

Mineralization and sorption of ^{14}C -glyphosate in samples from three soil types collected in El Espinal, Colombia

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Abstract

We studied the mineralization and sorption of ^{14}C -glyphosate in samples of three soil types from El Espinal municipality (Tolima) with different land use history: (1) soil subjected to pesticide use and sowing of annual crops (mainly rice) during 30 years; (2) soil in rest for five years with sown grassland, and (3) secondary semidry forest soil without record of pesticide use. The ^{14}C -glyphosate mineralization in the soil samples was effected in biometers. One hundred and six days after the incubation with the herbicide, 53%, 38% and 23% of the glyphosate was mineralized in the forest, grass and rice-crop soil samples, respectively. The glyphosate sorption phenomenon (adsorption-desorption) was adjusted to the Freundlich model. The $K_{d_{des}}$ values for forest, grassland and rice-crop soil samples were 17.00, 1.83 and 3.10, respectively. The low rate of glyphosate mineralization allowed us to infer that the glyphosate can become an environmental pollution problem due to its high persistence in the soil.

Key words: Herbicides, microorganisms, radiomarked, pollution, rice.

Mineralización y adsorción del ^{14}C -glifosato en muestras de tres tipos de suelo provenientes de El Espinal, Colombia

Resumen

Se estudió la adsorción y la mineralización de ^{14}C -glifosato en muestras de tres tipos de suelo provenientes del municipio de El Espinal, Tolima, con diferente historia de uso: (1) un suelo con historia de uso de pesticidas durante 30 años y cultivos anuales (principalmente arroz); (2) un suelo en receso de 5 años con pasto de corte, y (3) un suelo de bosque secundario semiseco sin registro de uso de plaguicidas. La mineralización del ^{14}C -glifosato en los suelos se efectuó en biómetros. A los 106 días de la incubación con el herbicida, 53 %, 38 % y 23 % del glifosato estaba mineralizado en los suelos de bosque, de pasto de corte y cultivo de arroz, respectivamente. El fenómeno de adsorción del glifosato (adsorción-desorción) se ajustó al modelo de Freundlich. Los valores $K_{d_{des}}$ para los suelos de bosque, pasto de corte y cultivo de arroz fueron 17,00, 1,83 y 3,10, respectivamente. La baja tasa de mineralización del glifosato permite inferir que este puede convertirse en un problema de contaminación del medio ambiente debido a su alta persistencia en el suelo.

Palabras clave: herbicidas, microorganismos, radiomarcaje, contaminación, arroz.

Introduction

Pesticide sorption in soils is regulated by the molecular properties of the pesticide and by the characteristics of the soil adsorbent surfaces, which determine the type of interactions established between pesticide molecules and soil adsorbent surfaces, relations that have been explained in detailed by Calvet, *et al.* (2005). Roy, *et al.*, (1989) and Feng & Thompson (1990) have emphasized that the complex nature of adsorption and the pH are two of the most important factors regulating sorption phenomena. In the first case, adsorption is very much related to organic matter contents and to the quantity and nature of clays. Thus, ion pesticides are highly adsorbed by soils rich in oxides and metallic

hydroxides while cationic pesticides are adsorbed by soils with high content of clays (montmorillonite and vermiculite, mainly) and organic matter (Calvet, *et al.*, 2005). Calvet, *et al.*, (2005) also explained how the type of interaction and the adsorption mechanism depend on pH levels in the case of ionic pesticides, acids and weak bases. In weak acids (like glyphosate), the proportion of the non-ionized molecules (with a higher K_{wo} than ionized molecules) increases when the pH decreases; so, the importance of the polar interactions

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in hydrogen binding and, therefore, of the interchange mechanism with water molecules, increase. When the pH increases, the proportion of ionizable molecules also increases, and hence, the electrostatic repulsion. With regard to the properties of compounds, four of these properties have influence on adsorption: the ionization (mentioned previously), the distribution of electric charges in the molecule, the molecular structure, and the partition characteristics (solubility in water, Kow, FBC and Koc); **Calvet, et al.**, (2005) have expounded the influence of molecular properties on adsorption in detail.

Ion interchange is among pesticides adsorption mechanisms. This exchange occurs between the organic cations present in the soil solution and the inorganic cations placed on the adsorbent surface. It is an index that measures the capacity of a pesticide molecule to be retained by the soil organic matter or by the clays. The cationic compounds can be adsorbed by the negative charges of the clays and the organic matter as if treated to another cation (interchange of cations). The anions are adsorbed mainly by the organic matter and by the iron and aluminium oxyhydroxides; in this case, the adsorption will be influenced by the pH (**Sprankle, et al.**, 1975b; **Hance, 1976; Nomura & Hilton, 1977; Roy, et al.**, 1989; **Feng & Thompson, 1990**).

Once the glyphosate is in contact with the soil, it is quickly bound to soil particles and inactivated (**Sprankle, et al.**, 1975a; **Roy, et al.**, 1989; **Feng & Thompson, 1990**). This adsorption occurs quickly, during the first hour after its application, but later it slows down (**Sprankle, et al.**, 1975b). Glyphosate molecules have several binding mechanisms to soil: they can bound to the oxydic fraction of soil or be adsorbed electrostatically by clay minerals and organic matter through the formation of hydrogen bridges (**Aubin & Smith, 1992; Haney, et al.**, 2000; **Veiga, et al.**, 2001). Additionally, glyphosate is a dipolar organic compound and, for this reason, it exhibits a quick and high rate of adsorption to iron and aluminium oxides and hydroxides, such as soil organic matter, as it has been evidenced in different studies (**Guarracino, et al.**, 1998; **Forlani, et al.**, 1999; **Prata, et al.**, 2000; **Haney, et al.**, 2000).

Other authors indicate that glyphosate adsorption increases when clays content and soil cation exchange capacity increase, and when soil pH and phosphorus content decrease (**Sprankle, et al.**, 1975a, b; **Hance, 1976; Nomura & Hilton, 1977; Rueppel, et al.**, 1977; **Glass, 1987**). Glyphosate binds to soil particles through the phosphonic acid group, competing for the sites that bind to inorganic phosphates in the soil (**Rueppel, et al.**, 1977).

Those glyphosate molecules that remain in the soil solution, without binding to soil constituents, are degraded at a constant and relatively quick rate by soil microorganisms.

Bound molecules are also degraded biologically at a constant but slow rate (**Nomura & Hilton, 1977; Rueppel, et al.**, 1977). Glyphosate, therefore, can persist in soils and in aquatic environments. **Sprankle, et al.**, 1975a; **Hance, 1976; Nomura & Hilton, 1977**). **Newton, et al.**, (1994) and **Veiga, et al.**, (2001) have corroborated that glyphosate fixed by adsorption is more persistent in soil because its degradation is slower than the herbicidal action in the soil solution, since the wastes bound to the solid fraction of soil are hardly accessible to the main herbicide-degrading microorganisms.

Glyphosate average life in soil has been calculated to be around two months, but this can vary from several weeks to years (**Nomura & Hilton, 1977; Rueppel, et al.**, 1977; **Roy, et al.**, 1989; **Feng & Thompson, 1990; Anton, 1993**). Although the strong adsorption of glyphosate to soil allows persistent wastes, these are immovable and there is no significant leaching. **Feng & Thompson (1990)** found that glyphosate wastes were present in the first 15 cm of the soil profile, while herbicide wastes were found at 35 cm of depth. However, in spite of glyphosate immobility in soil and its low leaching risk, studies by the French environment institute carried out between 2001 and 2002 found that glyphosate occupied the tenth place among those pesticides used in France that were polluting ground waters, and the fourth place among those polluting surface waters (**Calvet, et al.**, 2005).

Although glyphosate is not recommended for direct use in soil, a significant amount can reach the soil when it is used for the control of undesirable emerging plants (**Haney, et al.**, 2000). As it was indicated previously, an important characteristic of glyphosate is its capacity to be adsorbed by soil particles and to remain biologically inactive as herbicide, but the persistence of its phytotoxic activity for several weeks after its use has also been demonstrated (**Anton, 1993; Carlisle & Trevors, 1988; Feng & Thompson, 1990; Giesy, et al.**, 2000; **Heinonen-Tanski, 1989; Newton, et al.**, 1984; **Nomura & Hilton, 1977; Rueppel, et al.**, 1977; **Weber, 1994**).

This indicates that the function of adsorption in glyphosate bioavailability and biodegradation is not totally conclusive.

Microorganisms metabolize glyphosate under aerobic conditions transforming it to compounds such as AMPA, sarcosine, water, CO₂ and inorganic phosphorus (**Schuette, 1998**). In studies undertaken by **Haney, et al.**, (2002) it has been demonstrated that glyphosate addition to soil increases microbial activity measured by means of CO₂ evolution products of soil respiration. Besides, it was found that when the herbicide dose is increased, CO₂ production also raises; this allowed the authors to deduce that soil microbial communities proliferate by using the herbicide as energy source, and given that glyphosate degradation is mainly due

to microbial activity, a quick dissipation of the herbicide is to be expected. Mamy’s research work in France (2004) indicated that mineralization is the main cause of glyphosate dissipation; after 140 days of incubation at 28°C of a calcareous clayey loam soil treated with the herbicide, 70% of the parental compound had mineralized and 10% remained as bound, non-extractable wastes.

Glyphosate behaviour in soil has been very much studied in other latitudes, but there is lack of information regarding tropical soils, in general, and of Colombian soils, specifically. The main objective of our work was to study glyphosate sorption and mineralization in samples of three soil types from an agricultural area in the department of Tolima, Colombia.

Materials and methods

¹⁴C-glyphosate mineralization in the samples of the three soil types under microcosm conditions

The samples were collected from three areas in the municipality of El Espinal, Department of Tolima, subjected to different land uses: one had been dedicated to the sowing of annual crops (mainly rice) and subjected to continued use of pesticides for more than 30 years; a second sample came from a grassland area with no reported pesticide use during the previous five years, and the other sample came from a secondary forest with no reported use of pesticides. Each soil sample was homogenized and sieved with a 2 mm mesh. The experiment was conducted under microcosm conditions using 500 ml biometers. An amount of 100 g of soil was deposited on a non-sterilized dry base in each biometer and treated with a Roundup solution equivalent to 144 mg of glyphosate isopropylamine salt/kg of soil, plus one ¹⁴C-radiomarked solution (glyphosate isopropylamine salt, SIGMA; specific activity: 2.2 mCi/mmol to obtain an activity of approximately 2.2 x 10⁶ DPM (1 μCi) per 100 g of soil by biometer. The ¹⁴C-glyphosate quantity was equal to 0.0072 mg/kg of soil, i.e., a negligible amount. The herbicide mixture (commercial “cold” and ¹⁴C glyphosate “warm”) was homogenized with the soil by waving the treated soil in hermetically sealed plastic bags. This herbicide concentration was calculated based on a field dose of 3 l/ha of Roundup, and considering an extreme situation of the soil in which all the product fallen to the soil and remained in the first 2 mm soil layer (Roberts, et al., 2003). Repetitions were done in four biometers for each one of the soil samples for a total of 12 biometers.

Mineralization was measured taking the resulting ¹⁴CO₂ quantity produced by the mineralization of the radiomarked glyphosate, which was collected in a NaOH 0.3 N solution disposed on the lateral arm of the biometer. Biometers were disposed in an incubator (WTB Binder, BD 115 model), in the dark, and at 28 ± 2°C. Evaluations of the ¹⁴CO₂ were done

at days 1, 3, 6, 10, 15, 22, 36, 50, 64, 78, 92 and 106 after the beginning of the test. In each observation, the NaOH solution was changed and the ¹⁴CO₂ was determined in aliquots of 1,00 ml of the solution, later disposed in 20 ml glass vials, and then 10 ml of scintillation liquid for watery solutions was added (Ultra Gold DXR Packard BioScience), to be read in a scintillation counter (Beckman LS 5600). In the final stage of the experiment, a soil sample of 1 g was deposited in an oxide burner to determine the amount of ¹⁴C-glyphosate that stayed bound to the soil for balancing mass. Later on, we used the resulting data to produce ¹⁴C percentage curves opposing initial activity percentage to time.

¹⁴C-glyphosate adsorption – desorption in the samples from the three soil types

For this experiment we prepared six solutions (C1 to C6) of glyphosate isopropylamine salt (Roundup®) mixed with a radiomarked solution of ¹⁴C-glyphosate (glyphosate isopropylamine salt, SIGMA; specific activity: 2.2 mCi/mmol in CaCl₂ 0,01M for a final concentration of isopropylamine salt between 0.0144 and 0.0864 mg/kg of soil, and an activity of approximately 5x10⁴ DPM per 2 g of soil (Table 1). The initial activity of each of these solutions was measured by liquid scintillation using scintillation liquid for watery solutions (Ultra Gold DXR Packard BioScience) in the scintillation counter (Beckman LS 5600).

The experiment was conducted using 25 ml glass centrifuge tubes, which were salinized with dichlorodimethylsilane 5% in toluene to prevent the adsorption of pesticides on the tube walls (Harris, 1992; Pérez, et al., 2005); 2 g of dry soil were weighed in the air and 5 ml were added to the

Table 1. Mineralogical analysis of clays in the samples from the three soil types under study

Minerals	Grassland soil	Rice-crop soil	Forest soil
Constitution	Percentage		
Interstratified smectite (montmorillonite)	+ 6	+ 10	+ 7
Mica	----	----	----
Amphibol	+ 8	++17	++ 17
Caolinite	++ 24	++ 25	++ 20
Quartz	----	tr	----
Cristobalite	++ 22	++ 21	+++ 42
Feldspars	++ 16	----	+ 10
Chlorite	+ 6	+ 5	----
	+ 7	+ 7	tr
	+ 8	+ 13	tr

++++: Dominant (>50%); +++: Abundant (30-50%); ++: Common (15-30%); +: Present (5-15%); tr: Trace (<5%) ?; Doubtful
 Analysis by the Chemical Laboratory of Soils, Agustin Codazzi Geographical Institute, Bogotá, D.C., Colombia

respective solution with four repetitions by dilution per soil type. They were then put in an Eberbach horizontal agitator for 24 hours at 16 rpm, and the supernatant was isolated by centrifuging (MLW T 25 centrifuge) at 10.000 rpm for 15 min. The aliquot activity of 1.00 ml supernatant was then determined by means of liquid scintillation in a scintillation counter (Beckman LS5600).

Desorption was determined by carefully removing the remnant solution with a micropipette. Then, 5.00 ml of the CaCl_2 0,01M solution were added to each tube and were taken to an Eberbach horizontal agitator for 24 hours, after which they were centrifuged for 15 minutes and the ^{14}C activity present in the solution was determined, considering it as desorbed by the soil. A correction was required due to the small amount of the solution (1 ml, approximately), which was impossible to isolate from the soil because the tubes were weighed before and after adding the CaCl_2 0,01M solution (Valencia, 2005).

Glyphosate concentration in the solution at adsorption equilibrium point was determined by means of the following equation:

$$C_{ea} (\mu\text{g/ml}) = \frac{Aed (DPM) \times C_i (\mu\text{g/ml})}{Ai (DPM)}$$

where C_{ea} ($\mu\text{g/ml}$) is the glyphosate concentration in the solution at the desorption equilibrium point; Aed (DPM) is the solution activity at the desorption equilibrium point; C_i ($\mu\text{g/ml}$) is the total concentration in the initial dilution of glyphosate (cold + marked), and Ai (DPM) is the activity of the initial dilution of glyphosate.

The amount of glyphosate adsorbed by gram of soil was calculated as the difference between the initial quantity and the quantity at the adsorption equilibrium point by means of the following equation:

$$\mu\text{g glyphosate/g soil} = \frac{\text{Initial dilution quantity} - \text{Quantity in equilibrium}}{\text{Sample weight}}$$

$$\mu\text{g glyphosate/g soil} = \frac{C_n - \text{Concentration in equilibrium} \times \text{volume}}{\text{Sample weight (g)}}$$

Glyphosate concentration in the solution at the desorption equilibrium point was determined by means of the following equation:

$$C_{ed} (\mu\text{g/ml}) = \frac{Aed (DPM) \times C_i (\mu\text{g/ml})}{Ai (DPM)}$$

where C_{ed} ($\mu\text{g/ml}$) is the glyphosate concentration in the solution at the desorption equilibrium point; Aed (DPM)

is the solution activity at the desorption equilibrium point; C_i ($\mu\text{g/ml}$) is the total concentration in the initial dilution of glyphosate (cold + marked), and Ai (DPM) is the initial dilution activity of glyphosate.

The concentration of the remnant solution from the desorption experiment was calculated by means of the following equation:

$$C_r (\mu\text{g/ml}) = \frac{\text{Remnant volume (ml)} \times C_{ea} (\mu\text{g/ml})}{\text{Total volume (ml)}}$$

where C_r ($\mu\text{g/ml}$) is the glyphosate concentration in the remnant solution.

The glyphosate desorbed by gram of soil was calculated with the following equation:

$$\frac{\mu\text{g glyphosate}}{\text{g soil}} = \frac{C_{ed} (\mu\text{g/ml}) - C_r (\mu\text{g/ml})}{\text{Dry soil weight (g)}} \times \frac{\text{Total volume (ml)}}{\text{volume (ml)}}$$

Bound and extractable glyphosate were determined by means of the equation:

$$C_{(l+e)} (\mu\text{g/g}) = (\text{adsorbed glyphosate-desorbed glyphosate}) (\mu\text{g/g}),$$

where $C_{(l+e)}$ ($\mu\text{g/g}$) is the bound glyphosate+extractable by gram of soil.

After the soil was subjected to multiple extractions with organic solvent, it was possible to obtain the bound glyphosate, i.e., that one which is bound to the soil probably through strong chemical bindings. In this study, the bound and extractable wastes were determined jointly as the difference between the adsorbed and desorbed pesticide, and this fraction was called bound fraction.

Mass was balanced in order to establish the percentage of the substance that can be recovered after an adsorption-desorption experiment from the quantity of nominal substance present at the beginning of the process. Possible loss of pesticide was determined through the following equation:

$$B.M. (\%) = \frac{C_{ed}^* (\mu\text{g}) + (C_{l+e}^*) (\mu\text{g}) + C_{ed}^* (\mu\text{g}) + C_r^* (\mu\text{g})}{C_i^* (\mu\text{g})} \times 100,$$

where C_{ed}^* (μg) is the glyphosate quantity in the solution at the adsorption equilibrium point; (C_{l+e}^*) (μg) is the glyphosate quantity in the solution at the desorption equilibrium point; C_{ed}^* (μg) is the glyphosate quantity in the remnant solution; C_r^* (μg) is the bound and extractable glyphosate quantity, and C_i^* (μg) is the total quantity in the initial dilution of glyphosate (cold + marked).

The results of the adsorption, desorption, bound wastes and mass balance processes using glyphosate are shown in Table 2. The evaluation was made on all three types of soils at six levels of concentration.

Table 2. Preparation of initial diluted solutions of glyphosate for the adsorption-desorption experiment in the samples from the three soil types under study

Roundup® solution C _n	Concentration of ¹⁴ C (µg/ml acid glyphosate)	Roundup® concentration (µg/ml isopropylamine salt)	Total concentration (µg/ml)	Final concentration in the soil (µg)
C ₁	0.01	0.0044	0.0144	0.036
C ₂	0.01	0.0188	0.0288	0.072
C ₃	0.01	0.0332	0.0432	0.108
C ₄	0.01	0.0476	0.0576	0.144
C ₅	0.01	0.062	0.072	0.180
C ₆	0.01	0.0764	0.0864	0.216

C_n: Different total glyphosate concentrations

Table 3. Adsorption, desorption and bound waste of ¹⁴C-glyphosate in the samples from the three soil types under study

Soil	Solution	Concentration Cold +marked (µg/ml)	Activity Initial solution DPM	Adsorbed glyphosate			Desorbed glyphosate			Bound glyphosate (µg/g soil)	Mass balance (%)
				Activity (DPM)	Concentration (µg ml ⁻¹)	(µg/g of soil)	Activity (DPM)	Concentration (µg/ml)	(µg/g of soil)		
Rice crops	C1*	0.02	10,698.72	8,024.04	0.0190	0.0475	153.07	0.000199	0.000497	0.047038	66.2
	C2	0.03	10,718.85	8,039.14	0.0332	0.0816	134.20	0.000356	0.000875	0.080753	64.0
	C3	0.05	10,925.17	8,193.88	0.0475	0.1159	172.46	0.000639	0.001555	0.114347	62.1
	C4	0.06	10,635.04	7,976.28	0.0612	0.1456	158.23	0.000838	0.002007	0.143552	69.5
	C5	0.08	10,713.17	8,034.88	0.0752	0.1860	220.66	0.001411	0.003489	0.182547	61.3
	C6	0.09	10,752.44	8,064.33	0.0893	0.2125	221.88	0.001369	0.003265	0.209201	66.0
Forest	C1	0.02	10,698.72	5,349.36	0.0161	0.0389	899.27	0.000731	0.001769	0.037107	82.3
	C2	0.03	10,718.85	5,359.43	0.0271	0.0675	872.41	0.001389	0.003470	0.064074	75.0
	C3	0.05	10,925.17	5,462.59	0.0377	0.0938	927.34	0.002003	0.004999	0.088819	75.7
	C4	0.06	10,635.04	5,317.52	0.0466	0.1165	1,068.55	0.002915	0.007287	0.109186	79.2
	C5	0.08	10,713.17	5,356.59	0.0564	0.1366	1,106.16	0.003354	0.008155	0.128449	75.7
	C6	0.09	10,752.44	5,376.22	0.0648	0.1620	1,175.17	0.003892	0.009743	0.152290	73.1
Grassland	C1	0.02	10,698.72	2,674.68	0.0190	0.0480	129.93	0.000197	0.000486	0.047531	85.7
	C2	0.03	10,718.85	2,679.71	0.0332	0.0855	145.40	0.000334	0.000880	0.084605	80.4
	C3	0.05	10,925.17	2,731.29	0.0474	0.1229	154.82	0.000466	0.001209	0.121709	76.4
	C4	0.06	10,635.04	2,658.76	0.0609	0.1533	170.68	0.000610	0.001538	0.151761	79.9
	C5	0.08	10,713.17	2,678.29	0.0742	0.1978	197.87	0.000913	0.002433	0.195326	80.0
	C6	0.09	10,752.44	2,688.11	0.0867	0.2105	219.00	0.000928	0.002246	0.208244	77.5

*: Average values of 4 repetitions

Reading volume = 1 ml; extract volume = 5 ml; approximate soil weight = 2.00 g; contact time = 24 h

Using the data obtained, adsorption and desorption curves were drawn (Figures 2 and 3). Data was adjusted to the Langmuir and Freundlich models (United States Environmental Protection Agency - EPA, 1998), and the data thus obtained enabled us to calculate K_d values for each of the soil samples (Table 3). Data analyses were done with the STATGRAPHICS and the Statistical Analysis System for microcomputers (SAS Institute, 1988).

Results

¹⁴C-glyphosate mineralization in soil samples under microcosm conditions

The mineralogical analysis of clays is shown in Table 1. In forest soil, caoilinite was abundant (42%), followed by mica and montmorillonite with 20% and 17%. In rice soil, mica, caoilinite and montmorillonite were also found in a proportion

of 25%, 21% and 17%, respectively, while in grassland soil, the proportion of mica, caolinite and quartz minerals in the total content of clays was 24%, 22% and 16%, respectively. Rice and forest soils had the highest values of monmorillonite content (17%), which explains the adsorption capacity of glyphosate in these soils (Glass, 1987; Chorbadjian & Kogan, 2001). However, the dominant content of caolinite in forest soil samples (Table 1) resulted in low ion retention, and, therefore, also in a low glyphosate retention in this type of soil.

Glyphosate mineralization curves in the three soil types after 106 days allowed us to determine that they exhibited different metabolic activity (Figure 1). Glyphosate degradation during the first 36 days of incubation showed the same tendency in forest and grassland soils (Figure 1); however, after day 36, the mineralization rate in the forest soil was faster, and after 106 days of incubation, around 53% of the compound had been mineralized in this soil and 38% in the grassland soil (Figure 1). Regarding the rice-crop soil type, less than 25% of the herbicide had been transformed at the end of the experiment (day 106), and all through its duration, ¹⁴C-glyphosate mineralization was less than that of the forest and grassland soils (Figure 1).

The average life of glyphosate mineralization in each soil type was estimated by means of linear regression equations which considered the quantity of glyphosate in each soil (measured by means of the activity in DPM from the ¹⁴CO₂ product of the ¹⁴C-glyphosate transformation) in relation to the time (Figure 2). The equations for the three soils are as follows:

Rice-crop	$Y=0.0373X + 0.1051$	$R^2=0.9792$
Forest	$Y=0.0713X + 0.4906$	$R^2=0.9036$
Grassland	$Y=0.0572X - 0.1392$	$R^2=0.9036$,

where Y is glyphosate average life in the soil, and X, the ¹⁴C-CO₂ production.

Based on these equations, glyphosate average life was estimated as 94, 526 and 11,365 days for forest, grassland and rice-crop soils, respectively.

¹⁴C-glyphosate adsorption-desorption in the three soil types

Table 2 shows the results of glyphosate sorption in the soil. The values for bound wastes (bound and extractable) do not give an exact idea of the pesticide quantity that can be irreversibly retained in the soil, but it is a useful value for mass balancing. We found that mass balance average values were 65%, 77% and 80% for rice-crop, forest and grassland soils, respectively. Percentages less than 100% may be explained by a loss of material, especially in those soils with higher organic content, and by the presence

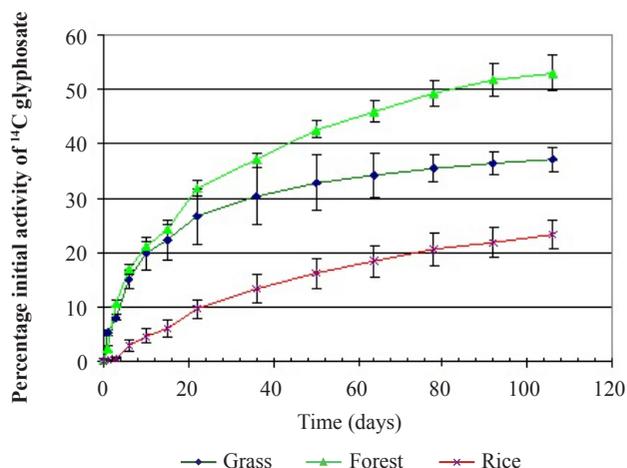


Figure 1. Accumulated ¹⁴C-glyphosate mineralization measured by means of the CO₂ evolution during 106 days under microcosm conditions

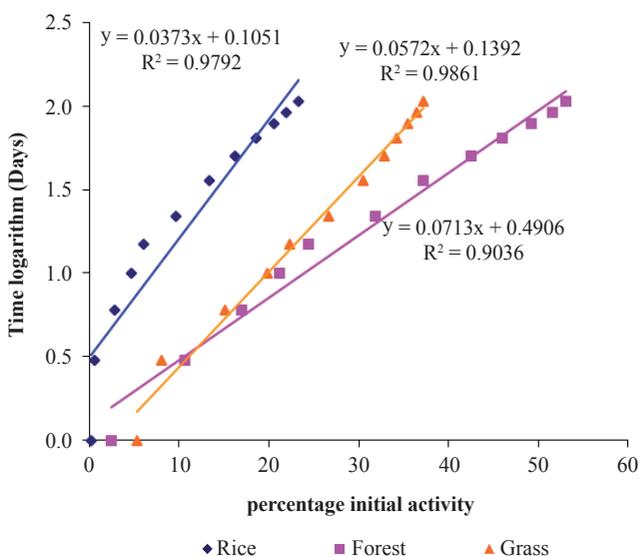


Figure 2. Linear regression equations of the activity in DPM (disintegrations per minute) of the ¹⁴CO₂ with regard to the time line

of roots less than 2 mm in size, which in spite of having been subjected to centrifuging, floated and, therefore, were eliminated in the solution. On the other hand, the existence of a third pseudo-phase (EPA, 1998) was not considered for colloids (size <0.2 μm), and this can have an important impact on the total adsorption mechanism of a substance in a soil suspension.

Adsorption-desorption was adjusted to the Freundlich's equation; Freundlich's lineal model is expressed as (Calvet, et al., 2005):

$$q_{ae} = KdC_e$$

Table 4. Adsorption and desorption of ¹⁴C-glyphosate adjusted to the Freundlich's model in the samples from the three soil types under study

Soils	Curve	r	m	b	Kd (ml g ⁻¹)	Koc (ml g ⁻¹)	1/n	H
Rice crops	Adsorption	0.999	0.70	1.23	17.05	1,311.5	0.70 _(ads)	0.72
	Desorption	0.999	0.97	0.22	1.66		0.97 _(des)	
Forest	Adsorption	0.984	0.47	0.49	3.10	134.8	0.47 _(ads)	0.74
	Desorption	0.984	0.76	-0.52	3.31		0.76 _(des)	
Grassland	Adsorption	0.998	0.66	0.26	1.83	42.6	0.66 _(ads)	0.74
	Desorption	0.997	0.89	-0.21	1.62		0.89 _(des)	

R: Regression coefficient; b: Intercept; m: Slope; Kd: Intercept antilog; Kd_(ads): Adsorption coefficient; Kd_(des): Desorption coefficient; K_{oc}: Normalized adsorption coefficient in relation to the organic carbon content from the adsorbent ($K_{oc} = Kd/f_{oc}$, where $f_{oc} = C_{org}/100$; $C_{org} = C_{org}$: the quantity from the adsorbent is expressed in g/100g); 1/n_(ads): Adsorption equation slope; 1/n_(des): Desorption equation slope; H: Hysteresis coefficient = $1/n_{(ads)}/1/n_{(des)}$ (Calvet, *et al.*, 2005; Autio, *et al.*, 2004; Mamy, *et al.*, 2005; Valencia, 2005)

where q_{ac} is the quantity adsorbed per mass unit from the adsorbent; C_e is the solution concentration at equilibrium point, and K_d is the distribution coefficient (or adsorption coefficient).

Table 4 shows the correlation coefficient of the isotherms, the adsorption and desorption coefficients, the normalized adsorption coefficients with organic carbon content in each soil, as well as the ¹⁴C glyphosate hysteresis coefficients.

Adequate correlation coefficients were obtained with the following values: For rice-crop soil type, 0.999_{ads}; 0.999_{des}; for forest soil, 0.984_{ads}; 0.984_{des}, and for grassland soil, 0.998_{ads}; 0.997_{des}. As can be observed in Table 4, the values of 1/n were not near 1, and, therefore, the Freundlich's adsorption and desorption constants ($K_{F_{ads}}$ and $K_{F_{des}}$) were not equal to the adsorption and desorption constants at the equilibrium point (K_{ads} and K_{des}), so it can be assumed that those constants varied along the isotherms in the rate of concentrations under study. K_{ads} and K_{des} values are not independent of the glyphosate concentration (EPA, 1998).

Glyphosate $K_{d_{ads}}$ in the rice-crop soil type ($K_{d_{ads}} = 17.05$) was greater in magnitude as compared to the values in forest soils ($K_{d_{ads}} = 3.10$) and in grassland soils ($K_{d_{ads}} = 1.83$). Regarding the $K_{d_{des}}$, the compound in forest soils tended to a higher desorption ($K_{d_{des}} = 3.31$) than that in rice soils ($K_{d_{des}} = 1.66$), and grassland soils ($K_{d_{des}} = 1.62$).

These results indicate that the herbicide in rice-crop soils was greatly adsorbed and once adsorbed, it was not released in the soil solution. In grassland soils, adsorption and desorption were equally low, whereas in forest soils there was a balance between adsorption and desorption. In forest soils, a higher glyphosate quantity could be expected in the liquid phase, and in rice-crop soils, in the solid phase. Regarding the K_{oc} (normalized adsorption coefficient based on the organic carbon content in the soil), this value in rice-crop soils, was almost 2 and 3 times higher than the values in grassland and forest soils, respectively. This would indicate that rice-crop

soils have a high affinity with glyphosate due to the organic matter (2.24%), although organic matter contents were higher in forest soils (3.97%) and in grassland soils (7.41%). However, other factors besides the organic matter contents or the interaction with organic matter could be controlling herbicide sorption in these soils.

Normal adsorption curves are shown in Figure 3 and desorption curves in Figure 4. Table 4 shows how rice-crop soil samples had the highest adsorption value with 17.05 ml/g, while grassland soil had the lowest, with a value of 1.83 ml/g, and forest soils exhibited the highest desorption values, with 3.31 ml/g.

Based on the glyphosate quantification obtained in the adsorption, desorption and binding phases, the recovery percentage itself was calculated by assigning an average recovery of 65, 77 and 80% to rice-crop, forest and grassland soils, respectively (Figure 5). Percentages below 100% may be explained by a loss of organic material on the surface solution, which was eliminated after centrifuging. On the other hand, the presence of colloids with size <0,2 μm in suspension were not considered, but they can have an important impact on glyphosate desorption, thus affecting recovery values.

Discussion

In laboratory conditions and by means of isotopic techniques, we were able to determine a relatively low glyphosate mineralization rate, which related directly to soil type. Mineralization rate in rice-crop soil samples with a long history of pesticide use, among them glyphosate, was the lowest, whereas the degradation rate in forest soils with no use of pesticides was the highest (Figure 1). After 106 days, the maximum value of accumulated mineralization for forest soil samples was 53% in average, 38% for grassland soil, and only 23% of the herbicide parental molecule was degraded in the rice-crop soil samples (Figure 1). Other authors have determined higher mineralization rates; Rueppel, *et al.*,

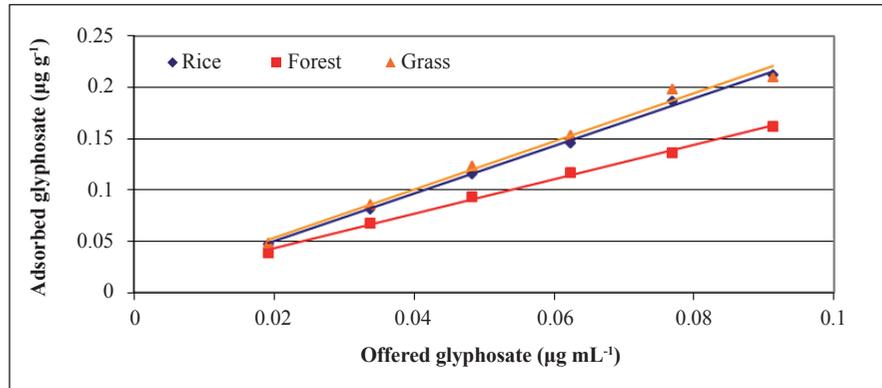


Figure 3. Natural curves of adsorption (3) for the rice-crop soil samples under evaluation (El Espinal, Colombia)

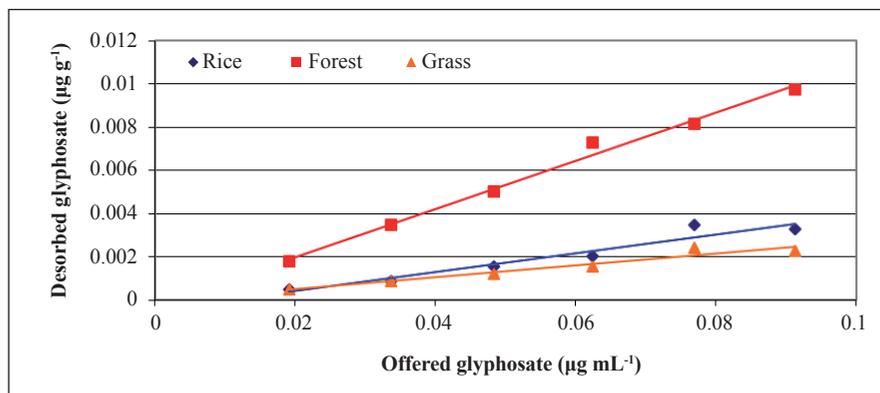


Figure 4. Natural curves of desorption (4) for the rice-crop soil samples under evaluation (El Espinal, Colombia)

(1977) and Moshier & Penner (1978) found that 28 days were required to degrade 45% to 48% of the original glyphosate in the soil.

Forest soil samples showed the strongest metabolic activity as compared to the other soils. This activity can be partially determined by the type of organisms present in the soil, which probably have adapted to “adverse” natural environments (land inclination, scarce humidity retention, forest vegetation cover with great species diversity, etc.). Many studies have demonstrated that glyphosate degradation in soil occurs mainly because of microbial activity. For example, **Vinther & Brinch’s** research work (2002) in Denmark indicated that the spatial variation of glyphosate mineralization follows a similar pattern to that of soil microbial activity. **Haney, et al.**, (2002) reported that the RoundupUltra® glyphosate formulation applied in soils from Georgia and Texas (USA) was quickly mineralized by the local microbial biota regardless of the soil type, content of clays, and CO or pH levels. **Moshier & Penner** (1978) have indicated that glyphosate is degraded by bacteria which vary in numbers and species depending on the soil, but many bacteria do

not degrade the glyphosate, and its complete degradation depend on the bacterial species present in a site. Due to the slow glyphosate degradation, microbiologists “think” that its biotransformation occurs by co-metabolism, which means that glyphosate would not be a source for bacteria growth and, therefore, it could accumulate in the soil even in the presence of bacteria with the capacity to degrade it (**Torstenson**, 1985).

The microbial decomposition of most organic herbicides follow a typical bacterial growth curve and all conditions favoring soil microbial biota growth (high temperature and humidity, high oxygen and organic matter content, high pH and high fertility), also favor herbicides decomposition in the soil. On the other hand, many studies indicate that soil properties and characteristics (in this case, the adsorbent) determine glyphosate adsorption rates in soil and, therefore, its availability in the soil solution to become a “target” for microorganisms.

Among those factors that have been proved to have an effect of increasing glyphosate adsorption degree in soil we can mention the following: high iron (Fe) contents (**Gerritse**,

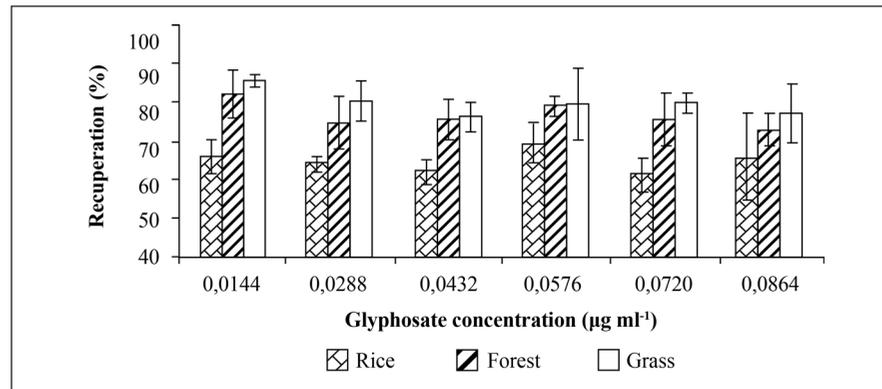


Figure 5. ^{14}C -glyphosate recovery percentage in the sorption process on rice-crop soil samples (El Espinal, Colombia)

et al., 1996), high aluminium (Al) contents (Sánchez, *et al.*, 1999), low phosphorus (P) contents (Sprankle, *et al.*, 1975a,b; Hance, 1976; Nomura & Hilton, 1977; Rueppel, *et al.*, 1977; Glass, 1987), high magnesium (Mg) contents (Sánchez, *et al.*, 1999), high content of clays (Sprankle, *et al.*, 1975a,b; Hance, 1976; Nomura & Hilton, 1977; Rueppel, *et al.*, 1977; Glass, 1987; Vinther & Brinch, 2002), as well as the type of clays, mainly montmorillonite (Calvet, *et al.*, 2005), and a high CO content (Gerritse, *et al.*, 1996; Vinther & Brinch, 2002; Haney, *et al.*, 2002). Other authors, however, have pointed out the opposite: high CO content reduces glyphosate adsorption (Gerritse, *et al.*, 1996) or it is not related with adsorption, but, instead, it depends on the montmorillonite quantity present in the soil (Calvet, *et al.*, 2005), on a high cation exchange capacity (CEC) (Sprankle, *et al.*, 1975a,b; Hance, 1976; Nomura & Hilton, 1977; Rueppel, *et al.*, 1977; Glass, 1987) and on low pH values (Sprankle, *et al.*, 1975a,b; Hance, 1976; Nomura & Hilton, 1977; Rueppel, *et al.*, 1977; Glass, 1987; Sánchez, *et al.*, 1999). Sánchez, *et al.*, (1999) have pointed out on their part that pH is the main factor in glyphosate adsorption. In brief, glyphosate adsorption depends on many soil characteristics, as well as on their interaction, and not only on the organic matter and clay contents as is generally accepted.

The K_d values obtained in this work were low (17.05; 3.10; 1.83 ml/g) compared to those registered in other studies. Sánchez, *et al.*, (1999) determined very variable K_d values for glyphosate in hydrotalcite (HT) and calcined hydrotalcite (HT500) that depended on the adsorption system pH; these values varied from 36.82 to 6,252 for HT and between 17,801 and 26,558 for HT500. Strabge-Hansen, *et al.*, (2004) reported glyphosate adsorption coefficients (K_d) between 62 and 164 l/kg in gravel and 410 l/kg in a sandy substrata soil from Denmark. Also in Denmark, Vinther & Brinch (2002) registered very variable K_d values in 51 samples from

a 120 m x 120 m sandy plot of soil, ranging from 93.9 to 457.2. These results indicate that even inside a small area, glyphosate adsorption to the soil varies a lot and depends on the spatial variation of the soil.

In our study, glyphosate mineralization in rice-crop soil samples was the lowest (23% after 106 days) in comparison with the other two soils. Rice-crop soil samples had a low CIC (6.2 meq/100g), acid pH (5.7), and a low CO content (1.3), but a high K_{oc} (1311.8), indicating a high affinity of the organic matter for the herbicide in this soil (Calvet, *et al.*, 2005), as well as the highest $K_{d_{ads}}$ value (17.05), a P content of 43.3 ppm and an intermediate Fe content of 51.4 ppm, all of which explains the high herbicide adsorption in rice-crop soil samples. $K_{d_{ads}}$ low values allow a relatively high glyphosate mineralization rate in the soil; when the $K_{d_{ads}}$ is high, on the contrary, the mineralization is low (Strabge-Hansen, *et al.*, 2004). Additionally, a low $K_{d_{des}}$ value was registered in rice-crop soil samples (1.66). All this means that high $K_{d_{ads}}$ and K_{oc} , besides low $K_{d_{des}}$ in rice-crop soil samples limited herbicide availability in the soil solution, fundamental condition for its mineralization through microbial activity, determining, therefore, a longer persistence in the soil.

With regard to grassland and forest soils, pH with a basic tendency (7.7), low Fe content and $K_{ads/des}$ values equivalent or near 3.0 in forest soil samples possibly explain a greater glyphosate availability in the soil liquid phase, and, therefore, its high mineralization rate in comparison with the others two soils. In grassland soil samples, other factors such as the high P content (90.8 ppm), the absence of montmorillonite in spite of the total content of clays, which was the highest (30.5%), the lowest K_{oc} value of the three soils (42.47), and the highest total organic matter content (7.41%) jointly explain the intermediate herbicide availability in the soil solution and, in consequence, the intermediate mineralization value. Carlisle & Trevors (1988) have indicated that glyphosate competes

for absorption sites with soil phosphates, which means that soils with low phosphorus contents tend to have higher adsorption rates of the herbicide.

Studies on glyphosate interaction with soil constituents indicate that the herbicide is quickly inactivated due to its adsorption to the soil, and that this inactivation or dissipation is not due to the chemical and biological degradation (Sprankle, *et al.*, 1975a). In these studies it was concluded that glyphosate adsorption increases as clays contents increase in the soil (Glass, 1987; Hensley, *et al.*, 1978; McConnell & Hossner, 1985; Miles & Moye, 1988). However, it seems that the type of clay is more important than the total content of clays for determining the adsorption of an ionic herbicide such as glyphosate (Sprankle, *et al.*, 1975a; McConnell & Hossner, 1985; Glass, 1987; Miles & Moye, 1988; Chorbadjian & Bogan, 2001), which would be the case in our study, as forest soil samples showed the lowest levels of adsorption, even though they contained more total clays than the rice-crop soil samples. However, forest soil had a caolinitic clays content of 42% of the total content of clays, which partly explains the low retention capacity of this soil.

In general, the estimated average life values in laboratory conditions, of 94, 526 and 11.365 days for forest, grassland and rice-crop soil samples, respectively, were higher than those reported in the literature, which vary from 4 (Schuette, 1998; European Commission-Health and Consumer Protection Directorate-General, 2002), to 40 and 47 days (Tu, *et al.*, 2001; European Commission-Health and Consumer Protection Directorate-General, 2002), 180 days (European Commission-Health and Consumer Protection Directorate-General, 2002) and up to extreme values of 1.699 days (European Commission-Health and Consumer Protection Directorate-General, 2002) and 8.322 days in sugar cane soils from Hawaii (Nomura & Hilton, 1977). On the other hand, average dissipation life values in the field were between 5 and 106 days (European Commission-Health and Consumer Protection Directorate-General, 2002).

The results of this work allow us to infer that glyphosate can constitute an environmental pollution problem due to its high persistence in the soil, as shown by the high average life values obtained in general, and in rice-crop soil samples specifically. In real field conditions, it would not be surprising to find glyphosate wastes due to its accumulation after continued and prolonged use, accumulation which does not occur from one day to the other, but after 20 or 30 years (Heimen, 2002). In the case of soils used for rice crops in Colombia, where glyphosate has been intensively applied in the last decade to control persistent undergrowth in pre-sowing, attention must be given to the probable accumulation of glyphosate wastes in the soil due to the estimated average life of 31 years.

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