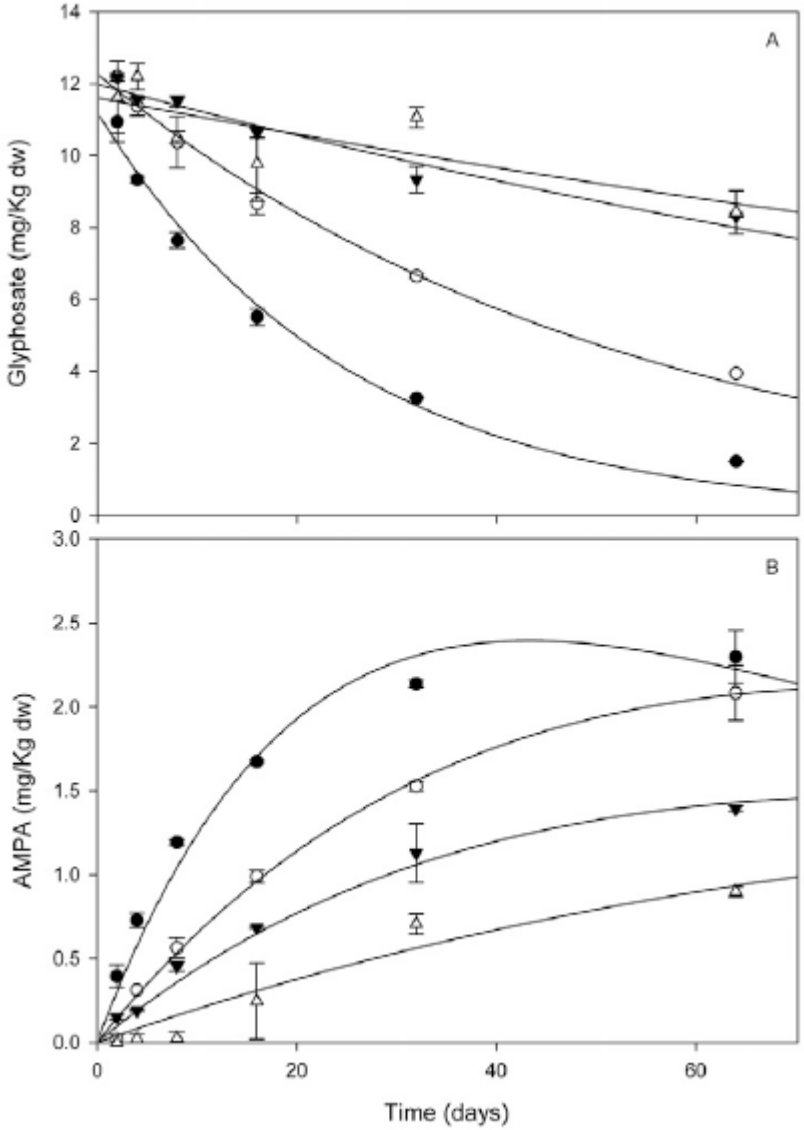


Table 4. Coefficients (± SE, n = 12) obtained by nonlinear regression for degradation of glyphosate according to first−order kinetics (Eq. [2]).

Soil	c_{00}†	k	R^2	$t_{1/2}$‡	DT_{90}§
	mg kg^{-1}	d^{-1}	–	d	d
Sand topsoil	11.21 ± 0.33	0.041 ± 0.003	0.978	16.9	56.2
Sand subsoil	12.27 ± 0.19	0.019 ± 0.001	0.985	36.5	121
Clay topsoil	11.99 ± 0.15	0.0063 ± 0.0005	0.948	110	365
Clay subsoil	11.61 ± 0.41	0.0046 ± 0.0014	0.562	151	501

† c_{00} = initial concentration of glyphosate; k = first-order rate coefficient for degradation; t1/2, = half-life; DT_{90} = time for 90% degradation.

‡ Calculated as ln(2)/k.

§ Calculated as ln(10)/k.

Table 5. Coefficients (± SE, n = 12) obtained by nonlinear regression for formation and degradation of aminomethylphosphonic acid (AMPA) according to Eq. [4].

Soil	k_1†	k_2	$t_{1/2}$‡	R^2
	d^{-1}		d	–
Sand topsoil	0.0216 ± 0.0011	0.0115 ± 0.0019	60.4	0.965
Sand subsoil	0.0092 ± 0.0005	0.0076 ± 0.0018	91.3	0.988
Clay topsoil	0.0063§ ± 0.0005	0.0199 ± 0.0013	34.9	0.973
Clay subsoil	0.0028 ± 0.0006	0.0071 ± 0.0087	97.6	0.901

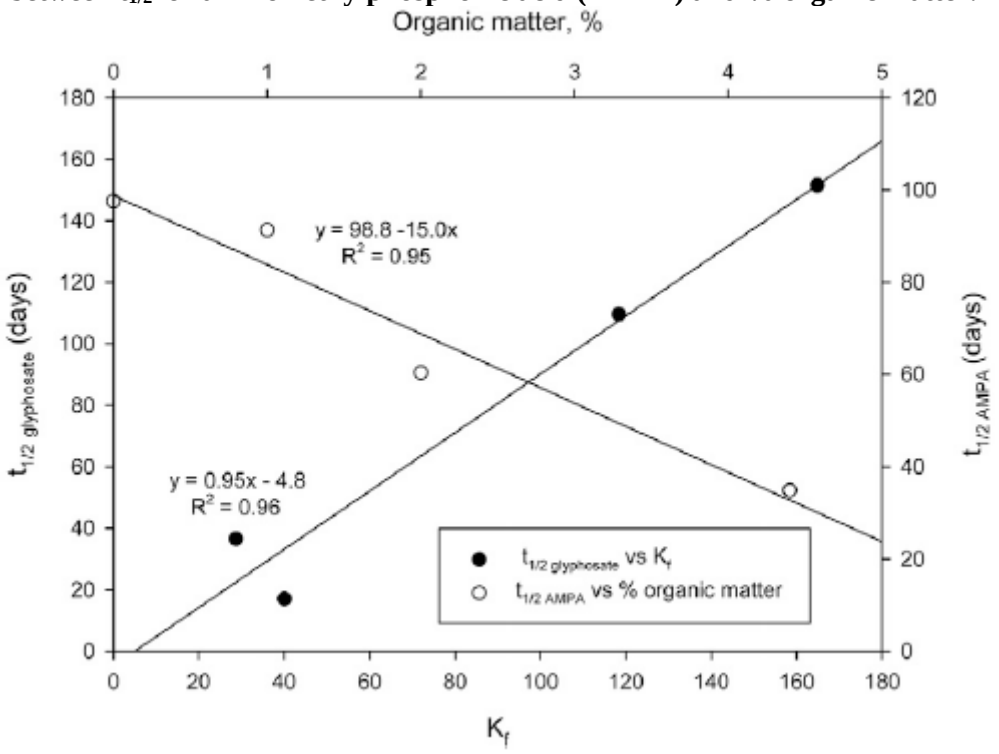
† k_1 = first-order rate coefficient for degradation of glyphosate to AMPA; k_2 = first-order rate coefficient for degradation of AMPA ; $t_{1/2}$ = half-life.

‡ Calculated as ln(2)/k_2.

§ In the clay topsoil, k_1 became 0.0073, i.e., larger than k (first-order rate coefficient for degradation; Table 4), leading to a formation fraction > 1, and was therefore set equal to k.

Glyphosate degraded relatively rapidly in the sand, with a half−life of 16.9 and 36.5 d in the topsoil and subsoil, respectively (Table 4). In the clay, very long half−life values of 110 and 151 d were obtained, and remarkable values of 365 and 500 d for 90 % degradation (DT_{90}). These half−life values are within the range previously reported for glyphosate degradation in agricultural soils (Giesy et al., 2000). There was a high correlation between half−life and K_f (Figure 3), suggesting that adsorption is important for the amount of glyphosate available in the soil water for degradation.

Figure 3. Relationship between half−life ($t_{1/2}$) for glyphosate and Freundlich adsorption coefficient (K_f), and between $t_{1/2}$ for aminomethylphosphonic acid (AMPA) and % organic matter.



The concentration of AMPA steadily increased during the incubation period of 64 d in all soils except the sand topsoil (Figure 2B), where it peaked after 43.4 d at 2.4 mg of AMPA/kg, representing 32.4 % of the initial amount of glyphosate added (Table 6). The degradation rate of AMPA, as quantified by k_2, gave a half−life of 35 to 98 d, with slower rates in the subsoil (Table 5). The correlation between these half−life values and the amount of organic matter was −0.973 (Figure 3), suggesting that increasing amounts of organic matter, or perhaps AMPA−degrading microorganisms dwelling there, increase degradation rates.

Table 6. Derived parameter values on fraction of glyphosate degraded to aminomethylphosphonic acid (AMPA) ($k1/k$), rate constant for formation of sarcosine (k_3), incubation time (t_{Amax}) at which the AMPA−concentration peaks (c_{Amax}), and c_{Amax} as fraction of initially added glyphosate.

Soil	k_1/k†	k_3‡	t_{Amax}§	c_{Amax}¶	c_{Amax} fraction#
	–	d^{-1}	d	mg kg^{-1}	%
Sand topsoil	0.53	0.0190	43.4	2.40	32.4
Sand subsoil	0.48	0.0098	80.4	2.12	26.2
Clay topsoil	1	0	84.7	1.47	18.6
Clay subsoil	0.61	0.0018	174	1.34	17.5

† Values of k (first-order rate coefficient for glyphosate degradation) from Eq. [2] (Table 4) and of k_1 (first-order rate coefficient for degradation of glyphosate to AMPA) from Eq. [4] (Table 5).

‡ $k_3 = k - k_1$.

§ Calculated according to Eq. [5].

¶ Obtained by inserting derived values of k, k_1, k_2, c_{GI}, and t_{Amax} into Eq. [4].

Calculated as $(c_{Amax} \times 1.52 \times 100)/c_{GI}$, where 1.52 is the stoichiometric factor for conversion of AMPA to glyphosate concentration.

The degradation of AMPA is reported to be slower than that of glyphosate (Giesy et al., 2000). In the Footprint database, AMPA is classified as persistent, with a typical half−life of 151 d, compared with 12 d for glyphosate (Footprint, 2009). The fact that AMPA is formed when glyphosate is degraded clearly means that the persistence of AMPA has to be equal to or longer than that of glyphosate. However, we did not find any previous study in which the degradation of AMPA was studied and compared with that of glyphosate in the same soil. In a study where glyphosate degraded with a half−life of 9 d, Simonsen et al. (2008) estimated a half−life of 32 d for AMPA from the descending part of data on AMPA residues. However, this is a worst−case scenario as these data represent the sum of AMPA formation from degradation of the glyphosate still present and AMPA degradation. This does not reveal how fast the AMPA molecule per se is degraded. Our data suggest that the AMPA degradation rate can be faster than that for glyphosate, for instance in soils with high clay content, which slows down glyphosate degradation, and high organic matter content, which stimulates AMPA degradation (Figure 3).

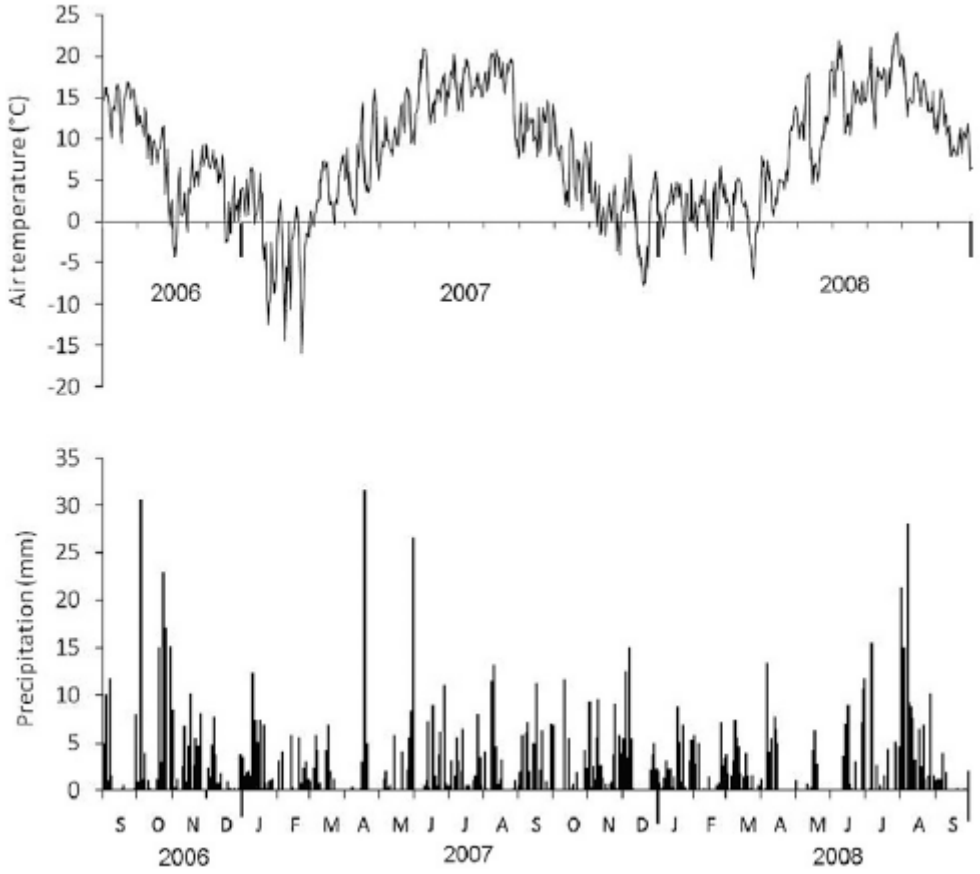
Microbial degradation is the main process controlling the disappearance of glyphosate in soil, and there are two well−described biological pathways for such degradation that give AMPA and sarcosine as the respective metabolites (Karpouzas and Singh, 2006; Borggaard and Gimsing, 2008). It has recently been shown that ligninolytic enzymes can also transform glyphosate into AMPA (Pizzul et al., 2009). Because AMPA is the only significant soil metabolite found in soil degradation studies, it is frequently suggested that metabolism of glyphosate in soil usually proceeds via the AMPA pathway (Giesy et al., 2000; Karpouzas and Singh, 2006). However, the fractions of AMPA formed in our study (48−100 %, Table 6) suggest that both pathways can be active in soil, with up to 52 % not following the AMPA pathway. Reasons for the sarcosine pathway not being considered significant in soil could be that soil residues of sarcosine are not determined in most studies and that sarcosine rapidly degrades to glycine (Karpouzas and Singh, 2006) in biologically active soil.

Precipitation and Drainage Conditions

Daily precipitation and average air temperatures at the lysimeter station are shown in Figure 4. Over the 2−yr study period (15 Sept. 2006−15 Sept. 2008), cumulative precipitation was 1192 mm, which, in combination with supplemental irrigation, resulted in a total water input to the lysimeters of 1214 mm. This total water input is slightly higher (10 %) than the long−term average precipitation for the Uppsala region (554 mm/yr). Average air temperature during the experimental period (7.8°C) was also higher than the long−term average at Uppsala (5.3°C).

A few weeks after glyphosate was applied, from 30 September onward, rain events were quite frequent (Figure 4), and precipitation totaled 232 mm by the end of 2006. This clearly created worst−case conditions for leaching of the herbicide, and the average amounts of leachate were 169 and 156 mm from the sand and clay soil, respectively, during this period. Peak weekly amounts of leachate, reaching 42 (sand) and 33 (clay) mm, occurred 8 wk after herbicide application. During 2007, precipitation was close to the normal for the area, although quite unevenly distributed. During periods with low evaporation (November, December, and January), monthly precipitation was about 60 mm, which was clearly above the average and increased the risk of leaching. In 2008, precipitation was again above normal, causing large amounts of leachate.

Figure 4. Average daily temperature (upper graph) and daily precipitation (lower graph) at the lysimeter site during the experimental period.



The cumulative amounts of leachate from the lysimeters each year are shown in Table 7. In total over the 2−yr period, the amount of leachate was 572 (± 17) mm from the sand and 461 (± 15) from the clay soil. In relation to water input, these amounts constituted 47 and 38 % of precipitation plus irrigation, which is considerably higher than in other similar leaching studies performed in Sweden (Bergström and Jokela, 2001).

Table 7. Water inputs to the lysimeters and mean annual amounts of leachate from the lysimeters to which glyphosate was applied (± SD; n = 2 for sand, n = 3 for clay)

Year	No. of days	Precipitation + irrigation	Leachate	
			Sand lysimeters	Clay lysimeters
		mm		
2006†	104	232	169 (± 1.4)	156 (± 4.7)
2007	365	550	229 (± 3.2)	158 (± 9.1)
2008‡	261	432§	174 (± 13)	148 (± 4.0)
Total	730	1214	572 (± 17)	461 (± 15)

† Measurements made during the period 18 September to 31 December.

‡ Measurements made during the period 1 January to 18 September.

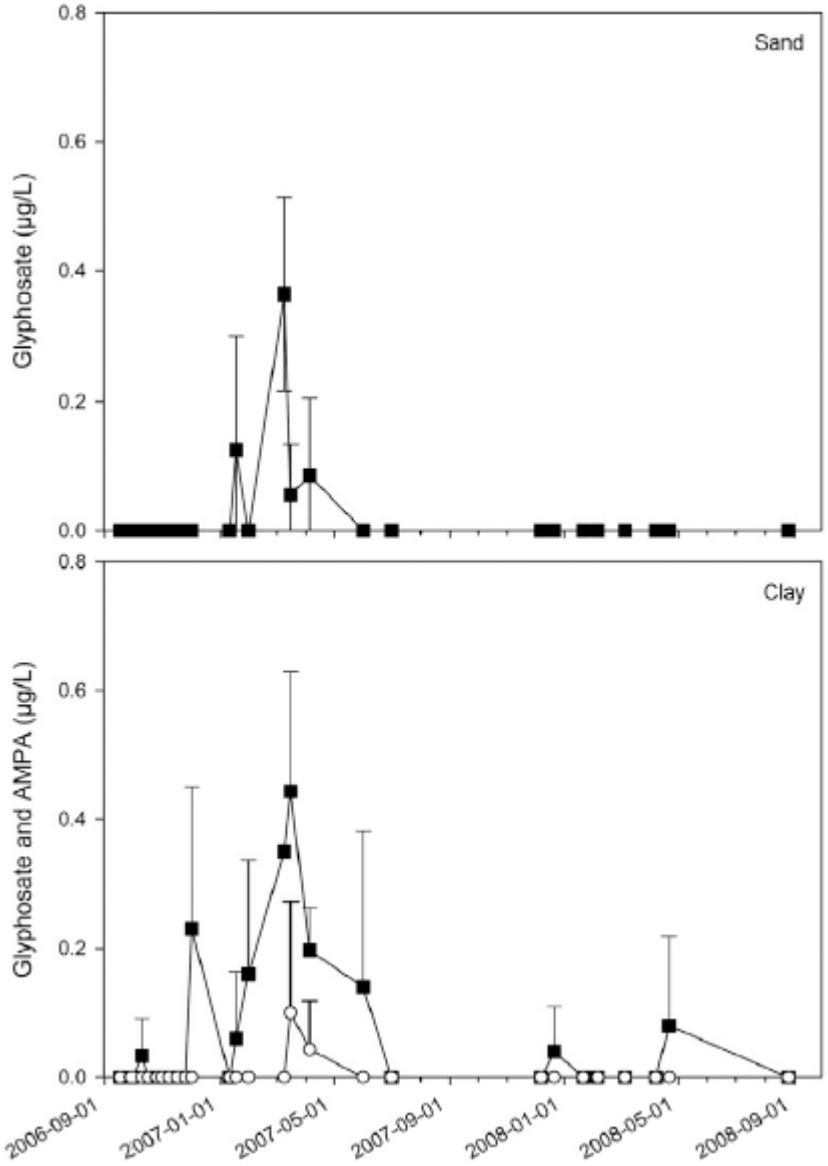
§ Includes 22 mm irrigation.

Leaching of Glyphosate and AMPA

Average concentrations of glyphosate and AMPA in leachate are shown in Figure 5. In the sand, the average peak concentration of glyphosate reached 0.36 µg/L in the beginning of March 2007, when temperatures were consistently above freezing, about 25 wk after pesticide application. During this period, the amount of leachate was about 250 mm (i.e., equivalent to 1.5 effective pore volumes).

Thereafter the glyphosate concentration decreased and the average concentration was below 0.1 µg/L from 16 March 2007 onward. This leaching pattern indicates limited preferential transport of the herbicide through the sand profile, although some preferential transport must have occurred considering the strong sorption of glyphosate (Table 3) and thereby expected large retardation. This is a flow behavior reported in several other leaching studies in sandy soils (e.g., Bergström and Shirmohammadi, 1999). The fact that the glyphosate peak occurred about 15 wk later than the corresponding bromide peak is a reflection of bromide being a nonreactive tracer. In the clay soil, the initial glyphosate peak occurred in the beginning of December and reached 0.23 µg/L after about 150 mm of water (i.e., equivalent to 0.8 effective pore volumes) had leached out of the soil columns. This considerably smaller amount of leachate suggests that glyphosate was partly transported through preferential flow paths in the clay profile, as was the case for bromide. This flow pattern has been documented earlier in this clay soil for reactive solutes (Djodjic et al., 1999; Bergström, 1995) and for nonreactive tracers (Bergström and Jarvis, 1993; Bergström and Shirmohammadi, 1999). However, the highest glyphosate peak (0.44 µg/L) in leachate from the clay soil coincided with that in the sand, i.e., in the beginning of March 2007. This glyphosate peak was washed out of the columns slightly earlier than the corresponding bromide peak, which was rather unexpected. Apart from preferential flow, another explanation could be that the highly water−soluble bromide diffused into micropores in the clay soil relatively soon after application and once in these pores it was largely protected from percolating water (Bergström and Stenström, 1998). From July 2007 onward, the average glyphosate concentration in clay soil leachate was <0.1 µg/L, although single samples had concentrations slightly exceeding the detection limit (0.1 µg/L). Average concentrations of AMPA in leachate were at or below 0.1 µg/L in both soils (Figure 5), with the highest concentration (0.30 µg/L) in a sample from one of the clay lysimeters. The average total amount of glyphosate that leached from the sand was 0.13 (± 0.03) g/ha and from the clay soil 0.28 (± 0.08) g/ha. These amounts correspond to 0.009 and 0.019 % of the amount of glyphosate applied to the soils. No leaching of AMPA occurred in the sand, whereas 0.03 g/ha leached in the clay soil. Total leaching of the ^{14}C applied in September 2006 was on average 0.31 % from the sand soil and 0.25 % from the clay. This shows that constituents other than glyphosate and AMPA that were not positively identified formed the major proportion of the total radioactivity in leachate. The leaching rates determined in this study are quite small compared with those in many other studies. For example, in a study performed by Al−Rajab et al. (2008), which included microlysimeters of three soils (clay loam, silty clay loam, and sandy loam), the amounts of glyphosate leached during 11 mo ranged between 0.11 and 0.28 % of the amount applied. However, there are also studies showing similar results to those obtained in the present experiment. In a study in France performed using lysimeters filled with calcareous soil (Landry et al., 2005), leaching of glyphosate was between 0.02 and 0.06 % of that applied after 680 mm of rainfall. Similarly, Cheah et al. (1997) recovered 0.04 to 0.07 % of applied glyphosate in lysimeter leachate after 200 mm of simulated rainfall. However, the conditions in all the above−mentioned studies were quite different from those in this study; the lysimeters were only 9.8 to 25 cm long, the experimental periods were considerably shorter (a few days to 1 yr), and the amounts of rainfall were much smaller (200 to 869 mm). These differences certainly have to be taken into account in a comparison of results.

Figure 5. Average concentrations of glyphosate (■) and aminomethylphosphonic acid (AMPA) (○) in the leachate (mean + SD, n = 2 for sand and n = 3 for clay). No AMPA was found in the leachate from sand.



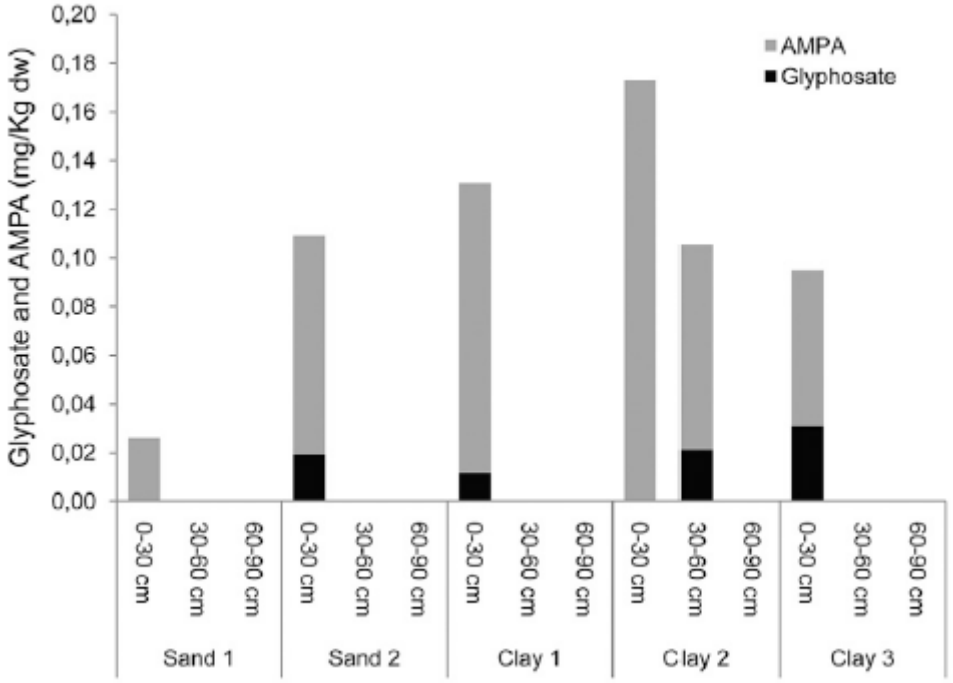
No glyphosate or AMPA was determined to be particle−bound, even though large quantities of particles were present in leachate from the clay soil. It is noteworthy that the particles were operationally defined as those being retained on a 0.22−µm glass−fiber filter. Some studies have shown that colloid−facilitated transport of glyphosate can occur. For example, de Jonge et al. (2000) showed in a study on lysimeters filled with undisturbed topsoil of a sandy loam that 1 to 27 % of leached glyphosate was particle−bound. Considering the overall low total concentrations of glyphosate in the present study (Figure 5), the particle−bound proportion would be below the detection limit (0.1 µg/L) if it constituted less than 25 % of what was leached. It is also important to bear in mind that topsoil lysimeters only include about 30 % of the profiles used in this study and may in fact, as indicated above, generate results that are quite atypical of results obtained in full−length lysimeters, such as those used here. The underlying subsoil can act as a sink or source for particles leaching through the soil profile.

The trend for glyphosate to leach in larger amounts from clay soils than from sandy soils is relatively well documented. In a Danish study, this was attributed to periods of high intensity rainfall shortly after application, when glyphosate was located on the soil surface and thereby exposed to rapid water transport in clay macropores extending up to the surface (Kjaer et al., 2003).

Residues of Glyphosate and AMPA in Soil

Residues of glyphosate and AMPA in the 0− to 30−, 30− to 60−, and 60− to 90−cm soil layers 748 d after application are shown in Figure 6. Residues were found in the 0− to 30−cm layer in all lysimeters and also in the 30− to 60−cm layer in one of the lysimeters with clay soil, possibly due to preferential flow in clay macropores and translocation in plant roots (Laitinen et al., 2007). No residues were found in the 60− to 90−cm layer in any of the lysimeters. Considering the worst−case conditions prevailing for leaching after application of glyphosate in the autumn of 2006, these results confirm the generally low mobility found for these compounds (Giesy et al., 2000; Vereecken, 2005).

Figure 6. Residues of glyphosate and aminomethylphosphonic acid (AMPA) in the 0− to 30−, 30− to 60−, and 60− to 90−cm soil layers 748 d after application. dw = dry weight.



No glyphosate was detected in one of the sand lysimeters, and 0.019 mg/kg remained at 0 to 30 cm in the other one. Low concentrations could be expected from the fast degradation in the sand topsoil (laboratory half−life 16.9 d). The concentrations of AMPA (0.026 and 0.090 mg/kg) remaining can be due to a combination of slow degradation (laboratory half-life 60.4 d) and continuous supply from degradation of remaining glyphosate. Related to the initial amount of glyphosate added, the remaining glyphosate residues represented 2.7 % and total residues of glyphosate + AMPA, calculated as glyphosate equivalents, represented 27 %.

In the clay soil, glyphosate and AMPA were found in all three lysimeters, probably due to very slow degradation of glyphosate in the topsoil and subsoil (Table 4), and thereby a long−term supply of AMPA, slow degradation of AMPA in the clay subsoil, and 100 % formation of AMPA from glyphosate degradation in the topsoil. Glyphosate residues represented 5.1 % and total residues 59 % of the initial amount of glyphosate added. Similar field persistence of glyphosate and AMPA residues was found in a sandy soil in Finland, where total residues in the 0− to 60−cm layer accounted for 72 % of the amount applied 20 mo after application (Laitinen et al., 2009).

Conclusion

The influence of adsorption on glyphosate degradation was confirmed, giving very slow degradation in the clay soil. The kinetics of AMPA residues suggest that although AMPA is always more persistent than glyphosate when formed from glyphosate, its degradation can be faster, for instance in soils with a high clay content, which slows down glyphosate degradation, and a high organic matter content, which stimulates AMPA degradation. The kinetics also suggest that apart from glyphosate being transformed to AMPA, the sarcosine pathway can be just as significant. The long persistence of glyphosate was also

confirmed in the lysimeter study, where glyphosate+AMPA residues constituted 59 % of the initial amount of glyphosate added to the clay soil 748 d after application. However, despite quite frequent rain events and large amounts of precipitation in the autumn and winter after application, these residues were mainly located in the topsoil, confirming the generally low mobility reported for these compounds. This conclusion is also supported by the small amounts of glyphosate and AMPA leached during the whole study period. Possible residues of glyphosate and AMPA due to transport on particles >0.22 µm were below the limit of detection (0.1 µg/L), and this does not appear to be an important transport mechanism in the soils included in this study.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The study describes a lysimeters experiment including derivation of sorption parameters and degradation data for glyphosate and AMPA in two Swedish agricultural soils. Chemical purity of the test substances is not reported, no mass balances or tabulated results per sample point are provided.

Lysimeter experiment: Not all required information is reported to check the validity of the study.

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

Sorption experiment: The experiment was conducted according the OECD 106 guideline for glyphosate. Not all required information is provided to check the validity of the study (see above, additionally LoD not sensitive enough).

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

Degradation experiment: The soil degradation of glyphosate and AMPA was considered. Not all required information is provided to check the validity of the study (see above, additionally mass of soil <50 g).

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

1. Information on the study

Data point:	KCA 7.1.4.3
Report author	Candela, L. Et al.
Report year	2010
Report title	Glyphosate transport through weathered granite soils under irrigated and non–irrigated conditions–Barcelona, Spain
Document No	The Science of the total environment, (2010), Vol. 408, No. 12, pp. 2509–16.
Guidelines followed in study	None
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 (Experimental conditions not sufficiently described to evaluate validity of the results)

2.Full summary of the study according to OECD format

The transport of Glyphosate ([N–phosphonomethyl] glycine), AMPA (aminomethylphosphonic acid, CH_6NO_3P), and Bromide (Br^-) has been studied, in the Mediterranean Maresme area of Spain, north of Barcelona, where groundwater is located at a depth of 5.5 m. The unsaturated zone of weathered granite soils was characterized in adjacent irrigated and non–irrigated experimental plots where 11 and 10 boreholes were drilled, respectively. At the non–irrigated plot, the first half of the period was affected by a persistent and intense rainfall. After 69 days of application, residues of Glyphosate up to 73.6 μg/g were detected till a depth of 0.5 m under irrigated conditions, AMPA, analyzed only in the irrigated plot was detected till a depth of 0.5 m. According to the retardation coefficient of Glyphosate as compared to that of Br^- for the topsoil and subsoil (80 and 83, respectively) and the maximum observed migration depth of Br^- (2.9 m) Glyphosate and AMPA should have been detected till a depth of 0.05 m only. Such migration could be related to the low content of organic matter and clays in the soils; recharge generated by irrigation and heavy rain, and possible preferential solute transport and/or colloidal mediated transport.

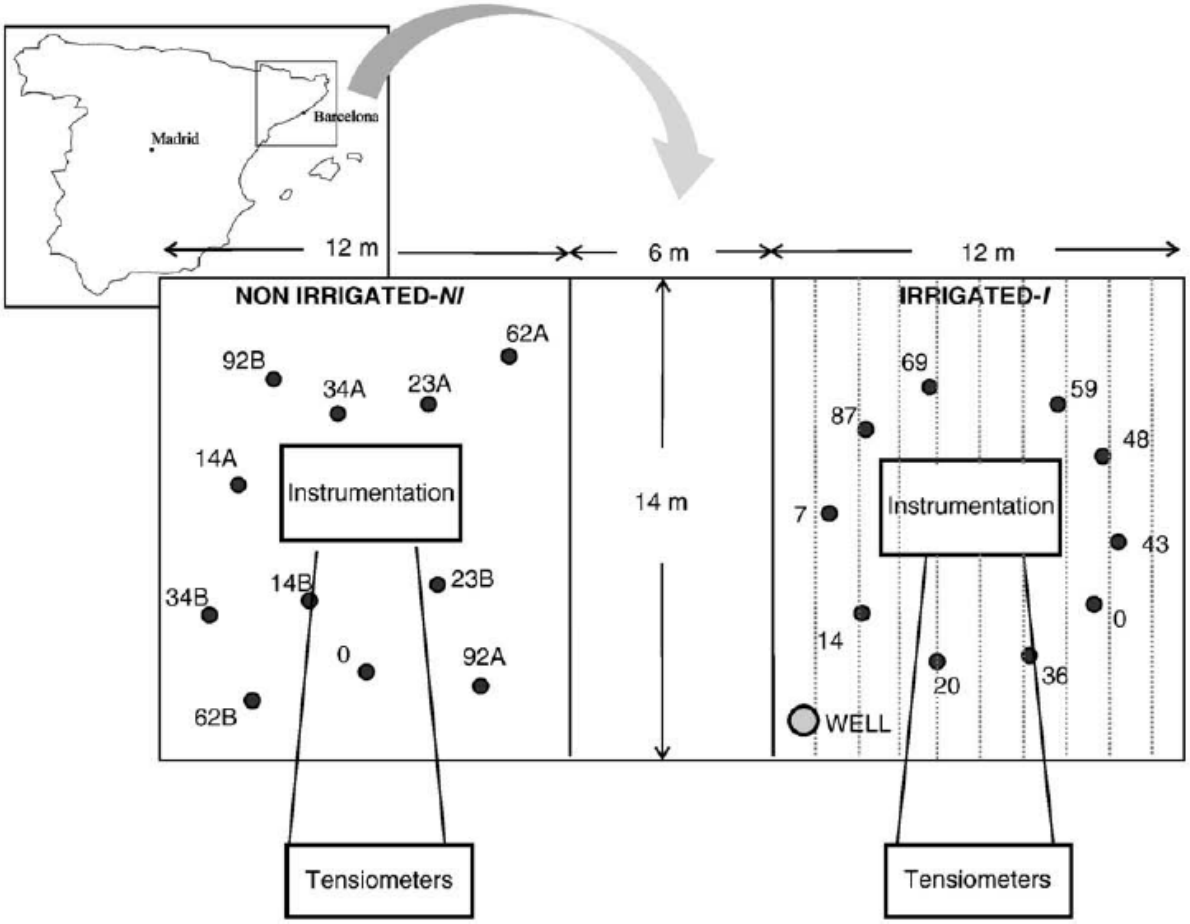
Materials and methods

Experimental site

The experimental site was located in a narrow coastal strip composed of weathered granite in the IRTA agricultural station of the Maresme region, located 30 km North of Barcelona– Spain (Figure 1). The area, under no–tillage farming, was only covered by a wheat crop to protect the soil from erosion for more than ten years. Groundwater was at a depth of 5.5 m; the hydrology of the study site has been described in detail by Guimerà et al. (1995).

Two individual plots of approximately 168 m^2 each, separated by a control area of 84 m^2, were selected. Initially, the weeds covering both plots were manually removed to allow installation of the irrigation and vadose zone monitoring equipment. Subsequently, the wheat cover was allowed to redevelop, prior to herbicide application. The upward–downward flux of water in the unsaturated zone was monitored by 7 tensiometers (Soilmoisture®). At the beginning of the experiment duplicate tensiometer sets were installed by manual drilling, in the middle of the plots, at a depth of 0.30, 0.60 and 0.90 m 106 and one tensiometer was installed at a depth of 1.20 m (Figure 1). Instrumentation remained in place until the end of the experimental activities.

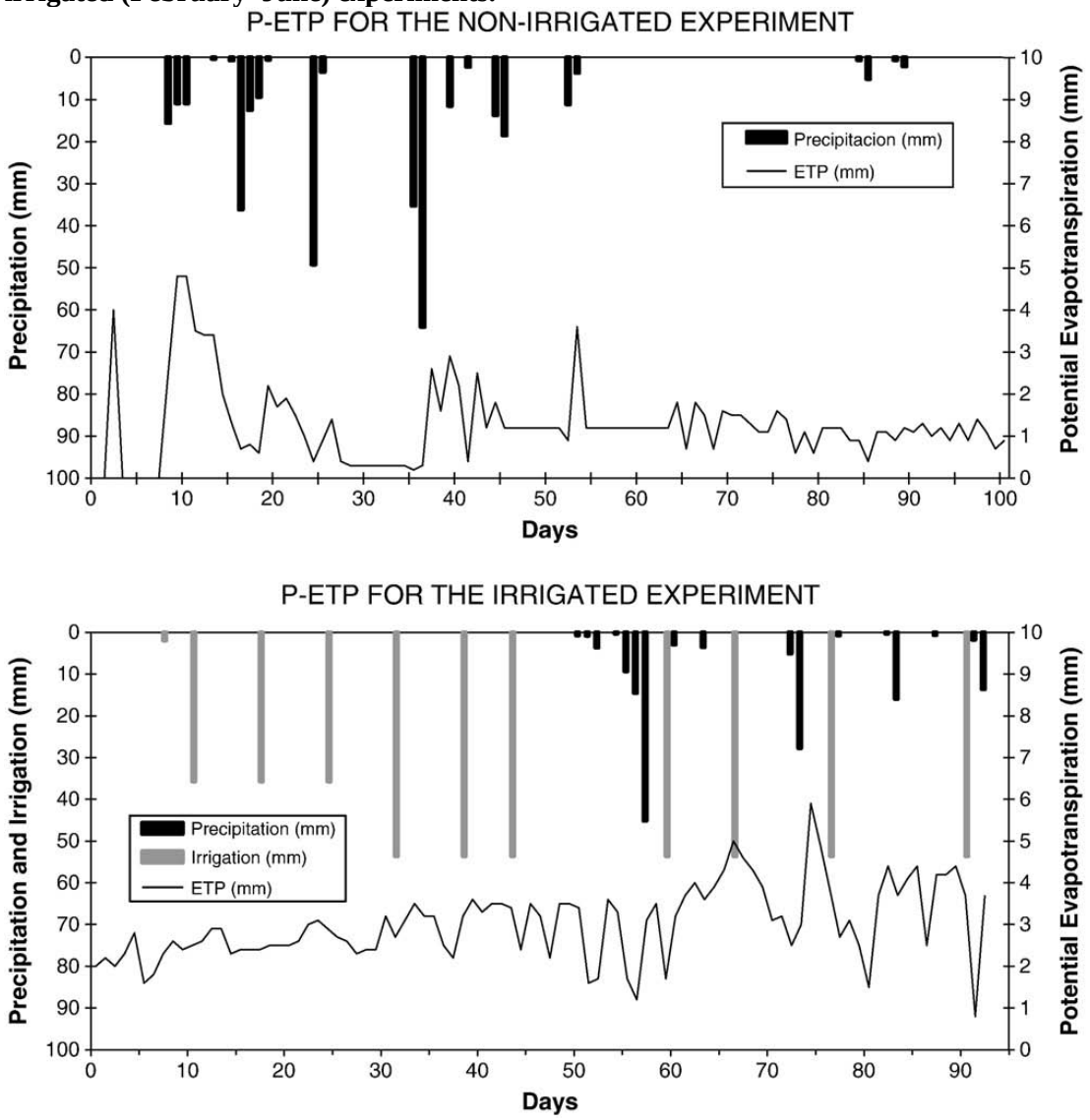
Figure 1. Study area location and non–irrigated (NI) and irrigated (I) experimental plots. The location of in situ field instrumentation and drillings is shown for the two sites; 0 denotes location of background drillings; duplicate drillings for *NI* are denoted as A and B (e.g., 1A and 1B). The vertical dashed lines (in *I*) denote the location of soak bands. The location of a groundwater well is also shown.



For initial characterization of the unsaturated zone profile, before starting the experiments, two boreholes were drilled (location denoted as "0" in the non–irrigated–*NI* and irrigated–*I* plots) and undisturbed soil samples were taken down to 4.50 m. In both plots soil matrix characterization and the monitoring of pore water content was performed by destructive sampling. The amount of pesticide in the vadose zone at given times was determined in undisturbed soil sampling. Groundwater quality was monitored in an existing pumping well in plot *I* (Figure 1).

Precipitation amounts for both experimental periods were provided by the IRTA meteorological station. Irrigation was based on soak bands that were installed in the *I* plot (Figure 1) with a separation of 0.30 m in order to obtain a uniform spatial distribution of water. Two irrigation doses of 36 mm per week, were applied during two hours for the first three weeks of the study period (March I-14 to I-27). Subsequently, the amount of irrigation increased to 53 mm per week (see Figure 2).

Figure 2. Precipitation and evapotranspiration during the non-irrigated (September–December) and irrigated (February–June) experiments.



Application of Glyphosate and bromide

Both Glyphosate (*Roundup*®, 36 % p/v, Montsanto Europe S.A.), and bromide (NaBr; conservative tracer) were applied under non–irrigated (*NI*) and irrigated (*I*) conditions (Figure 1). The first field experiment, non–irrigated, was conducted during the rainy season (September to December, 1994) and sampling and monitoring activities extended over 92 days. During the second field experiment, irrigated, that lasted 87 days, the area was irrigated from February to May (1995).

Glyphosate, along with a solution of NaBr, was applied on the soil surface on September 13 on the *NI* plot and on March 7 on the *I* plot using an automated spray system to ensure uniformity. The pesticide and bromide solutions were prepared at the study site before application. The concentrations of BrNa solutions for the *NI* and *I* plots were of 20 g/L and 17 g/L of BrNa respectively. Glyphosate solution was prepared by mixing 400 cm^3 and 420 cm^3 of a commercial 36 % (p/v) Glyphosate EC formulation with 20 and 21 L of groundwater for the *NI* and *I* plots, respectively. This procedure of pesticide application follows standard agricultural practice in the Maresme area.

Vadose zone soil and water sampling methodology

Soil samples were obtained with a hollow–stem auger after pesticide and bromide application in both field plots. A random sampling scheme with duplicate soil cores was applied in the non–irrigated area (Figure 1; *NI*–0(A,B) to 92 (A,B)) where undisturbed soil cores were taken at 0.20 m intervals till a

depth of 1 m, and at 0.50 m intervals below it (Figure 1). Due to field and experimental constraints a circular sampling pattern and single cores, where undisturbed soil samples were obtained at 0.20 m intervals, was applied in the irrigated experimental plot (Figure 1; *I*–0 to 69). To prevent possible contamination from overlying layers, two samples of the soil to be analyzed were taken from the inner part of each core, after discarding the top and the bottom portions of it. One sample was carefully wrapped in aluminum foil, and frozen until pesticide and Br laboratory analyses. The other one was used for the determination of volumetric water content, saturated and unsaturated hydraulic conductivity, and bulk density (following ASTM 1993 standards) clay content and clay type (RX diffraction) and organic matter content. Also pH, CEC and Al and Fe oxides were determined in samples following standard techniques described in Melo (1996) and Candela et al. (2007).

During the field experiments (Tables 1A and 1B), groundwater samples, soil cores and soil–water potential measurements from the tensiometers were obtained after each rain or irrigation episode. Groundwater samples were obtained with a bailer from the existing open well where also the depth of the water table was monitored. Due to analytical constrains, the concentration of AMPA was monitored in the irrigated plot only.

Table 1A. Sampling dates and precipitation amounts for the non–irrigated (NI) experiment conducted in 1994 (September–December).

Sampling survey	NI-0 Background	NI-14	NI-23	NI-34	NI-62	NI-92
Drilling date	Sep 6[a]	Sep 27	Oct 6	Oct 17	Nov 14	Dec14
Precipitation (mm)[b]	–	52.6	110.5	49.1	7.2	–
Days after glyphosate and NaBr application[c]	−7	14	23	34	62	92

[a] September 6 (*NI*-0), soil profile characterization.
[b] Cumulative values for the time interval between sampling. Total precipitation: 219.4 mm.
[c] Glyphosate and NaBr application on September 13.

Table 1B. Sampling dates and precipitation amounts for the irrigated (I) experiment conducted in 1995 (February–June).

Sampling survey	*I*-0 background	I-7	I-14	I-20	I-36	I-43	I-48	I-59	I-69	I-87
Drilling date	Feb 28[a]	Mar 14	Mar 21	Mar 27	Apr 12	Apr 19	Apr 24	May 5	May 15	Jun 2
Irrigation mm/week[b]	36	36	36	53	53	53	53	53	53	53
Precipitation (mm)[b]	–	–	–	–	–	0.7	4.7	75.3	32.8	32.9
Days after glyphosate and NaBr application[c]	−7	7	14	20	36	43	48	59	69	87

[a] February 28 (*I*-0), soil profile characterization.
[b] Total irrigation: 479 mm. Total precipitation: 146.4 mm. Precipitation (January–March 15) 8.6 mm.
[c] Glyphosate and NaBr application on March 7.

The total length of the sampled soil cores in each survey was determined according to: (a) the depth of penetration of water through the unsaturated zone as predicted from in situ tensiometer readings, (b) the hydraulic conductivity of soil samples as determined in the laboratory, (c) the predicted theoretical depth reached by the center of mass of Br^-, and (d) the retardation factor, R (Ghodrati and Jury, 1992) of glyphosate as determined in batch experiments for soils and sediments of the area. However, as a safety measure, soil drillings and sampling depths were always greater than the calculated theoretical depth of penetration of Glyphosate.

Chemical analyses

Chemical analysis of glyphosate and AMPA residues in soil and water samples was performed using an HPLC method (Hewtlett Packard, HPLC ChemStation G1034A) based on reversed–phase chromatography, with flourescent detection using pre-column derivatization with FMOC (9–fluorenylmethylchloroformate) to give the flourescent derivative. The liquid chromatography coupled column (LC–LC) methodology described by Sancho et al. (1996) was used to confirm the presence of glyphosate and AMPA residues in positive samples. The LC–LC technique presents several advantages, such as improved sensitivity, selectivity, and sample throughput. The detection limit of glyphosate and

AMPA was 6 ng/g and 4 ng/g for soil, and 0.15 µg/L and 0.1 µg/L for water samples respectively, with extraction efficiency greater than 95 % for both analytes. Bromide content was determined by ionic chromatography (VYDAC column) and the detection limit was 0.1 ng/g.

Results

Soil properties

The soil profile, a Typic Xerorthent (Soil Survey Staff, 1999), is very homogeneous and consists of medium to coarse sand size with low clay content (clay, 5 %; silt, 20 %; sand, 75 %). The clay fraction is mainly composed of smectite, illite and kaolinite. The soil had no visual structure except for the presence of a coarse sand layer at 1.50–1.90 m and granite debris at a depth of about 4.50 m. However, according to physico–chemical properties a top soil layer and a subsoil horizon may be distinguished. The soil chemical properties determined from samples at a depth of 0–0.20 m (top soil) and 0.70–1 m (subsoil horizon) respectively, are: cation exchange capacity (CEC), 5.2 and 4.6 meq.100/g; pH (1:1 in H_2O), 7.9 and 7.3; organic matter 1.1 and 0.09 (%); P 0.2 mg.100/g (top soil), total Fe_2O_3, 1.92 and 5.43 g.100/g; and total Al_2O_3 1.75 and 7.22 g.100/g. Average values of soil bulk density from field samples were 1.65 and 1.7 g/cm^3 for the top soil and subsoil, respectively. Residues of Glyphosate and Br^- were not detected along vadose zone profile before the experiments (*I*–0 and *NI*–0; Figure 3 A and B).

Figure 3A. volumetric content of water, bromide and glyphosate in the different soil profiles for the non–irrigated plot (September–December 1994). *NI*–0: soil profile prior to pesticide and bromide application. The high water content level (0.20–0.14 cm^3/cm^3) at a depth of 1.5 m reflects the presence of a coarse sand layer (LoD: 6 ng/g Glyphosate; 4 ng/g AMPA; 0.1 ng/g Br).

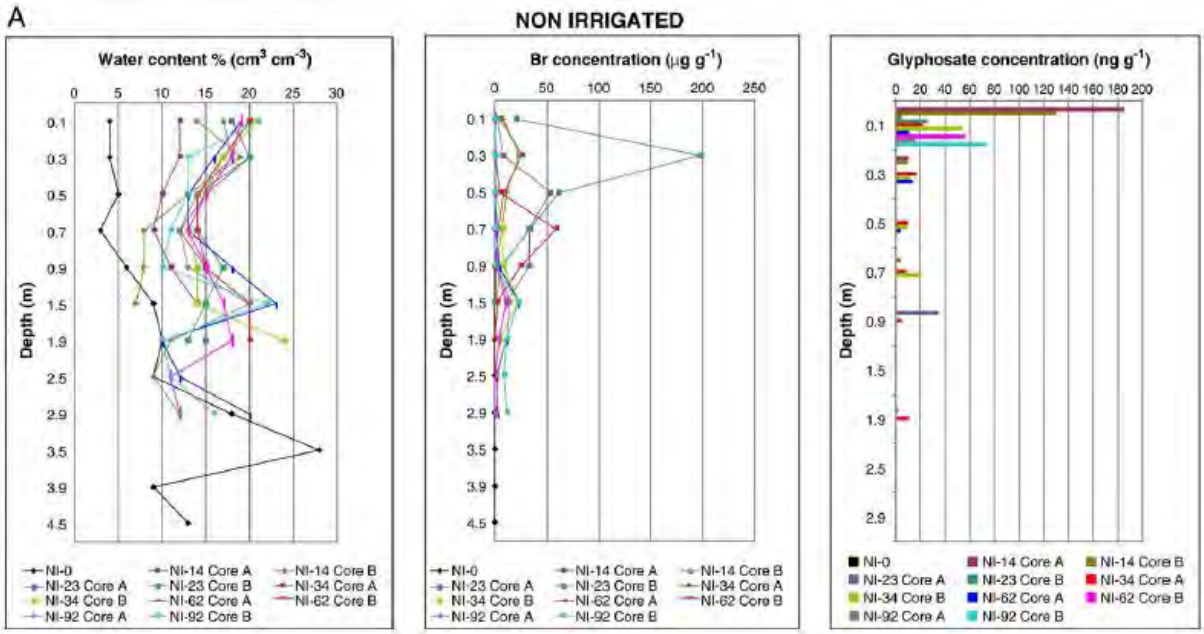
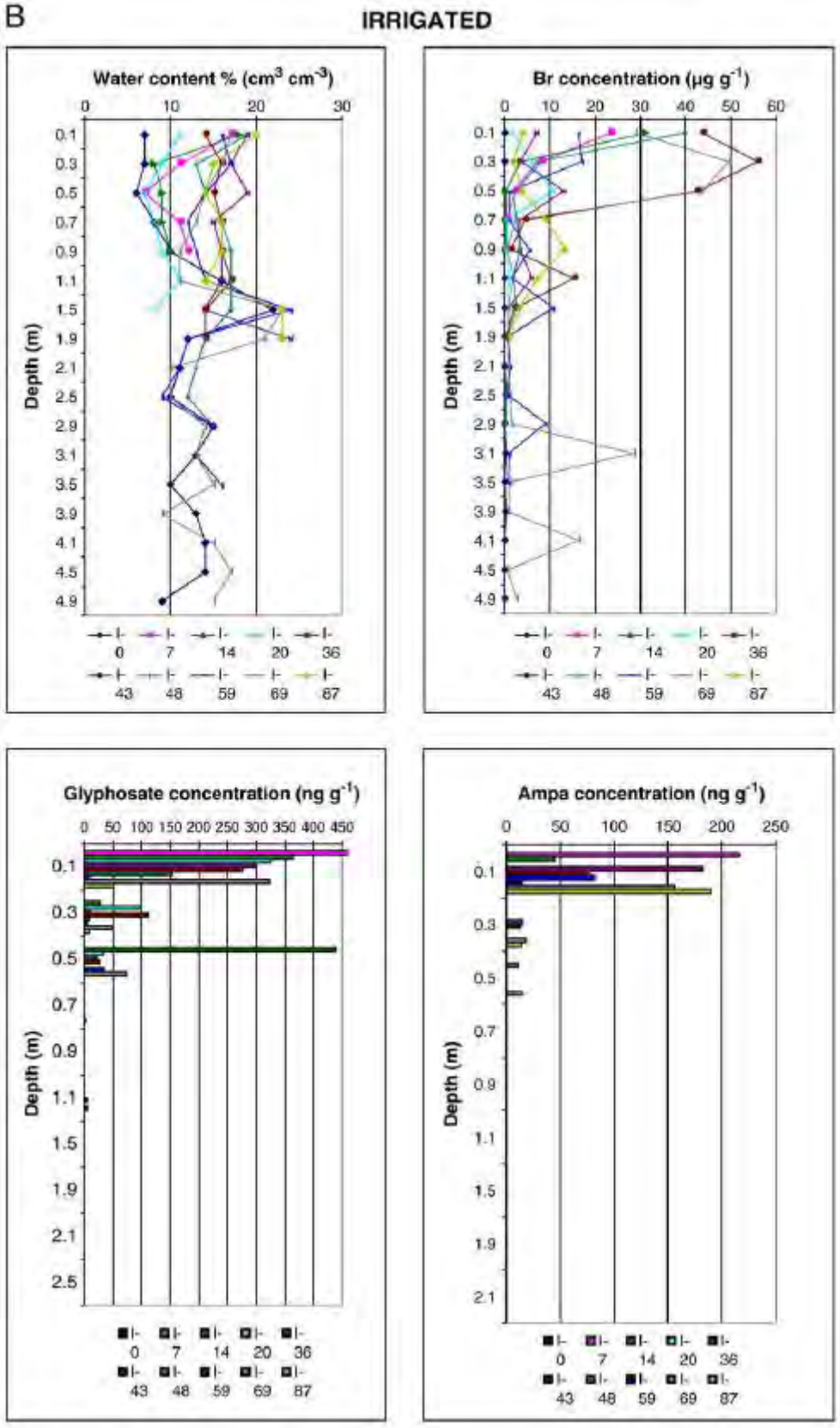


Figure 3B. volumetric content of water and bromide in the different soil profiles for the irrigated experiment (March–June 1995). *I*–0: soil profile prior to pesticide and bromide application. The high water content level (0.22–0.15 cm^3/cm^3) at a depth of 1.5 m reflects the presence of a coarse sand layer. (LoD: 6 ng/g Glyphosate; 4 ng/g AMPA; 0.1 ng/g Br).



Non-irrigated plot

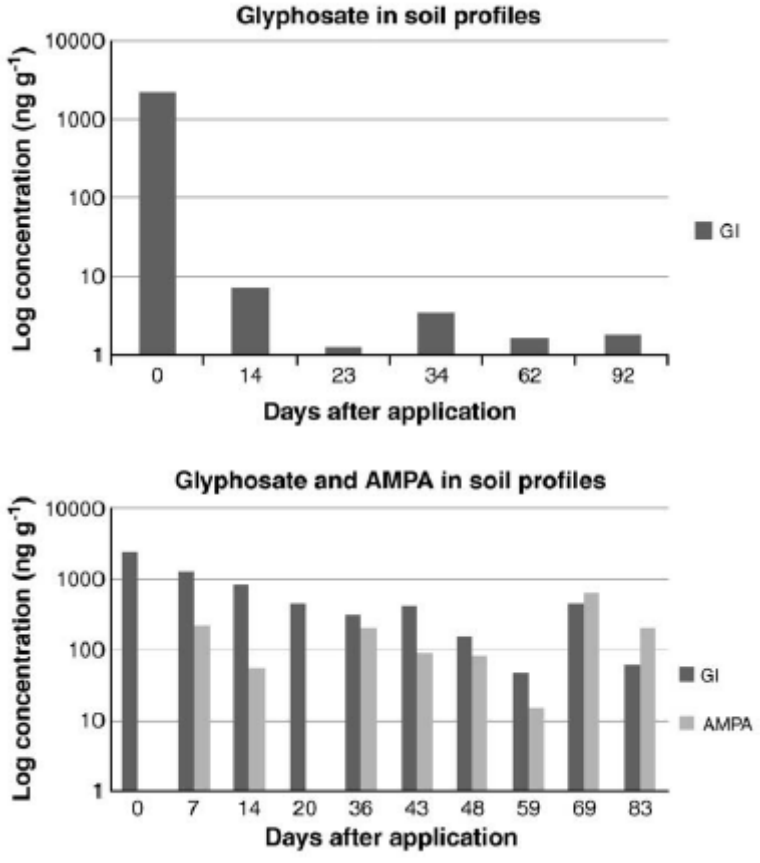
During the non–irrigated experiment the average temperature was 14.7°C, rainfall accounted for

219.4 mm, and more than 50 % of the total precipitation (163 mm) was due to three storm events in September and October. For this same time period, evapotranspiration was 118.9 mm (see Figure 2). In this plot the water content till a depth of 1.5 m was extremely low (6 % background average) due to lack of precipitation and high temperature during the summer period (*NI*, Figure 3A). After the first rain event, a week after Br and pesticide application, the movement of the wetting front is clearly observed (*NI*-14). In the upper 1.50 m the water content increases up to 20 % at the end of the experimental period and soil–water content seems to stabilize after 34 days (*NI*–34). The greatest water content along the profile was observed in the coarse sand layer at 1.5 m depth.

Maximum concentration of Glyphosate in the unsaturated zone were detected at a depth 0–0.30 m, except for *NI–23A* and *NI–34B* where residues were also detected at a depth of 0.9 and 0.7 m, respectively. The depth of penetration in individual cores varied widely. Glyphosate residues were also detected along the unsaturated zone, at concentration below the detection limit (LoD), up to a depth of 0.90 and 1.90 m after 23 and 34 days of application. After 14 days, the residual amount was 7 % of the total applied mass. After 23 days and till the end of the experiment, residual amounts account for 1 %. Glyphosate half–life (or half concentration time) calculated from in situ experimental values was 7 days, although it may be even lower considering that the first sampling campaign was undertaken after 14 days of pesticide application.

Figure 4 presents the amount of pesticide remaining in the soil profile till the end of the experiment for each core and sampled borehole. Mass estimation refers to the initial applied dose. A rapid initial dissipation phase, followed by a slower one is observed after 23 days. Degradation rate, estimated from logarithmic pesticide concentration vs. time (best fit equation) was 1.52 days. However, the small value of the correlation coefficient obtained ($R^2 = 0.4$) indicates the low accuracy of the calculations and the associated uncertainty.

Figure 4. Residual mass of glyphosate and AMPA remaining in soil profile as a function of time. Non–irrigated and irrigated experiment.



Irrigated plot

In the irrigated plot experiment carried out during springtime, the total amount of water applied was three times higher than that of the NI plot as precipitation accounted for 146.4 mm and irrigation for 483 mm. The average temperature was of 12.3°C, and evapotranspiration (266.6 mm) was greater than in the non–irrigated experiment. The background average water content in the soil profile up to 1.5 m was 10.9 %. From *I–36* (when the irrigation dose is increased), until the end of the experiment the soil profile water content is quite constant (Figure 3B, water content). The increase in water content at 1.50–1.90 m is due to the presence of a coarse sand layer.

As shown in Figure 3B, maximum concentration of Glyphosate was always detected between the first 0–0.5 m of the soil profile and concentration values were greater than those found in the non–irrigated plot. Residues of Glyphosate (below LoD) were still found at 1.50 m after 69 days of application and continued to be detected after 87 days. Residual amount of Glyphosate in soil profile after 14 days was 34 % of the applied dose, being reduced to 2 % after 59 day, and up to the end of the experiment (Figure 4). Field half–life (or half concentration time) was around 7 days and estimated degradation rate was 0.04 days ($R^2 = 0.6$).

Glyphosate and bromide were not detected in groundwater samples obtained with a bailer along all the monitoring periods.

Discussion

For the non–irrigated experiment (NI) Br concentration along the soil profile was clearly affected by the rain episodes, and was detected up to a depth of 150 cm after 14 days of application, implying a flow velocity of 10 cm/day calculated according to Burns (1975). The observed deficit at *NI–14* profile (55 % recovery of applied dose) could be attributed to the uptake of bromide by plants (Kung, 1990a). After decomposition of plant residues, bromide may return to the soil and can be accounted for as an external input in the bulk mass balance. In the irrigated plot (Figure 3B) tracer distribution over depth is fully controlled by irrigation dose, and Br^- concentration presents lower variability.

As shown in the results of both field experiments, concentrations of Glyphosate were detected, much deeper than expected according to the distribution coefficients calculated for the surface soil ($K_f = 93$), and subsoil ($K_f = 154$) in batch experiments (Melo, 1996; Candela et al., 2007). The retardation factor (R, Ghodrati and Jury, 1992) of Glyphosate, as compared to that of Br for the topsoil and subsoil, is 80 and 83, respectively. Considering a worst–case scenario (R = 80) and the maximum migration depth of Br (2.90 m; and 4.90 m Figure 3A, B), then, the maximum transport depth of Glyphosate should have been 0.05 m only. We hypothesize that the deep transfer of both glyphosate and AMPA can be the result of: (a) preferential transport along the unsaturated zone (Kung, 1990b; Van den Bosch et al., 1999; Scorza et al., 2004; Coppola et al., 2009), and/or (b) colloidal mediated transport of both components (Vereecken, 2005; Borggaard and Gimsing, 2008), a process that can be inferred from their relatively large K_f values.

The mobility of strongly adsorbing compounds as Glyphosate (Veiga et al., 2001; Kjaer et al., 2005; Vereecken, 2005, among others) has already been shown for pesticides such as propiconazole and fempropimorph (Krongvang et al., 2004), regardless of how strongly they were found to be adsorbed under equilibrium conditions in the laboratory. For the two experiments reported here, the observed differences in soil profile distribution, and rate of degradation are probably conditioned by climatic factors prevailing during the experiments (autumn and springtime), agricultural practices (dryland–irrigated), inherent variability of soil spatial parameters, land cover and roughness of soil surface.

At the *NI* experiment the presence of glyphosate at greater depth than expected may be the consequence of rainfall events. In the *NI–34* profile, Glyphosate was detected along all the sampled profile showing a high concentration (20.3 ng/g) at 0.70 m although according to batch experiments (Candela et al., 2007), after 34 days the pesticide should have been retained in the upper part of the soil. The high precipitation registered immediately after pesticide application could induce a rapid flux of water through the unsaturated zone, inhibiting adsorption onto soil particles. This process could be favored by

the amount of Glyphosate available and the initial low water content in soil before rain which could promote the existence of preferential solute transport. In sandy soils with no visible structure in the top 1 m, preferential flow appears to be dependent on soil moisture and water flow tends to be channeled through low moisture zones. This effect has been observed by Kladivko et al. (1999) and Nolan et al. (2008). Previous laboratory soil column experiments carried out with the same soils and pesticide demonstrated the importance of non–equilibrium sorption under flow conditions. Mass loss is larger for longer residence times associated either to low pore–water velocity or long soil column lengths.

Mobility of AMPA is lower than Glyphosate and residues were only detected in the 0–0.30 m interval. Considering the molecular weight of both compounds, a 0.6 ratio glyphosate/AMPA concentration in soil and water samples could be expected. However, AMPA concentrations detected in soil samples only accounted for 15 % of glyphosate degradation (Figure 4). A slower glyphosate/AMPA transformation over time, or even AMPA degradation could explain the missing amount of herbicide. The analysis on dissipation of Glyphosate and AMPA formation was not the objective of this research and the available data are not sufficient to assess the importance of biological and chemical transformation of Glyphosate. Analysis of AMPA formation (0.08 days according to best fit equation) are highly uncertain due to the low correlation coefficient obtained ($R^2 = 0.295$).

Very little is known about the nature and kinetics of this process (Grunewald et al., 2001), therefore, to gain insight into it, soil microbiological activity and the fast mineralization of both Glyphosate and AMPA should be the subject of future research.

Based on the non–reacting behavior of Br and the reduced mobility of pesticide induced by adsorption, estimation of glyphosate percentage found 3 times deeper than predicted, calculated following the Ghodrati and Jury (1992) approach, would account for 18 % and 28 %for the non–irrigated and irrigated areas, respectively (Table 2). Note that in the non–irrigated area the transport of the pesticide is clearly influenced by the two rain events (NI–34 and NI–62; Figure 2A), a phenomenon not observed in the irrigated plot where water infiltration is mainly conditioned by continuous irrigation.

Table 2. Percentage of glyphosate found three times deeper than predicted (ZG) for the different soil profiles considering achievement of equilibrium adsorption.

Non irrigated (NI)			Irrigated (I)		
Profile	$3Z_C$ (cm)	PF (%)	Profile	$3Z_C$ (cm)	PF (%)
NI-14A	0.6	5	I-7	0.2	0
N1-14B	0.6	9	I-14	0.1	56
NI-23A	1.0	0	I-20	0.6	28
NI-23B	1.0	0	I-36	0.2	10
NI-34A	1.4	64	I-43	0.6	33
NI-34B	2.4	43	I-48	0.2	5
NI-62A	3.0	62	I-59	0.6	82
NI-62B	3.0	0	I-69	0.6	28
NI-92A	3.4	0	I-87	1.3	15
NI-92B	3.8	0			
Average (%)		18	Average (%)		28

Z_C : theoretical depth to the center of mass of the pesticide (Ghodrati and Jury, 1992). PF: preferential flow; $Z_C = Z_{Br}/R$, where R is the retardation factor and Z_{Br} the depth reached by the center of mass of a pulse of the conservative tracer (Br). Center of mass depth of tracer (Z_{Br}) was estimated at each sampling profile (see Fig 3).

Although in both plots detectable amounts of tracer and pesticide have been found in the soil profile, direct comparison of results is not possible as they are conditioned by climatic parameters and water application regime. The experiment under non–irrigated conditions was undertaken in autumn; the most important aspect of the precipitation pattern is its concentration in a few unevenly distributed events of heavy storms, characteristics of the Mediterranean environment. Evapotranspiration presents a decreasing trend and water content in soil profile was low at the beginning of the exercise. For the irrigated experiment, spring climatic conditions prevail and evapotranspiration is much higher than in autumn. The weekly applied irrigation dose controls water content in soil presenting a more uniform distribution along the soil profile and experimental period.

As far as the authors are aware, such deep penetration of Glyphosate has not been reported from field studies for granite soils, such as the studied ones in the Maresme area of Spain.

Conclusion

Glyhosate is commonly considered a pesticide strongly sorbed on soils, presenting a low risk for groundwater pollution due to the phosphonate functional group strong adsorption to clay minerals, Fe and Al–oxides and OM according to laboratory experiments. A problem is whether pesticide parameters measured in the laboratory are representative for predicting pesticide behaviour under field conditions. Field investigation and monitoring of pesticide leaching present the complexity of profiling pesticide concentration in soil and the difficulty of sampling pesticide migration through preferential flow paths.

As shown in the field experiments described above, Glyphosate deep leaching in a weathered granite soil profile was observed under natural field conditions regardless of the irrigated or non–irrigated conditions and climatic season. Laboratory miscible displacement experiments performed with the same soils showed that Glyhosate adsorption in soils is essentially a kinetic process and depends on the pore water velocity and residence time of soil solutions. If flow velocities are slow and enough time is given to react with the soil matrix, surface complexation and precipitation takes place. Complexation with iron and aluminum oxides, transition metals or alkaline–earth metals has been reported in literature (Sprankle et al., 1975; Vereecken, 2005). Since Glyphosate adsorption is not an instantaneous process, needing time to attain equilibrium conditions, under heavy rain or irrigation just after its application on soil surface, it could leach more than predicted.

Given the typical conditions of the Maresme region vadose zone, highly permeable medium–coarse sand with low organic matter and clay content and containing Al, Fe, oxides and hydroxides, the principal mechanism affecting Glyphosate transport through the vadose zone may not be chemical equilibrium with the solid matrix alone. At field scale two possible explanations accounting for physical non–equilibrium will be of decisive importance on the transport of pesticide through the vadose zone. Since the phosphate compound of the molecule can be strongly adsorbed by Al, Fe, oxides and hydroxides, organic matter and humic acids of colloidal size, transport of colloid–bound Glyphosate and AMPA and also, preferential flow pathways driven by rainfall events or water application dose is likely. Presence of Glyphosate residues below detection level at depth up to 1.10 and 1.9 m in the irrigated and non–irrigated plot suggests that the pesticide may migrate into deep soil layers. This observation emphasizes the potential risk of Glyphosate transport to groundwater.

At field scale, the half–life was found to be shorter than 7 days in both experiments, much shorter than values reported in the literature (47 days average). This can be attributed to the fact that under field conditions a multitude of factors and processes contribute to herbicide disappearance, while laboratory studies are generally designed to study one of these processes. It is important to note here that these results are conditioned by the low correlation coefficient obtained (best fit equation). From the limited information obtained during the experimental study, AMPA accumulation in soil from pesticide degradation accounts for 15 % of the initial herbicide application. The parent compound transformation rate is always superior to the above–mentioned rate, leading to the conclusion that Glyphosate/AMPA transformation is a slow process or rapid AMPA degradation has occurred. Whether the transformation of the herbicide during the course of the experiment is basically controlled by chemical or biochemical processes is unknown and more research is needed regarding microbiological transformation.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The study describes a leaching experiment with glyphosate in an agricultural area in Spain. Leaching over a period of several months in spring and in autumn was observed under irrigated and un–irrigated conditions. Glyphosate and AMPA were found in in deeper soil layers than expected from the calculations based on a tracer experiment. However, only assumptions for reasons of deeper leaching

were provided, and based on the provided information (i.e. soil structure etc.) no profound explanation can be established. Duration of the study is in addition not long enough to evaluate the leaching behavior for a long–time perspective. Details regarding field sampling and sample handling practices and analysis are not sufficient to classify the study as fully reliable.

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	Cassigneul, A. et al.
Report year	2016
Report title	Fate of glyphosate and degradates in cover crop residues and underlying soil: A laboratory study
Document No	Science of the Total Environment 545–546 (2016) 582–590
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 (literature publication)
Acceptability/Reliability:	Reliable with restrictions (Not all information reported to check validity of experiment against current guidelines, not all parameters reported to evaluate kinetic behavior)

2.Full summary of the study according to OECD format

The increasing use of cover crops (CC) may lead to an increase in glyphosate application for their destruction. Sorption and degradation of ^{14}C–glyphosate on and within 4 decaying CC–amended soils were compared to its fate in a bare soil. ^{14}C–Glyphosate and its metabolites distribution between mineralized, water–soluble, NH_4OH–soluble and non–extractable fractions was determined at 5 dates during a 20°C/84–d period. The presence of CC extends ^{14}C–glyphosate degradation half–life from 7 to 28 days depending on the CC. ^{14}C–Glyphosate dissipation occurred mainly through mineralization in soils and through mineralization and bound residue formation in decaying CC. Differences in sorption and degradation levels were attributed to differences in composition and availability to microorganisms. CC– and soil–specific dissipation patterns were established with the help of explicit relationships between extractability and microbial activity.

Materials and Methods

Soil and mulch sampling
Common vetch (*Vicia sativa*), white mustard (*Sinapis alba*), hybrid ryegrass (*Lolium hybridum*) and a mixture of common vetch + oat (*Avena sativa*) were grown as cover crops (CC) on the Lamothe INP–EI Purpan experimental station (near Toulouse, SW France) on a clay loam soil from June to September 2012. Prior to this cover crop, the whole field had grown a durum wheat–sunflower rotation without glyphosate application for more than 10 years. Aerial parts of the 4 cover crops were collected, dried at 40°C and cut into 1 cm square pieces. The underlying 0–5 cm topsoil was collected, sieved (5 mm) and stored at 4°C. CC–associated soils were sampled on each CC plot to record any possible plant–specific soil–borne microbial populations.

Herbicides
Both experiments were conducted with a mixture of technical–grade and [phosphonomethyl–^{14}C] glyphosate (Sigma–Aldrich), prepared in 0.01 M $CaCl_2$. Specific radioactivity and radiochemical purities of GLY were 81.4 MBq/mmol and 98.8 %, respectively.

Experiment 1: glyphosate adsorption on decaying cover crop residues
Incubations and CC characterization/description
CC– were subjected to accelerated decomposition in the dark for 6, 28 or 56 days at 28°C and in non–limiting moisture conditions. Each CC–was moistened and placed on top of its associated soil in a plastic tray (24 ∗ 37 ∗ 7 cm). Soils had been previously brought to field capacity (pF 2.5). At days 0, 6, 28 and 56 of the incubation, CC were dried, ground and analyzed (i) in duplicate for their carbon and nitrogen

content and (ii) on a single aliquot for their biochemical composition as assessed by Van Soest fractionation (Van Soest and Robertson, 1979). CC and soils characteristics are described in Table 1.

Table 1 Cover crops (a) and associated soils (b) characteristics at different incubation times. OM: organic matter, SOL: water–soluble, NDF: neutral detergent fiber soluble, HEM: hemicellulose–like, CEL: cellulose–like, LIC: lignin–like, C: carbon, N: nitrogen.

(a)

CC	Incubation Time (days)	OM	SOL	NDF	HEM	CEL	LIC	C ($mg \cdot g^{-1}$)	N ($mg \cdot g^{-1}$)	C/N
Vetch + oat	0	89.1	31.3	7.1	21.1	33.7	6.8	427.6	34.7	12.3
	6	81.0	29.4	12.0	12.7	38.7	7.1	427.5	34.8	12.3
	28	73.8	27.8	18.7	16.2	23.7	13.6	370.6	37.7	9.8
	56	75.8	18.8	24.3	18.2	23.5	15.2	374.0	36.5	10.3
Vetch	0	88.6	27.4	18.5	13.9	31.1	9.1	432.0	44.3	9.8
	6	66.8	29.6	13.0	11.6	33.9	11.9	352.7	27.9	12.6
	28	62.8	19.0	20.1	14.7	23.8	22.3	321.2	33.5	9.6
	56	54.2	7.4	30.3	11.5	27.1	23.6	290.3	29.9	9.7
White mustard	0	64.0	14.4	23.0	24.3	31.4	6.9	391.9	40.4	9.7
	6	62.8	35.9	24.7	7.8	22.1	9.6	323.4	29.5	11.0
	28	63.9	16.1	19.8	14.8	26.8	22.5	317.6	32.8	9.7
	56	69.9	23.1	20.8	15.2	25.5	15.4	336.1	30.5	11.0
Ryegrass	0	87.8	37.7	3.1	28.8	26.8	3.6	423.9	34.9	12.1
	6	72.1	24.4	26.1	15.1	18.2	16.1	377.6	44.6	8.5
	28	74.9	39.5	9.6	14.2	28.1	8.6	382.3	33.9	11.3
	56	72.6	23.5	27.3	15.7	19.2	14.3	380.9	43.2	8.8

(b)

CC	pH	OM (%)	C/N	N (%)	Organic C (%)	CEC (méq/100 g)	$CaCO_3$ (%)	CaO exangeable ($mg \cdot kg^{-1}$)	K_2O exangeable ($mg \cdot kg^{-1}$)	MgO exangeable ($mg \cdot kg^{-1}$)	P_2O_5 Olsen ($mg \cdot kg^{-1}$)
Vetch + oat	7.4	2.1	8.2	0.148	1.21	19.5	<0.1	4671	158	734	29.4
Vetch	7.5	1.8	7.4	0.138	1.02	16.2	<0.1	4255	153	582	20.3
White mustard	7.4	1.8	7.5	0.138	1.04	17.5	<0.1	4721	147	631	33.3
Ryegrass	7.4	2.2	9.2	0.140	1.28	17.0	<0.1	4387	184	645	24.0

Sorption characterization

Sorption of glyphosate onto CC residues and soil was determined using a batch equilibration technique, as detailed in Cassigneul et al. (2015). The sorbent:glyphosate solution ratio was 1:9 (g/mL) for soil and 1:5.8 for CC residues. Amounts of sorbed glyphosate were described using the partition coefficient K_d (L/kg) and the normalized organic carbon content K_d i.e. K_{oc} (L/kg OC)

Experiment 2: glyphosate degradation in microcosms of soil and cover crop residues

Microcosm setup/construction/description – Microcosms, i.e. cylinders containing soil (118 g dw) covered by CC mulch (2.5 g dw), were set up as detailed in Aslam et al. (2014). The amount of mulch corresponds to 8 t/ha of biomass in the field, soil and mulch densities being 1.2 g/cm^3 and 0.05 g/cm^3 respectively. This amount of biomass was chosen to ensure a sufficient soil coverage given our objectives. After determination of their retention curve using pressure plates, water content of both soil and mulch was brought to field capacity (pF 2.5) in order to ensure water availability to microorganisms. Microcosms were placed in a 2 L hermetically sealed jar and incubated in the dark (20 ± 1°C). To maintain constant soil moisture, a 10 mL vial filled with deionized water was placed in each jar and water content was adjusted weekly by weighing and adding water as necessary. Two 84–days incubations were performed, both with treatments including a bare soil (control) and 4 studied CC amended soils, but with and without ^{14}C–glyphosate application. The aim was to characterize separately (i) glyphosate fate in 'soil + mulch' and (ii) carbon mineralization from mulch. Each treatment was repeated thrice.

Organic C mineralization – CO_2–C produced by soil respiration and mulch decomposition was trapped in a vial containing 20 mL of 0.1 M NaOH, which was replaced weekly throughout the incubation. From a 1 mL aliquot, CO_2–C was analyzed by colorimetry on a continuous flow–analyzer. Net mineralization of CC carbon was calculated by subtracting the mineralization measured in the control soil treatment from that of the CC–amended treatment, and expressing the difference as a percentage of the initially–introduced organic carbon content.

Degradation study: pesticide monitoring in soil and mulch samples – At day 0, the recommended rate of glyphosate (2 L/ha) was applied at the microcosm surface (soil or mulch) in 2 mL of aqueous solution

with a micropipette. The water volume thus added had been subtracted from the total amount of water that had to be added to reach the targeted water content.

Mineralized fraction – $^{14}CO_2$–C originating from glyphosate mineralization in the mulch and/or underlying soil was trapped by the same procedure as total CO_2–C. The vials containing 20 mL of 0.1 M NaOH were replaced weekly throughout the incubation.

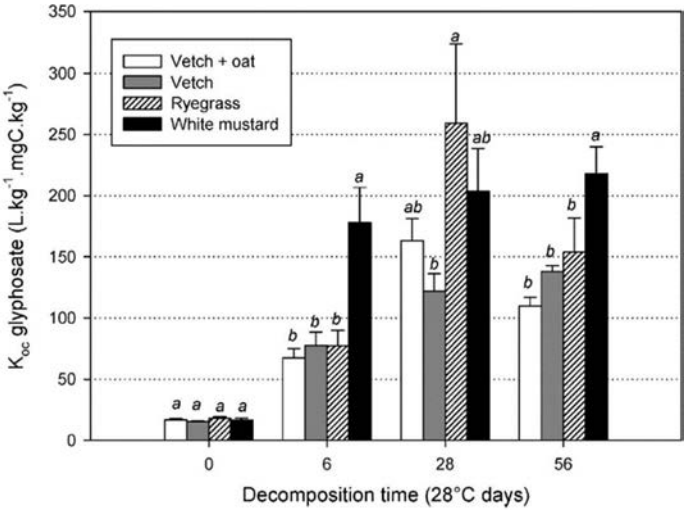


Figure 1. Sorption of glyphosate on cover crop residues. Letters correspond to LSD grouping within a single incubation time.

Extractable fractions – At 0, 7, 22, 49 and 84 DAT (days after treatment), microcosms were destructively sampled. Soils (top 1 cm) and mulches were separately submitted to 4 sequential extractions. Substrates were placed in polypropylene tubes containing solvent and shaken in a rotary shaker for 24 h in the dark at room temperature. The substrate:solvent ratio was 1:20 (g/g) and 1:3 (g/g) for mulches and soils respectively. Extractions were performed first with $CaCl_2$ (0.01 M) and then 3 times with NH_4OH (0.1 M), providing access to the weakly–sorbed and to the strongly–sorbed ^{14}C–glyphosate. Between each extraction, tubes were centrifuged for 10 min at 10,000 g and 6000 g for the mulch and the soil respectively. Supernatants were sampled for radioactivity counting, and the remaining volumes were stored at 4°C until HPLC analysis.

Non–extractable fraction – CC or soil material pellets remaining after the last extraction were oven–dried for 72 h (40°C) and ground for 10 min (Retsch GmbH, Germany). Duplicate aliquots of 500 mg were burnt in a Sample Oxidizer 307 where evolved $^{14}CO_2$ was trapped in a scintillation vial containing Oxysolve T. The vial was immediately subjected to scintillation counting.

Analytical determinations – Radioactivity content in the liquid samples was measured by scintillation counting from a 1 mL aliquot mixed with 10 mL of scintillation liquid (Ultima Gold™ XR, Perkin Elmer, USA), using a Packard Tri–Card counter (GMI, Inc., USA). To prevent a chemiluminescence reaction, NaOH and NH_4OH scintillation vials were submitted to a 24 h period in the dark prior to counting. A blank sample containing solvent or NaOH solution was inserted in each counting series. To determine the amount of glyphosate and metabolites, soil and mulch extracts containing sufficient radioactivity (83.3 Bq/mL) were previously filtered, concentrated by evaporation under vacuum at 50°C (Rotavapor ®, Büchi, Switzerland), and centrifuged to ensure maximum particle removal. Samples of NH_4OH extracts included the extracts of the 3 successive extractions. HPLC analysis was performed coupled with a Flexar (PerkinElmer, USA) coupled with a radioactive flow detector (Radiomatic Flow Scintillation Analyzer 150TR, PerkinElmer, USA). Samples (200–500 µL) were injected into an Allsep ™ A–2 anion exchanger column (100 mm × 4.6 mm, 7 µm, Grace Davison Discovery Science, USA) preceded by a GA–1 Anion guard column (7.5 × 4.6 mm, Grace Davison Discovery Science, USA) to ensure an efficient separation, eluted with a KH_2PO_4 solution (0.34 g/L) adjusted to pH 2 with a 85 %

H_3PO_4 solution. The mobile phase flow was 10^{-3} L/min. Under these conditions, the retention time was 3–5 min for GLY and 1–3 min for its main metabolite.

Glyphosate in the extracts was identified by comparison with the standard solution on the basis of retention time. Other detected peaks were considered as "main metabolite" (MM) or "unidentified" (UI) peaks. The main metabolite was suspected to be AMPA from previous experience with the same analytical method but, in the absence of a radiolabeled standard, this could not be verified in this particular experiment. The area of each peak was integrated (Chromera ® chromatography Data System, PerkinElmer) and expressed as a percentage of initial radioactivity applied in the microcosm.

Degradation half–life modeling.
The percentage of glyphosate in the extractable fraction in the microcosm (corresponding to the extractable fraction in the mulch + the extractable fraction in the underlying soil) was fitted to a single first–order kinetic model with untransformed data, $C(t) = C_0e–kt$ where C(t) is the measured concentration in glyphosate at time t, C_0 is the initial concentration measured immediately after application, and k is the first–order rate constant (day^{-1}). Following this model, the degradation half–life (DT_{50}), time (days) for 50 % disappearance of the initial amount of glyphosate, was calculated for $C = C_0/2$ and corresponds to the ln 2/k ratio.

Data analysis
Data handling and modeling – The radioactivity measured in the different glyphosate fractions was expressed as a percentage of the initially applied radioactivity. Cumulative net CO_2–C and $^{14}CO_2$–C mineralization were fitted to an exponential model that describes mineralization at incubation time t as $a_{MIN} * (1–\exp.(–k_{MIN} * t))$. The parameters α_{MIN} and k_{MIN} describe the maximum % C mineralized, and the rate at which it is reached, respectively. The kinetics data for extractable and non–extractable fractions proportions were fitted to an exponential model with 2 ($y = a_{EXT} * \exp.(–k_{EXT} * t)$) and 3 ($y = y_0 + a_{NER} * (1–\exp.(–k_{NER} * t))$) parameters respectively. In the former case, α_{EXT} is the initial extractable proportion and k_{EXT} the rate of decrease, and in the latter case y_0 is the initial NER proportion, a_{NER} the direction of variation, increase or decrease in NER and k_{NER} the rate of NER variation.

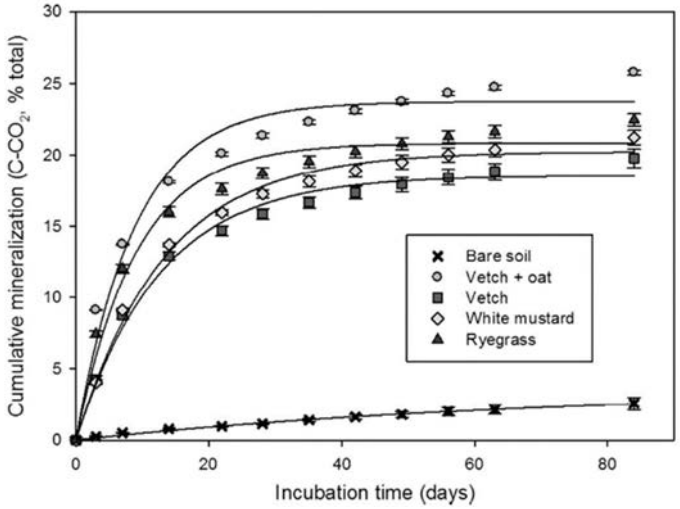


Figure 2. Organic carbon mineralization. Error bars represent the standard error of the mean of 3 replicates.

Statistical analysis
Analyses of variance were performed to ascertain whether each glyphosate fraction proportion was influenced by the incubation time, the treatment or the compartment (soil or mulch) at/on which it was measured. Then, for each fraction, a Fisher's LSD test was used to rank the treatments or compartments. Additionally, an analysis of the correlations between the different glyphosate fractions was carried out at the column and compartment level, with treatments considered together and alone. Parameters of the

different kinetic models were also subjected to analysis of variance and post–hoc LSD Fisher test to rank the different treatments, with a level of significance set at 0.05.

Results

Adsorption

Sorption was significantly higher on soil than on cover crop residues (Figure 1). K_d was 53 and 8 times higher on soil than on fresh and decomposed (56–d) CC residues, respectively. Furthermore, the statistical analysis performed within the CC revealed a significant effect of both decomposition degree and CC type. Sorption increased with the decomposition degree of cover crops ($p < 0.0001$), K_d and K_{oc} for decomposed CC (56 d) being on average 8 or 9 times higher than those measured on fresh CC (0 d). K_d was significantly higher on white mustard than on other CC for 6– and 56–d old CC, being 57 L/kg and 75 L/kg while other CC averaged 28 and 50 L/kg, respectively. For 28–d old CC, K_d was significantly higher on ryegrass (99 L/kg) than on vetch (39 L/kg), other CC being intermediate (66 L/kg). In CC, the analysis of correlations between sorption coefficients and organic matter descriptors did not show any significant relations for K_d. K_{oc} was inversely correlated with the hemicellulose–like fraction ($r = -0.55$, $p < 0.05$).

Degradation study

Mulch characteristics during incubation – During the whole incubation period, 2.6 % of the microcosms' carbon content was mineralized from the bare soil. In CC–amended soils, carbon mineralization ranged from 19 % (vetch) to 25 % (vetch + oat) (Figure 2), corresponding to a weight loss of approximately 35 %. Water content remained constant, being 17 % (w:w) in the soil and 60–72 % according to the mulch (data not shown).

Variability across intercepting material, plant type and time – Glyphosate recovery in the microcosms averaged 90 % of the initially applied dose (Figure 3). ^{14}C glyphosate fractions were significantly influenced by time, intercepting material (i.e. decaying residue or soil) and plant type (i.e. cover crop species).

Mineralized fraction – Glyphosate mineralization started immediately after application, without any lag phase. It fitted the chosen exponential model well ($R^2 > 0.95$), with parameter k_{MIN}, the speed at which the maximum is reached, and a, the maximum value reached. Treatments differed significantly from each other for the parameter a_{MIN} (Table 2), with a higher cumulative glyphosate mineralization in the bare soil microcosms, compared to the mineralisation in the CC–amended microcosms, with values ranging from 13.0 to 15.8 % (Table 2). Analysis of the plant type effect showed that the maximum mineralized glyphosate was reached significantly faster in ryegrass (Table 2).

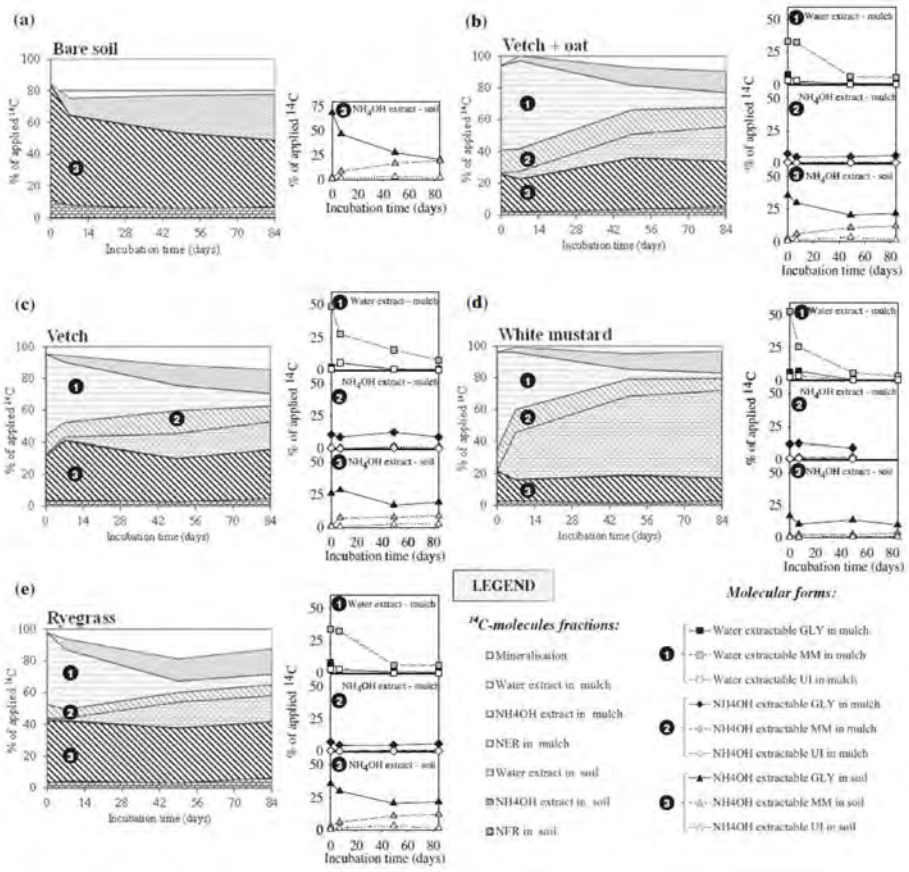


Figure 3. Fate of glyphosate in the microcosms. Letters indicate the treatment (a: bare soil, b: vetch + oat, c: vetch, d: white mustard, e: ryegrass) and numbers 1, 2 or 3 indicate the fraction within which molecular forms were analyzed (water- or NH_4OH–extractable in mulch and/or soil). Results are expressed as % of applied ^{14}C.

Extractable fraction – Total extractable fraction (corresponding to the water and NH_4OH extracts) was well fitted by the chosen exponential model, with $R^2 > 0.8$ and $R^2 = 0.65$ for CC–amended and bare soil treatments respectively. The extractable fraction decreased over time for all treatments (Figure 3). Differences were observed between the treatments, extractability falling faster in white mustard than in other treatments (param b_{EXT}) (Table 2). At the end of the experiment, significantly less glyphosate was extractable in the white mustard treatment. The extractable fraction is separated into a water–extractable and an ammonia–extractable fraction, for which more details of molecular forms are given below. The water–extractable fraction decreased rapidly from 52.7 ± 3.4 to 7.0 ± 1.3 % of the applied ^{14}C between 0 DAT and 84 DAT in the mulch compartment (Figure 3, fraction ❶) while it remained low in the soil compartment (<1 % of applied ^{14}C). A larger proportion of ^{14}C was extracted with water in the vetch + oat microcosms, until 49 days of incubation (Figure 3b). In mulches, more metabolites than glyphosate (GLY) were found in the water extracts. Both GLY and its main metabolite decreased during incubation, averaging 7.9 ± 2.7 % to 0.9 ± 0.3 % and 45.0 ± 4.7 % to 6.7 ± 1.2 % of the applied ^{14}C between 0 DAT and 84 DAT, respectively. The ammonia–extractable fraction remained stable with time in the mulch compartment and varied with no clear trend in the soil compartment while it decreased during incubation in the bare soil treatment (Figure 3, fractions ❷ and ❸). In the bare soil treatment, GLY proportions decreased from 70 to 20 % of the applied dose between 0 DAT and 84 DAT, whereas MM proportions increased from 2 to 19 % in the same period (Figure 3a). In all CC–amended treatments, the GLY proportion decreased from an average of 26.5 ± 4.7 % to 14.4 ± 2.1 % and from 11.9 ± 0.1 % to 8.8 ± 1.3 % in soil and in mulch compartments between 0 DAT and 84 DAT, respectively. Meanwhile, MM proportion (i) increased from 1.5 ± 0.3 % to 8.5 ± 1.9 % in soils and (ii) averaged 1.01 ± 0.15 % in mulches.

Table 2 Fraction–dynamics model parameters. Letters correspond to LSD groups

Fraction	Treatment	y_0			$a_{i\ (i\ =\ MIN,\ EXT,\ NER)}$			$k_{i\ (i\ =\ MIN,\ EXT,\ NER)}$		
		Value	Effect of mulch vs. bare soil	Effect of plant type	Value	Effect of mulch vs. bare soil	Effect of plant type	Value	Effect of mulch vs. bare soil	Effect of plant type
Mineralized fraction	Bare soil	–	–	–	27.1 ± 0.9	a	–	0.06 ± 0.01	ab	–
$y = a * (1\text{-exp.}(-kt))$	Vetch + oat	–	–	–	15.0 ± 2.5	b	ns	0.04 ± 0.01	b	b
	Vetch	–	–	–	15.8 ± 1.4	b	ns	0.04 ± 0.01	b	b
	White mustard	–	–	–	13.0 ± 0.4	b	ns	0.04 ± 0.01	b	b
	Ryegrass	–	–	–	15.1 ± 0.2	b	ns	0.08 ± 0.01	a	a
Extractable fraction	Bare soil	–	–	–	66.5 ± 0.6	b	–	0.01 ± 0.00	b	–
$y = a * \exp.(-kt)$	Vetch + oat	–	–	–	92.2 ± 0.5	a	ns	0.00 ± 0.00	b	b
	Vetch	–	–	–	89.6 ± 0.8	ab	ns	0.01 ± 0.00	b	b
	White mustard	–	–	–	84.9 ± 7.8	ab	ns	0.02 ± 0.00	a	a
	Ryegrass	–	–	–	89.0 ± 0.8	ab	ns	0.01 ± 0.00	b	b
Non-extractable fraction	Bare soil	8.9 ± 1.0	a	–	−2.7 ± 0.9	c	–	0.12 ± 0.04	a	–
$y = y_0 + a * (1\text{-exp.}(-kt))$	Vetch + oat	4.1 ± 1.0	b	ns	59.8 ± 27.7	a	ns	0.01 ± 0.01	b	b
	Vetch	3.1 ± 0.7	b	ns	28.2 ± 6.3	b	ns	0.02 ± 0.01	b	b
	White mustard	3.4 ± 1.0	b	ns	53.2 ± 2.3	ab	ns	0.05 ± 0.01	b	a
	Ryegrass	3.6 ± 0.9	b	ns	31.1 ± 5.9	ab	ns	0.02 ± 0.01	b	b

Non–extractable fraction – The NER fraction increased with time for CC–amended treatments, especially in the mulch compartment. On the contrary, NER decreased in the bare soil from 11 to 7 % of the applied dose between 0 and 84 DAT (a <0, Table 2). By comparison, in the soil compartment below the mulch NER increased from 3 to 5 % or remained constant (white mustard) (Figure 3, bricks symbols). At the end of the experiment, three statistical groups differing in their NER proportions were distinguished: (i) white mustard with 59.7 ± 2.8 %, (ii) the 3 other mulches with 27.2 ± 0.8 % and (iii) bare soil with 9.0 ± 1.1 % of the initially applied ^{14}C. The modeling of NER formation showed that NER formation rate was significantly greater in white mustard (k_{NER} parameter) than in other mulches (Table 2).

Glyphosate degradation half–life – In presence of a cover crop mulch, glyphosate degradation half–life was longer than in bare soil, being respectively 28–47 days and 20 days (Table 3). DT_{50} values showed that glyphosate persistence was increased in the presence of a mulch layer at the soil surface, whatever the type of mulch.

Table 3 Glyphosate half–life (DT_{50}) calculated from fitting of experimental data to $C = C_0.e^{-kt}$ model. Data are mean ± standard–error.

Treatment	DT_{50} (days)	LSD group	R^2
Bare soil	21 ± 1	*b*	0.91
Vetch + oat	28 ± 10	*ab*	0.80
Vetch	47 ± 4	*a*	0.74
White mustard	43	*ab*	0.77
Ryegrass	38 ± 5	*ab*	0.73

Correlation between processes – Considering all treatments, glyphosate mineralization was (i) positively correlated with carbon mineralization (r = 0.80 for CC– amended and r = 0.99 for bare soil) and non–extractable fraction (r = 0.54 for CC–amended and r = 0.99 for bare soil, p <0.05); (ii) negatively correlated with water–extractable fraction (r = −0.69 for CC–amended and r = −0.97 for bare soil, p <0.01). Furthermore, NER fraction was (i) positively correlated with mineralized glyphosate fraction (r >0.96, p <0.01) and carbon mineralization (r = 0.55 for CC–amended and r = 0.99 for bare soil, p <0.05) and (ii) negatively correlated with water–extractable fraction (r <−0.96, p <0.05) in bare soil and all CC–treatments except vetch. Vetch specific correlations were found between the ammonia–extractable fraction and carbon (r = −0.98) and glyphosate (r = −0.99) mineralization. At the compartment level, the analysis revealed a correlation of NER formation either with water–extractable fraction in mulch (r = −0.98) or with ammonia extractable fraction in soil (r = −0.94).

Discussion

Glyphosate fate depends on the intercepting material

After application, glyphosate fate presented specificities according to the intercepting material, i.e. soil or CC mulch. It was much strongly retained by soil than by mulch, being mainly extractable with ammonia and with water, respectively. These results are in agreement with the sorption measurements (Figure 1) and are mainly explained by the sorption affinity of glyphosate to soil mineral constituents (clays, oxides). Furthermore, despite a high microbial activity in the mulches, reflected by the carbon mineralization (Figure 2), glyphosate mineralization in the presence of mulch was lowered as compared to the bare soil treatment. These observations partly suggest a difference in glyphosate accessibility to microorganisms in the two compartments. In soil, although glyphosate is strongly retained, as stated above, the herbicide remains accessible for a complete biological degradation. These results are in agreement with those of Schnurer et al. (2006) who observed biodegradation of soil–sorbed glyphosate. In mulches, the absence of change in the molecular forms with time in the ammonia extracts (Figure 3, fraction ❷) suggests that mulch–sorbed molecules were not available for microorganisms. In contrast, soluble glyphosate and its degradates are available in the mulch–water extracts, as shown by the decrease in their respective proportion (Figure 3, fraction ❶). However, microbial populations that colonize the decaying mulch are not as efficient as soil microbial population in mineralizing glyphosate. NER formation is generally considered to be the result of either microbial incorporation of pesticide, physical entrapment in the nanoporosity, chemical stabilization by bounding, or diffusion to less accessible sites during a long period of contact. In this study, NER formation was positively correlated to glyphosate mineralization by microorganisms in both soil and mulch compartments. As the mulch compartment is prone to a higher microbial activity (Figure 2), NER formation is clearly one of the main dissipation pathways of glyphosate in mulches, while it is a minor pathway for soils. In our study, NER proportion was negatively correlated either to sorbed (ammonia extracted) glyphosate fraction in soil or to soluble (water extracted) glyphosate fraction in mulches. In CC mulches, the decrease with time in soluble glyphosate is combined with a weak mineralization and its nearly constant sorbed proportion (recovery of glyphosate in the ammonia fraction). This supports the hypothesis of a direct transfer from the ‘soluble’ to the ‘NER’ fraction.

Glyphosate fate as influenced by the nature of the intercepting plant material

Glyphosate fate in the mulch compartment is similar whatever the mulch, i.e. the time evolutions of different fractions are generally similar. However, two of the four cover crop species stand out from the others. Glyphosate was less mineralized in ryegrass than in other cover crops, which we cannot explain, and NER formation is maximal in white mustard. This latter result was not expected but can be explained in view of the results of the sorption study where white mustard was the mulch which maximized sorption at day 6 and 56.

Glyphosate fate in cover crop residues and environmental risk assessment

In this study, glyphosate fate was studied at a fine scale by considering several fractions. The results can be interpreted at a broader scale by considering only two fractions: (i) the dissipated glyphosate i.e. the glyphosate mineralized as CO_2 and immobilized as NER; and (ii) the available glyphosate and metabolites i.e. the molecules which remain available and could be leached in field conditions. At this scale, except for the white mustard treatment, both dissipated and available glyphosate were statistically the same in all treatments. This does not lead to the conclusion that glyphosate fate is not influenced by the presence of a cover crop since (i) dissipation pathways are treatment–specific, i.e. mineralization and metabolites (AMPA) formation are greater in bare soil and more non–extractable residues are formed in CC–amended treatments; and (ii) the NER formation pathway in mulch is time–dependent, leading to a potential decrease in availability of glyphosate in CC–amended treatment. According to the mechanisms potentially involved in NER formation routes we have proposed for mulch, such release cannot be excluded. The extent to which these results can be extrapolated to field conditions will be determined by (i) weather conditions, especially during the time between application and the first rain and the temperature; (ii) agricultural practices, especially cover crop incorporation and fertilization; and (iii) mulch biomass, coverage, and contact with soil as well as soil type.

Conclusions

This study aimed at evaluating the effects of a mulch of cover crop residues located at the soil surface on the environmental behavior of glyphosate. In the presence of a cover crop mulch, glyphosate and its metabolite remained mainly water–soluble, but with time, a higher proportion of the herbicide became non–extractable. Unlike in soil conditions, bound residue formation was the main process involved in glyphosate dissipation in cover crop mulches. Variations in the intensity of each process were observed among the four cover crop residues studied, but remained unexplained by the biochemical composition of the residues. Finally, degradation half–life of glyphosate was increased with all type of mulches. Such results suggest a greater risk of glyphosate transfer by leaching in conservation agriculture systems.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The article describes the degradation and sorption of Glyphosate to soil considering cover crops. The article is well described and provides potential endpoints for degradation and sorption. However, the available information does not allow to check the validity against current guidelines, and not enough parameters are provided to evaluate the kinetic behavior.

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