

Levels of glyphosate in surface waters, sediments and soils associated with direct sowing soybean cultivation in north pampasic region of Argentina

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Glyphosate concentrations in the environment from a region where little information exists about this and intensive cultivation activities predominate in large areas.

Abstract

Levels of glyphosate were determined in water, soil and sediment samples from a transgenic soybean cultivation area located near to tributaries streams of the Pergamino–Arrecifes system in the north of the Province of Buenos Aires, Argentina. Field work took into account both the pesticide application and the rains occurring after applications. The pesticide was analysed by HPLC-UV detection, previous derivatization with 9-fluorenylmethylchloroformate (FMOC-Cl). In addition, SoilFug multimedia model was used to analyse the environmental distribution of the pesticides. In the field, levels of glyphosate in waters ranged from 0.10 to 0.70 mg/L, while in sediments and soils values were between 0.5 and 5.0 mg/Kg. Temporal variation of glyphosate levels depended directly on the time of application and the rain events. The results obtained from the application of the model are in accordance with the values found in the field.

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1. Introduction

Soybean production in Argentina has increased over the last decade, currently with 10 000 000 hectares of sowed area. A total of 95% of this area corresponds to a transgenic variety of glyphosate tolerant soybean, which is cultivated by direct sowing (Pengue, 2005).

Glyphosate (*N*-(phosphonomethyl)glycine) is a broad-spectrum herbicide used to control a wide range of pests and is the active principle in Roundup[®], a product widely applied in the regional agriculture practice. Glyphosate itself is an acid, but it is commonly used in salt form, most commonly the isopropylamine salt. It is a polar, highly water soluble substance that makes complexes easily. It binds tightly to the soil particles, reaching a persistence of up to 170 days and an usual half life of 45–60 days (EXTOXNET, 1996; Vereecken, 2005). Its wide use has led to controversy regarding its possible effect on the environment. However, no monitoring studies assessing the possible effects of the pesticide on the ecosystem have been recorded in the region. For this reason, is very

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important to count with methods to determine glyphosate concentration, to carry out the monitoring of this herbicide in the exposed areas. Owing to its physicochemical properties, it is not easy to establish simple methodologies to extract and determine residues of the herbicide. GC or HPLC techniques require the analyte derivatization, either to favour its volatilisation or to improve the UV or fluorescence detection (Sancho et al., 1996; Stalikas and Konidari, 2001; Veiga et al., 2001).

Within the frame of a general study of the pesticide impact assessment associated with the direct sowing, were started studies to develop suitable analysis techniques for the determination of glyphosate residues in environmental samples, and to optimize the application of contaminants distribution models to predict the levels of this herbicide in the same samples for an specific described situation (Camilión et al., 2003; Martín et al., 2003; Marino and Ronco, 2005; Peruzzo et al., 2003).

This paper present a study located in an area belonging to Pergamino–Arrecifes system, Province of Buenos Aires, where the aquatic and terrestrial systems are submitted to a direct influence by the crop. The procedure includes optimization of the methodology for the analysis of glyphosate by HPLC-UV, previous derivatization with FMOC-Cl. Glyphosate contents were determined in water, sediment and soil samples. In addition, with specific information of the studied area, the physicochemical properties of glyphosate, the concentration of the applied pesticide and the rain events, expected concentrations in the environment were estimated with the multimedia model “SoilFug”.

2. Materials and methods

2.1. Area of study

Located in the north of the Province of Buenos Aires, this area presents the highest agricultural production in Argentina. The region is known as the “Pampa Ondulada Bonaerense” and is characterized by undulations cut by creeks, gullies, and rivers that originate well drained soils, rich in organic matter (Fidalgo et al., 1975; Camilión et al., 2003). Recent studies indicate a 3% organic matter in surface with a pH 5.5 value. This soil is highly susceptible to

hydic erosion and would provide input material to the watercourse. The material accumulates in drag bottoms or is carried directly to the streams. Sediment has higher content of clay than surface soil. Downstream the water-column lost a significant portion of the colloids. In fact, suspended matter is around 29.4 mg/L and downstream it decreases to 20.2 mg/L, and the content of organic carbon in this fractions were between 1.6 mgC/L and 0.6 mgC/L, respectively. A change in the tenor of clay is also observed. These clay particles, which are transported in suspension towards the wetland zone, have greater ability to retain toxic (Camilión et al., 2003; Carriquiriborde et al., 2007).

Sampling sites were selected according to two detail scales:

- A first-order stream of the Pergamino–Arrecifes system. After flowing through the cultivation area, the water stream forms a wetland. Sampling sites are located as follows: Site 1 (S1), adjacent to the soybean cultivation area; Site 2 (S2), approximately to 150 m downstream from the limit of the cultivation area; and Site 5 (S5), 1.5 Km downstream, at wetland (Fig. 1B).
- Four surface streams: “Arroyo del Medio” and “Arroyo Ramallo”, “Río Arrecifes” and “Río Areco”.

Fig. 1 shows the location of the sampling sites in a map of the studied area.

2.2. Sowing event, applications and precipitations

Fig. 2 shows the sowing event and the herbicide applications as well as the precipitations occurring during the studied period. Sowing of soybean (S) was done on 13/12/03; a dose of 1.5 kg/ha glyphosate were applied on 07/01/04 (A1) and a dose of 1.0 kg/ha glyphosate on 21/02/04 (A2). Soybean sowing and herbicide application dates were decided by the field manager, based on the weather conditions. It is important to note that the methodology of application involved sprinkling of the formulation using ground equipment. Precipitation data recorded during the period was provided by INTA (National Institute of Farming Technology)-Estación Experimental Agropecuaria Pergamino (Pergamino Experimental Farming Station).

2.3. Sampling

For the development of the sampling in the first-order stream, the glyphosate applications and the rain events were considered. The first sampling was realized before sowing in order to determine the base level of glyphosate in the field before the agriculture activities (samples SB). Additional sampling were realized after each herbicide application (samples SA) and after the first significant rain event (samples SR) after each glyphosate application. Creeks were periodically surveyed during the study period (samples C), without

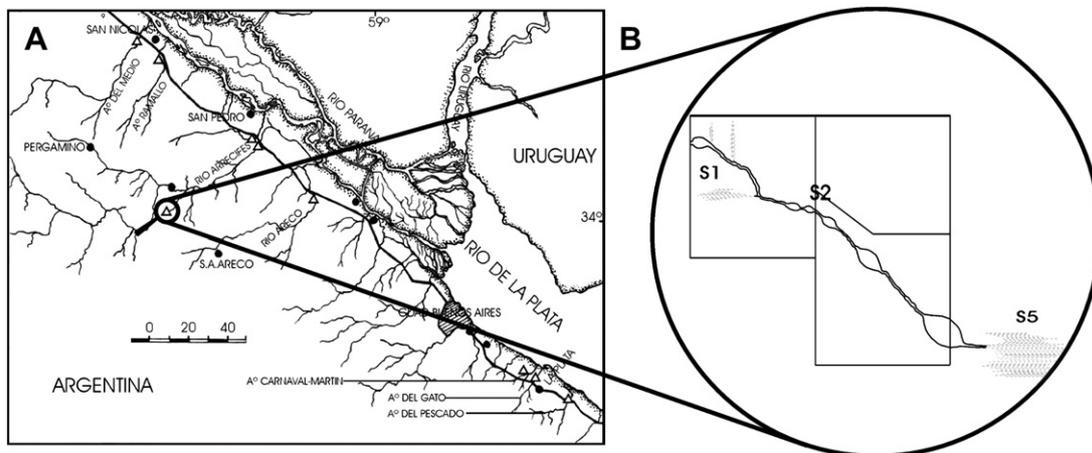


Fig. 1. A) Map of the region: studied area and sampling sites (Δ); and B) diagram of a first-order stream and sampling sites.

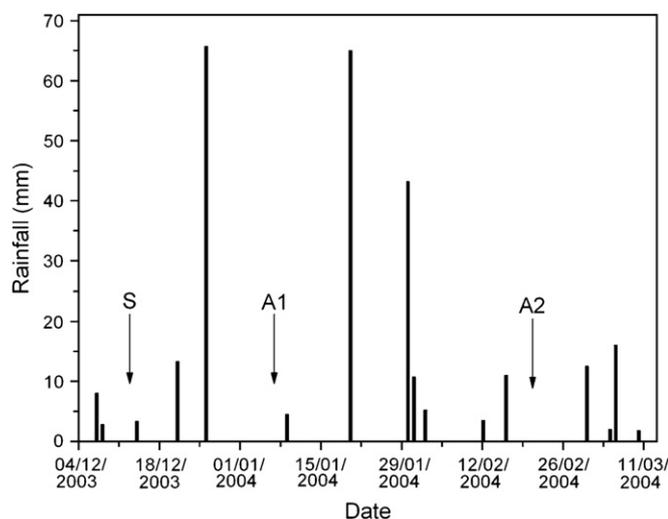


Fig. 2. Precipitations, sowing event (S) and first and second herbicide applications in the field (A1 and A2, respectively).

taking into account agricultural activities or rain events. Table 1 shows chronology and sampling details of above mentioned.

Sediments, water and soil samples were taken from each sampling site according to standardized methodologies. (EPA, 2007) Samples were stored in polypropylene containers at -20°C until analysed.

2.4. Chemicals

A glyphosate standard of high purity was obtained from SENASA (National service of Health and Agroalimentary Quality, Ministry of Production, Argentina). Chloroform (CHCl_3 , analytical grade, J.T. Baker), acetonitrile (CH_3CN , pesticide quality, J.T. Baker), potassium phosphate monobasic (KH_2PO_4 , analytical grade, Merck), phosphoric acid (H_3PO_4 , analytical grade, Merck) and FMOC-Cl (analytical grade, Riedel de-Haen) were used. Deionised milliQ water with a resistivity of 18.3 M was applied.

2.5. Glyphosate analysis

Determination of this herbicide in water was done on samples filtered through $0.45\ \mu\text{m}$ membrane (cellulose acetate). In soils and sediments, the herbicide was determined after extraction of 15 g of sample with KH_2PO_4 0.1 M; agitation (15 min); centrifugation (3500 rpm; 10 min) and filtration through

Whatman No. 1 filter paper. The extraction was repeated twice on solid residue, obtaining a 25 ml extract from each sample. Extracts were filtered through $0.45\ \mu\text{m}$ membrane (cellulose acetate). The derivatization was carried out as follows: 0.25 ml of borate buffer 5% and 0.30 ml of FMOC-Cl 2 mM in CHCl_3 were added to 1 ml of sample (water sample or extract of solid sample). After 24 h of reaction at 40°C in dark, reaction was stopped, added 0.30 ml of H_3PO_4 (2%) and kept in fridge until analysed. The derivatized product (Gly-FMOC) was analyzed by HPLC (Beckman, System Gold 126, detector 166 UV). Chromatographic conditions: Supelco RP 18 column ($5\ \mu\text{m}$ particle size, length \times I.D.: 25 cm \times 10 mm); mobil phase 0.05 M phosphate buffer (pH 5.5): acetonitrile (65:35); flow: 0.8 ml/min; UV detection: 206 nm. The method was adapted and optimised from literature. Recovery of the complete analytical technique was assessed by laboratory fortified sample method (Sancho et al., 1996; APHA, 1998; Stalikas and Konidari 2001; Veiga et al., 2001).

2.6. Analysis of the behaviour of pesticides following the ‘‘SoilFug’’ multimedia model

Estimation of the distribution of the applied pesticides in the system in study was done by the ‘‘SoilFug’’ multimedia model (Canadian Environmental Modelling Centre, 1996). The use of the SoilFug model is suggested for the calculation of predicted environmental concentrations in water, since it generally produces acceptable results from a relatively small set of input data, most of which is generally available (Di Guardo et al, 1994a,b). This method assesses the degradation, evaporation, leaching and runoff of a pesticide applied to a surface soil and consequently its potential impact on nearby water bodies considering the properties of the system in study regarding soil, pesticide and characteristics of the application events (number of events, time of application, dose and rains). The model was loaded with adjusted parameters from runoff and leaching tests at laboratory scale conducted during the development of the research project (see Table 2) (Peruzzo et al., 2003). The scaling applied to the real situation and the rain and application events recorded specially for this case were considered for the application of this model to the field situation.

3. Results and discussion

The method optimised for the detection of glyphosate in environmental samples shows the following reference parameters: LOQ = 0.10 mg/L (level of quantitation) and LOD = 0.04 mg/L (level of detection) for water samples, and LOQ = 0.25 mg/Kg and LOD = 0.10 mg/Kg for sediment and soil samples. Recoveries of 86% were obtained for glyphosate in water by the laboratory fortified sample method (APHA, 1998). This method has the additional advantage of being a simple procedure which uses analytical equipment normally available in standard chemical analysis laboratories.

Table 1
Identification of samples taken from the study area

Sampling	Date	Description
SB	03/09/03	Sites of the Pergamino–Arrecifes system, before sowing (soil, sediment, water)
C1	12/11/03	First sampling of creeks (water, sediment)
SA1	07/01/04	Sites of the Pergamino–Arrecifes system, after the first application (soil, sediment, water)
C2	07/01/04	Second sampling of creeks (water, sediment)
SR1	23/01/04	Sites of the Pergamino–Arrecifes system, after the first significant rain event, after the first application (soil, on sediment, water)
SA2	22/02/04	Sites of the Pergamino–Arrecifes system, after the second application (soil, sediment, water)
SR2	02/03/04	Sites of the Pergamino–Arrecifes system, after the second significant rain event, after the second application (soil, sediment, water)
C3	05/03/04	Third sampling of creeks (water, sediment)

Table 2
Parameters used in the SoilFug model

Glyphosate properties		Land properties	
Molecular weight (g/mol)	169.08	Basin area (Ha)	118
Water solubility (g/m^3)	12000	Temperature ($^{\circ}\text{C}$)	25
Vapor pressure (Pa)	0.00001	Soil depth (m)	0.3
Log K_{ow}	-2.8	Diffusive depth (m)	0.25
Half-life (d)	47	Volume fraction of air	0.2
Mineral water partition coefficient (L/Kg)	3.25E^{-6}	Volume fraction of water	0.3
		Mass fraction of organic carbon	0.016

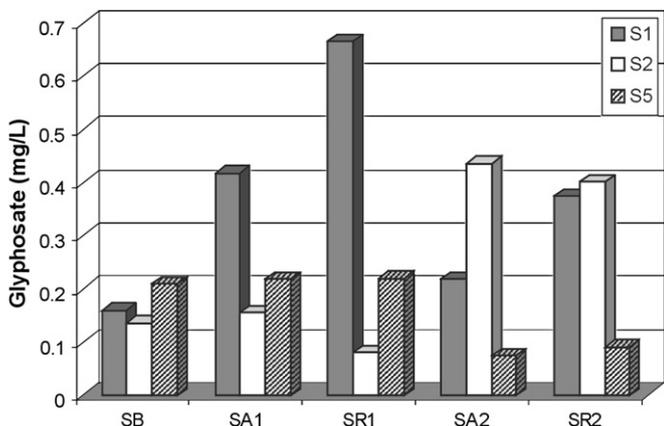


Fig. 3. Glyphosate concentration in water samples of sites near to the cultivation area (S1, S2 y S3) before the agriculture activities (SB), after the herbicide application (SA) and after the first significant rain event (SR).

Results of water, sediment and soil samples obtained from the sampling cruises are provided in Figs. 3, 4 and 5, respectively.

Glyphosate concentrations in waters ranged from 0.10 to 0.70 mg/L. A significant increase in the herbicide concentration was observed in correspondence with the rain events. These results revealed the drift of glyphosate due to precipitations and their related leaching in accordance with its high solubility in water and its associativity to the soil particles which are drifted by runoff. The increase in concentration in the body water is not so pronounced in the rain event after the second application, probably due to its lower dosage and to the lower intensity of the second rainfall (12 mm) in comparison with the first one (65 mm). In this case, rain events play a notable role, transporting the glyphosate present in the soil towards the water stream through the mechanisms of dilution or drift of the surface material by runoff.

Regarding sediment samples, an increase in the glyphosate concentration is observed in the sites near cultivation areas (S1 and S2) in both applications, providing evidence of the direct

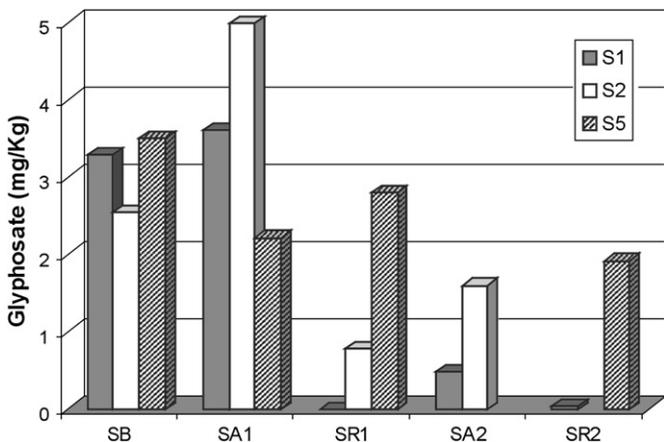


Fig. 4. Glyphosate concentration in sediment samples of sites near to the cultivation area (S1, S2 y S3) before the agriculture activities (SB), after the herbicide application (SA) and after the first significant rain event (SR).

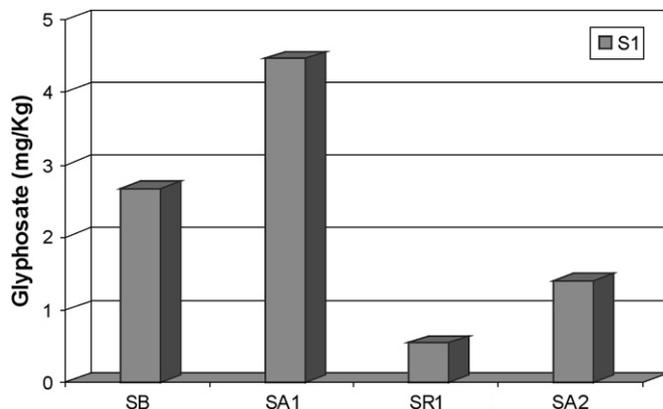


Fig. 5. Glyphosate concentration in soil samples of sites near to the cultivation area (S1, S2 y S3) before the agriculture activities (SB), after the herbicide application (SA) and after the first significant rain event (SR).

influence of these applications and maintaining proportionality with the dosage used in each case. With precipitations, a decrease in the concentration is observed in these sites, in correspondence with the situation of a low-flow water stream, which increases notably due to the precipitation effect drifting (washing) the upper portions of the sediment layer.

The wetland (S5) presents a lower fluctuation, possibly due to the accumulation of suspended solids containing the herbicide which are drifted from upstream sites by the stream water and retained in this place. This behaviour could be explained by considering the high affinity of glyphosate with soil and sediment, either in the soil organic matter fraction or in its mineral fraction (clay and/or amorphous iron and aluminium oxides), and consequently with the suspended solids (EXTOX-NET, 1996; Sancho et al., 1996; Yu and Zhou, 2005)

In soils, a higher concentration is observed in the samples corresponding to the applications, being concentration more significant in the first application than in the second one, probably due to the difference in the applied doses in each case and to herbicide interception by the soybean, which provides a greater foliar cover in the second application, and is unevenly distributed (Newton et al., 1994; Veiga et al., 2001; Landry et al., 2005). As expected, a decline in herbicide concentration is observed after rain events.

It must be pointed out that a high basal level of glyphosate was observed in the samples conducted before soybean seed sowing, this could be related to the herbicide applications performed before the direct sowing, which are done to wash and prepare the field in this cultivation method.

Table 3
Glyphosate concentration in water and sediment samples of the surface streams

Sampling	Areco		Ramallo		Arrecifes		Medio
	Water (mg/L)	Sed (mg/Kg)	Water (mg/L)	Sed (mg/Kg)	Water (mg/L)	Sed (mg/Kg)	Water (mg/L)
C1	0.26	0.16	1.15	1.15	0.28	1.38	0.37
C2	0.23	0.24	1.85	LD	LD	LD	0.14
C3	0.33	0.56	LD	LD	0.10	1.38	0.10

Table 4
Values estimated for S1 by the SoilFug model

Sampling	Water (mg/L)		Soil (mg/kg)	
	Estimated	Measured	Estimated	Measured
SB	1.27	0.418	0.33	4.45
SR1	0.60	0.66	0.22	0.53
SR2	0.87	0.37	0.24	–

Results of the analysis of the water and sediment samples of the four surface streams are given in Table 3. The levels of concentration found are lower than those recorded in the sites of cultivation due to the higher flow of the studied streams.

For the S1 (cultivation site), the values of the expected concentrations in the environment obtained by the “SoilFug” model, adjusted with parameters previously optimised in runoff and leaching tests done at laboratory scale (Peruzzo et al., 2003), show a good concordance with the results observed in the field (Table 4). It must be pointed out that the model calculates the pesticide concentration after a rain event, for this reason it does not estimate the initial concentrations in the field. In this sense, for the estimation of glyphosate levels in SA1, the rain event occurring after the A1 application (10/01/04; 4.5 mm) was used.

In water, the accordance is very good for the first rain event. This is possibly associated with the remarkable level of precipitation recorded (65 mm), being this the main contribution to the creek’s flow as runoff water. For this reason, the glyphosate concentration estimated by this model (runoff water) is representative of its concentration in the water stream. This situation is not repeated in the second case considered (SR2), in which the rain event recorded is significantly smaller (15 mm), probably by dilution of glyphosate in the receiving water body. In the case of soil, although the model presents accordance within the order of a magnitude, this underestimates the expected concentration when heavy rains occur. The light rain recorded and the high value of glyphosate in soils before the application, somehow explain the differences observed between the calculated values and those determined experimentally. However, this predictive tool provides a relevant global vision of the compartmentalization of the pesticides, contributing with valuable information when planning future samplings.

4. Conclusion

The optimised method shows an adequate sensitivity for the detection of glyphosate in water, soil and sediment samples by a simple procedure, useful in areas where agricultural practices by direct sowing of transgenic crops are conducted.

Regarding the levels detected in the environment, a direct relationship between the glyphosate application and its detection in soils is observed, in which the levels of the herbicide increase proportionally to the dosage of the applications. The rains cause the decrease in glyphosate concentration in this environmental compartment.

In waters and sediments, this behaviour is noticeable for the applications. However, the concentration variations are not so marked like in soil. Likewise, the flow increased by rain causes the transport of the herbicide from the direct area of influence to the downstream sites, increasing the glyphosate levels in these points.

The obtained values by the application of the SoilFug model are consistent with the experimental results, achieving a good accordance for the cases in which the precipitation is abundant.

Finally, the contribution of this preliminary study is pointed out because there is little information in this region reporting pesticides concentrations in the environment, where intensive cultivation activities predominate in large areas.

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