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Degradation of ^{14}C-glyphosate and aminomethylphosphonic acid (AMPA) in three agricultural soils

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Abstract

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.

Key words: glyphosate; aminomethylphosphonic acid (AMPA); mineralization; degradation; soil
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Introduction

Glyphosate [N-(phosphonomethyl)glycine, $C_3H_8NO_5P$] is a broad-spectrum, post-emergence and non-selective systemic herbicide. It is the active ingredient in Roundup and other weed-killing formulations especially used in agriculture and gardens maintenance. Upon its application for weed control, some amount of glyphosate reaches the soil where a part is adsorbed by the soil constituents, while other part remains in the soil solution. Depending on this distribution, the biological activity and physico-chemical properties of the medium, various biochemical and/or chemical reactions will participate and lead to the transformation and the mineralization of pesticide (Grébil et al., 2001; Al-Rajab et al., 2008).

The relatively rapid degradation of glyphosate has the advantage of limiting its role in polluting the environment, especially the solution of soil and water resources. However, this degradation could increase the pollution risk by its metabolites: aminomethylphosphonic acid (AMPA) and/or sarcosine (Al-Rajab et al., 2008; Landry et al., 2005). The complete mineralization of the active ingredient is the only way to oppose any contamination of the environment (Malik et al., 1989; Borggaard and Gimsing, 2008). Similarly, the interactions of pesticide-soil and the diffusion process lead to the formation of non-extractable residues trapped in areas not accessible to water flowing through the soil. The formation of non-extractable residues which ensures a stabilization of product in the soil can also be effective against the short-term water pollution.

Many studies, however, suggest the possibility of a slow remobilization of these residues, which could explain the low pollution level of groundwater by some pesticides at long-term. In addition, an assessment of the intensity of each process that affect the glyphosate in different types of soil is likely to give an indication of their capacity to impede or facilitate the pollution of water depending on the climatic conditions met after treatment. Research on the combined effects of mineralization, degradation, stabilization and remobilization of glyphosate in the soil is scarce (Laitinen et al., 2006; Roy et al., 1989).

In this article, the objectives of this study were to examine the fate of glyphosate in three agricultural soils which are very different in their physicochemical properties in

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order to assess its potential to contaminate each soil. For this end, we followed at the same time its mineralization, degradation, evolution of non-extractable residues and its availability to water.

1 Materials and methods

1.1 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.

1.2 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) (Jacquin and Florentin, 1988). None of these soils had ever been exposed to glyphosate. Soil types were classified as rendzic leptosol, fluvic cambisol, and stagnic luvisol (Batjes, 1998) 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.

1.3 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.

1.3.1 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.

1.3.2 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).

1.3.3 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-driered 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.

1.4 Analysis

1.4.1 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

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.

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.

1.4.2 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-NH_2 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.

1.5 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).

1.6 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.

2 Results and discussion

2.1 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 (Fig. 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. Comparable results were also observed by Haney et al. (2000) and Weaver et al. (2007).

2.2 Mineralization 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 rate of glyphosate degradation after its application on soil (Fig. 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,

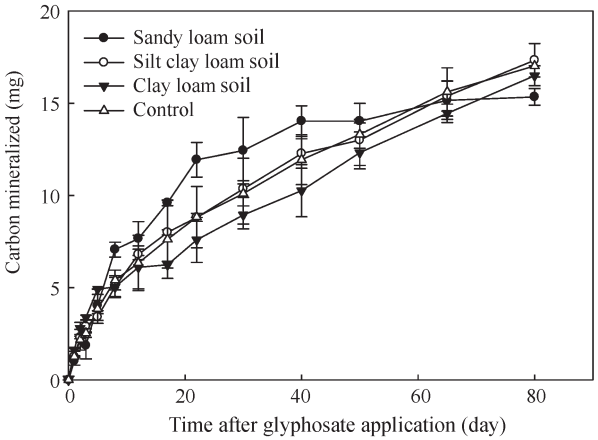

Fig. 1 Mineralization activity of microflora of three soils (clay loam, sandy loam and silt clay loam soils). The control is the average of mineralization activity for the three untreated soils.

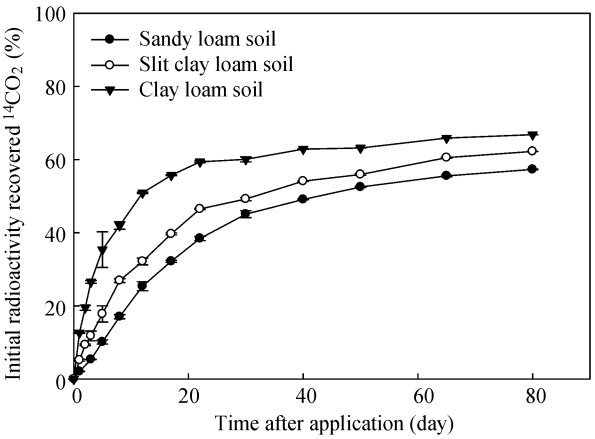

Fig. 2 Mineralization of ^{14}C-glyphosate to $^{14}CO_2$ in three soils incubated at 20°C.

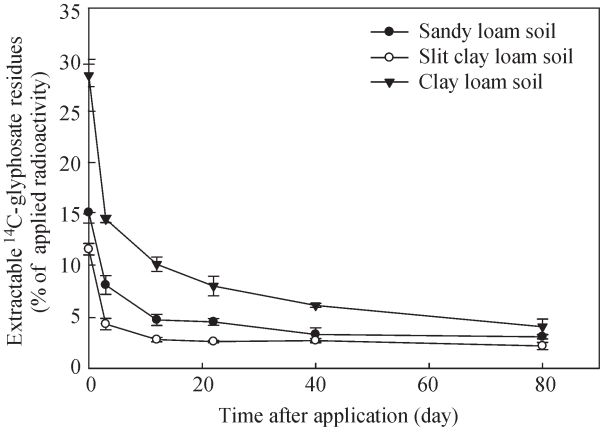

Fig. 3 Evolution of extractable ^{14}C-glyphosate residues with H_2O from the three soils during incubation at 20°C.

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.

2.3 Glyphosate degradation products

2.3.1 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 produce a 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 (Fig. 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.

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 (Fig. 4). In fact, in the sandy loam soil of K_f = 34, the percentage of glyphosate ^{14}C-phosphonomethyl extracted at T0, immediately after treatment, was (81.9 ± 0.55)% of the initial amount applied (Fig. 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 K_f = 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 K_f 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

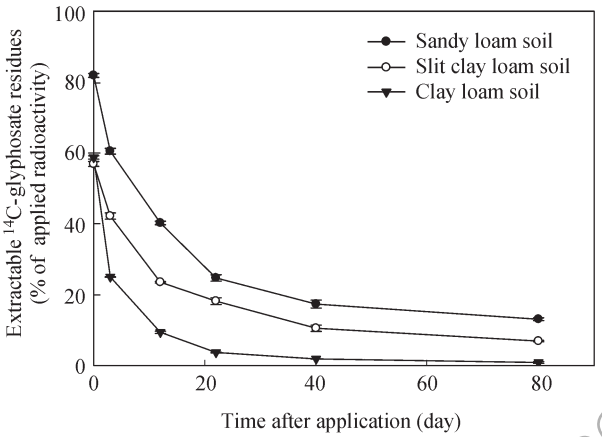

Fig. 4 Evolution of extractable ^{14}C-glyphosate residues with KH_2PO_4 from the three soils during incubation at 20°C.

and the rate that non-extractable residues become available for mineralization and extraction. A similar behaviour of extractable residues of glyphosate over time was reported (Getenga and Kengara, 2004; Mile and Moye, 1988).

2.3.2 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.

In the case of silt clay loam soil, the percentage of AMPA in the extracted residues was higher than that in sandy loam soil during the first incubation period. It reached 20.3% and 74.4% of residues after 3 and 22 days of incubation, respectively. Thereafter, this percentage increased gradually until the end of the experiment after 80 days, it reached 85.1% of extracted residues. In contrast, for the clay loam soil, in which glyphosate was mineralized faster than in the two other brown soils, AMPA represents 48.5% of extracted residues after 3 days of incubation, 88.0% after 22 days and 99.1% by the end of the experiment. Our results are consistent, to some extent, with those obtained by Cheah et al. (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 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 (Table 4). These values are consistent with the 6 to 9 days half-lives previously reported 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 et al. (1998). However, much longer half-lives have also been reported by Getenga and Kengara (2004), who reported that the half-life of glyphosate was 85.6 days in a clay soil incubated at 30°C.

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 (Fig. 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 (Fig. 5).

2.4 Non-extractable glyphosate residues

The non-extractable residues represent the fraction which can not be extracted from the soil by the series of KH_2PO_4 extractions (exhaustive extraction) (Fig. 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

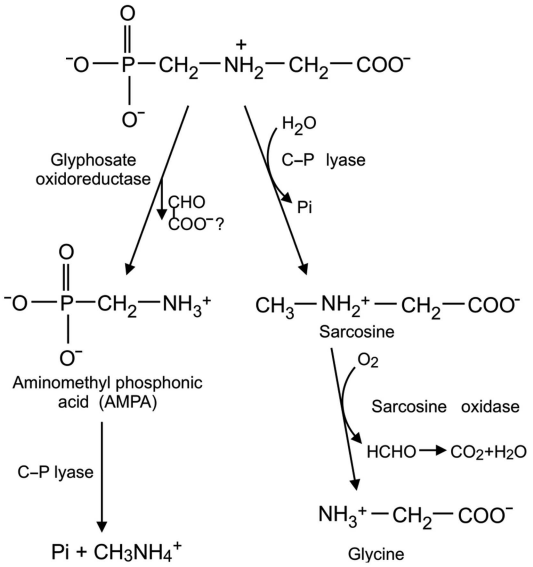

Fig. 5 Microbial degradation of glyphosate in soil through sarcosine or AMPA (Liu et al., 1991).

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.

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, unlike other pesticides such as atrazine where the rate of non-extractable residues increases gradually over dozens of days (Winkelmann and Klaine, 1991).

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) (Agritox, 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.

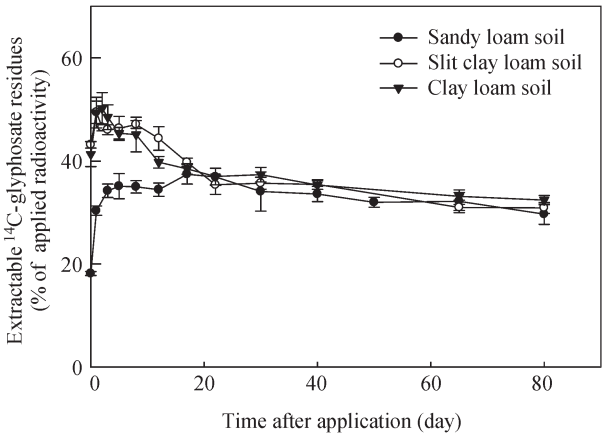

Fig. 6 Evolution of non-extractable residues in three soils during incubation time at 20°C.

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.

3 Conclusions

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.

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