



# Herbicides in surface and underground water from Argentina

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## INTRODUCTION

Argentina is a major agricultural producer of soybean, corn, wheat, sunflower, sorghum among other extensive crops. In the last two decades, many different transgenic crops were introduced, leading to a steady increase in the use of pesticides mainly herbicides, currently surpassing 30 million hectares the areas cultivated under no tillage. A selected group of herbicides such as glyphosate (N-(phosphonomethyl) glycine), atrazine (1-Chloro-3-ethylamino-5-isopropylamino-2,4,6-triazine), 2,4 D (2,4 Dichlorophenoxy-acetic acid), glufosinate (ammonium(S)-2-amino-4-[hydroxyl(methyl) phosphinoyl] butyrate) are in the top rank of use in this country. The most representative case is undoubtedly the use of glyphosate resistant soybeans that involved annual loads of about 200,000 tons of this herbicide since 1995. To evaluate the occurrence and impact of these environmentally concerned pesticides, widely used in the central region of Argentina (Provinces of Santa Fe and Entre Ríos), several field studies were carried on. The work comprised the analysis of over 500 surface and underground water samples for glyphosate, AMPA, glufosinate, atrazine and 2,4 D.

## ANALYTICAL APPROACHES

### ANALYTICAL METHOD

A simplified analytical tool for the residue determinations was developed. Sample preparation was based on a) solid phase extraction for 2,4 D and atrazine, and b) FMOC derivatization followed by liquid-liquid partition for glyphosate, AMPA and glufosinate determination.

#### GLYPHOSATE

##### SAMPLE PREPARATION

- 3 mL sample
- 6M HCl (pH~1)
- Isotopes <sup>13</sup>C, <sup>15</sup>N Gly and AMPA
- 6M KOH (pH~7)
- 40 mM Borate buffer (pH~9)

##### DERIVATIZATION

- 6 g/L FMOCl
- Acetonitrile
- 2 h reaction

##### CLEAN UP

- L-L partition with DCM

#### ATRAZINE AND 2,4 D

##### SAMPLE PREPARATION

- Cartridges SPE: 250 mg C<sub>18</sub>
- Conditioning:
  - 3 mL MeOH
  - 3 mL distilled H<sub>2</sub>O
  - 3 mL distilled H<sub>2</sub>O + orthophosphoric acid (pH~3)
- 100 mL sample + orthophosphoric acid (pH~3)
- Elution: 2 mL MeOH

FIGURE 1. Analytical method.

### LC-ESI-MS/MS

The methodology development assays involved also the optimization of UHPLC-MS/MS variables to achieve satisfactory analyte identification, confirmation and sensitivity. The whole methodology fits adequately with current purposes of environmental evaluation.

#### Glyphosate, AMPA and Glufosinate

ANALYTE	PARENT (m/z) (Cone)	PRODUCT 1 (m/z) (CE)	PRODUCT 2 (m/z) (CE)
Glyphosate-FMOC	392.0 (20 V)	88.1 (30 V)	214.1 (10 V)
AMPA-FMOC	334.0 (20 V)	112.1 (15 V)	179.1 (20 V)
Glufosinate-FMOC	404.0 (30 V)	136.1 (25 V)	208.2 (10 V)
Gly 2-C13 N15-FMOC	395.0 (20 V)	91.1 (30 V)	217.1 (10 V)
AMPA C13 N15-FMOC	336.0 (20 V)	114.1 (15 V)	181.1 (20 V)

TABLE 1. Parameters MS/MS of glyphosate, AMPA and glufosinate.

**UHPLC SYSTEM Waters Acquity®**  
 Mobile Phase A: H<sub>2</sub>O/MeCN 98:2 + 0.1 % FA  
 Mobile Phase B: MeCN + 0.1 % FA  
 Flow: 0.35 mL/min  
 Injection volume: 10 µL  
 Column: ACQUITY UPLC® HSS C18 1.8 µm, 2.1x100 mm  
 Column temperature: 40 °C

**Waters TQD SYSTEM**  
 Function: MRM ES+  
 Source Temperature: 140 °C  
 Desolvation Temperature: 500 °C Cone gas flow: 15 L/h  
 Capillary: 1 Kv Desolvation gas flow: 600 L/h  
 Dwell time: 0.01 sec  
 Software: Masslynx 4.1

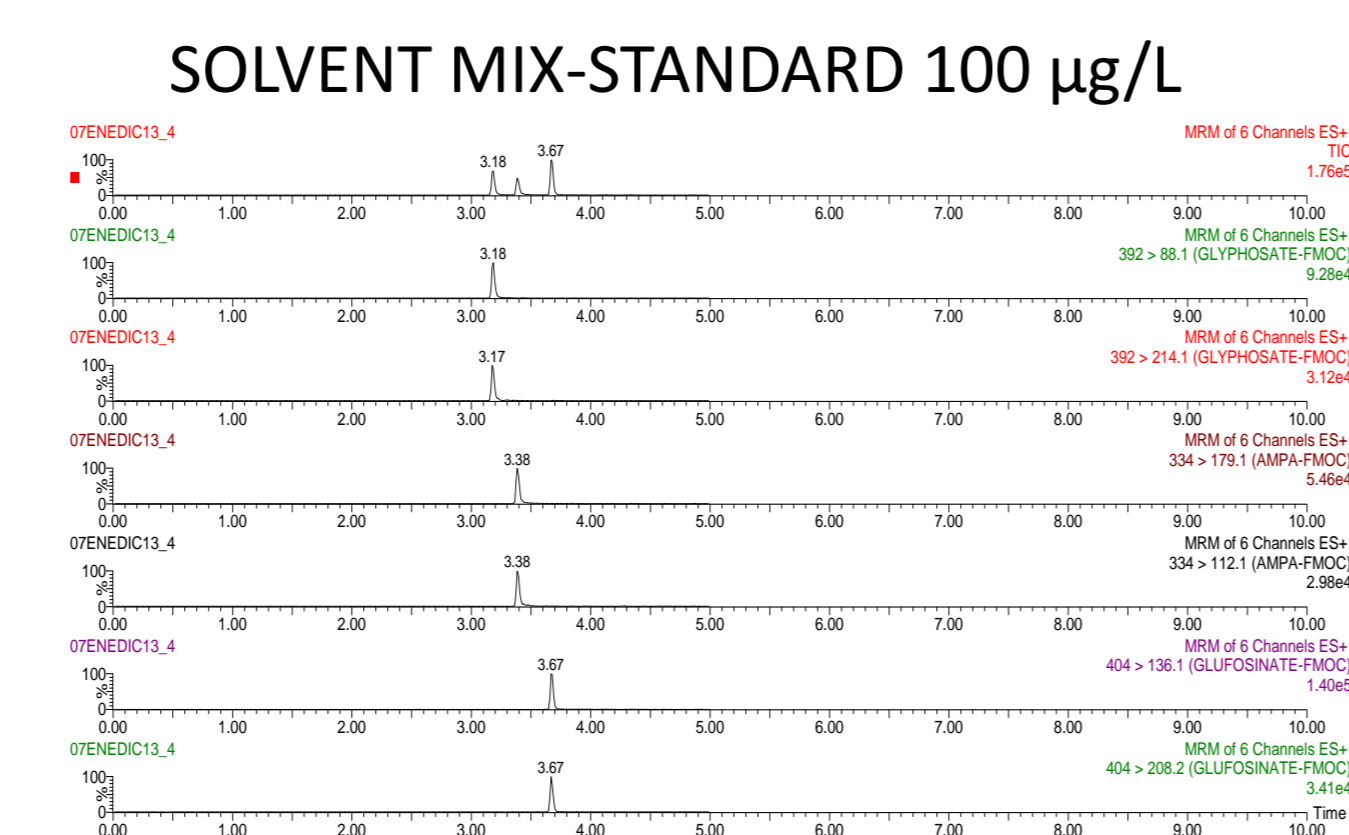


FIGURE 2. TIC and MRM chromatograms of glyphosate-FMOC, AMPA-FMOC and glufosinate-FMOC from a solvent mix-standard solution.

#### Atrazine and 2,4 D

ANALYTE	PARENT (m/z) (Cone)	PRODUCT 1 (m/z) (CE)	PRODUCT 2 (m/z) (CE)
Atrazine	216.1 (33 V)	96.01 (23 V)	174.1 (18 V)
2,4 D	219.0 (25 V)	125.0 (30 V)	161.0 (15 V)

TABLE 2. Parameters MS/MS of atrazine and 2,4 D.

**UHPLC SYSTEM Waters Acquity®**  
 Mobile Phase A: H<sub>2</sub>O/MeCN 98:2 + 5mM FNH<sub>4</sub> + 0.1 % FA  
 Mobile Phase B: MeOH + 5mM FNH<sub>4</sub> + 0.1 % FA  
 Flow: 0.35 mL/min  
 Injection volume: 10 µL  
 Column: ACQUITY UPLC® BEH Shield C18 1.8 µm, 2.1x100 mm  
 Column temperature: 40 °C

**Waters TQD SYSTEM**  
 Function: MRM ES+ (atrazine) and ES- (2,4 D)  
 Source Temperature: 120 °C  
 Desolvation Temperature: 350 °C Cone gas flow: 150 L/h  
 Capillary: 1 Kv Desolvation gas flow: 800 L/h  
 Dwell time: 0.008 sec  
 Software: Masslynx 4.1

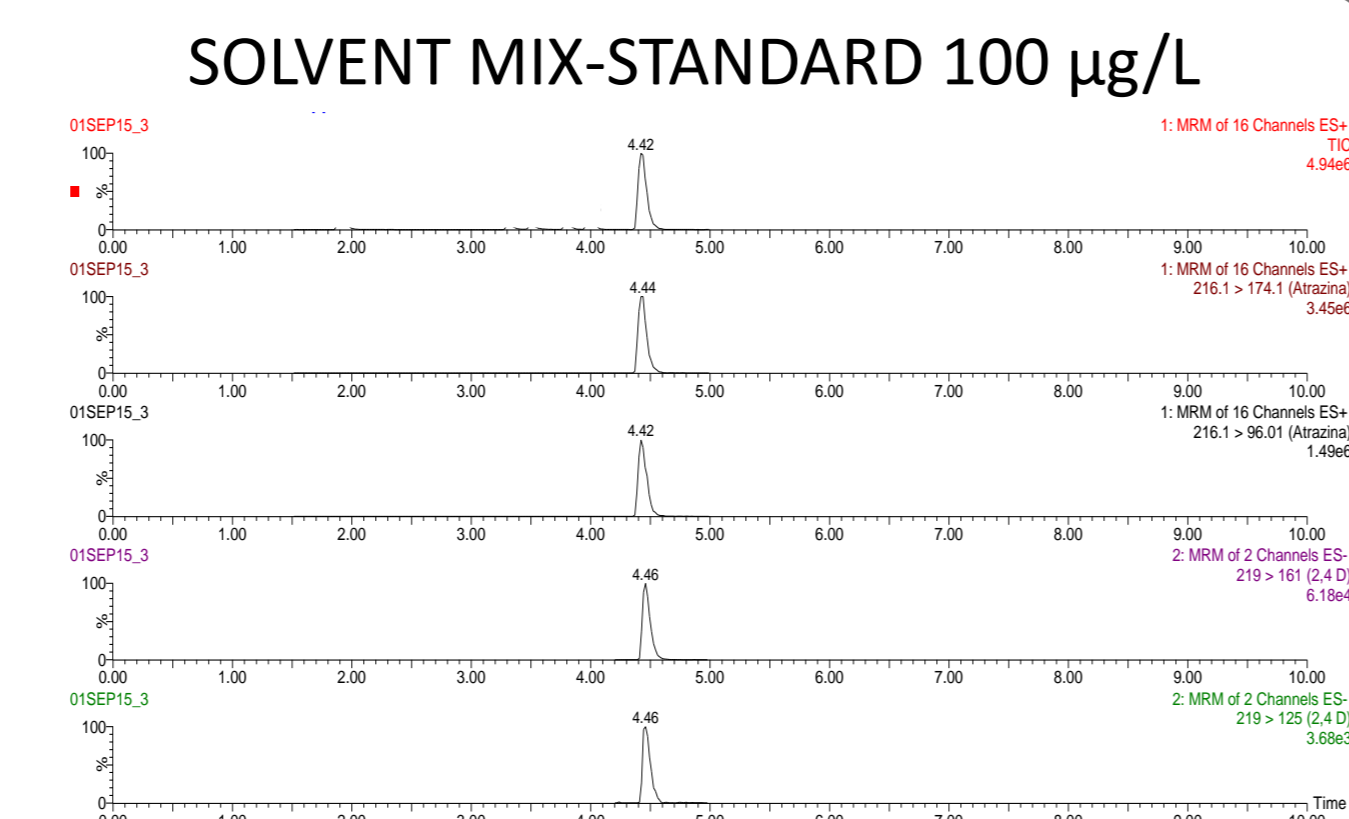


FIGURE 3. TIC and MRM chromatograms of atrazine (ES+) and 2,4 D (ES-) from a solvent mix-standard solution.

## RESULTS

### VALIDATION

The method was validated following SANCO/12571/2013 guidelines and satisfactory performance parameters were obtained:

ANALYTE	RECOVERIES 100 µg/L	RSD %	LOD µg/L	LOQ µg/L
Glyphosate	96-124 %	8	0.2	0.6
AMPA	80-90 %	10	0.1	0.2
Glufosinate	71-115 %	13	0.01	0.1
Atrazine	70-85 %	6	0.0004	0.002
2,4 D	75-87 %	5	0.004	0.02

TABLE 3. Validation parameters.

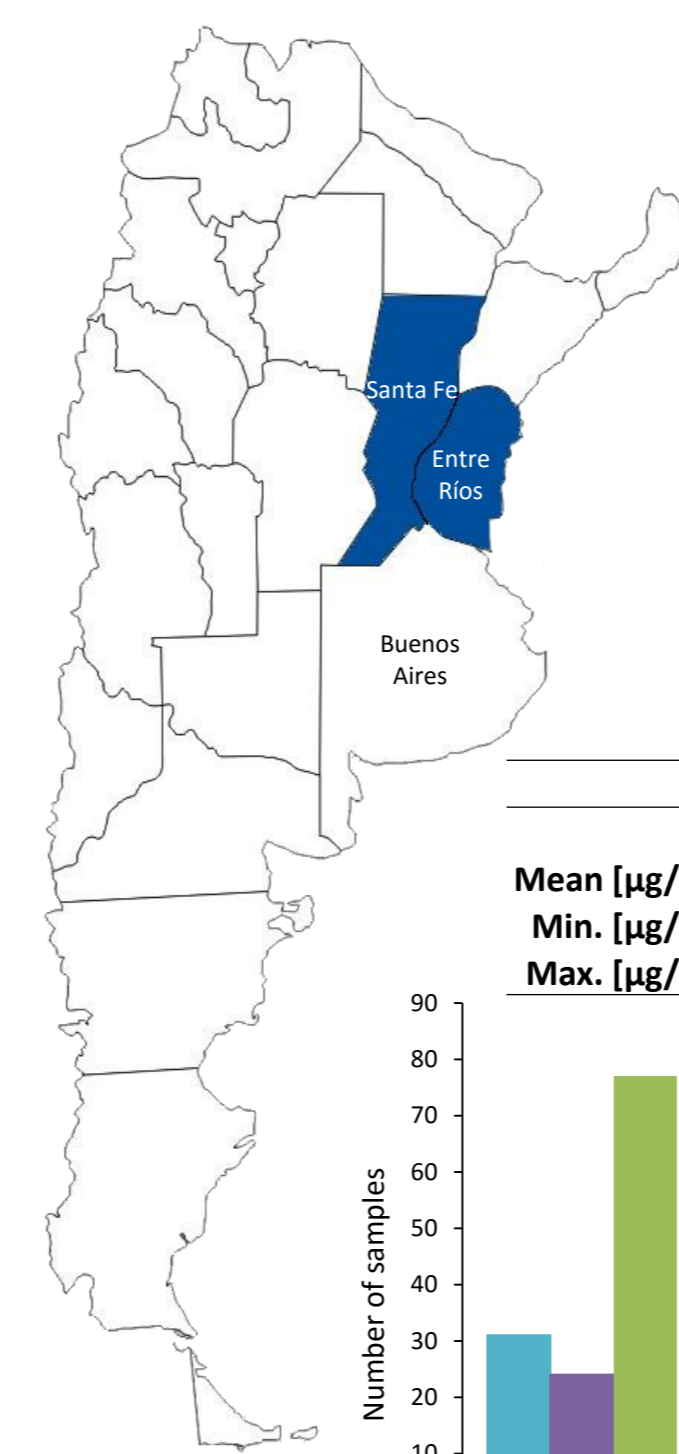
### SAMPLES

Surface waters (rivers, streams and channels) and groundwater from several regional areas of Santa Fe and Entre Ríos provinces were satisfactorily analyzed with the present method.

#### SURFACE WATER

##### • Santa Fe

Samples were collected in the central region of Santa Fe Province (Departments of San Justo, La Capital, Las Colonias, Castellanos). Surface water samples were under enforcement, regulatory control or legal purposes requested for analysis by the Environmental Secretary of Santa Fe Province.



	Glyphosate	AMPA	Glufosinate
n	132	132	83
Mean [µg/L]	196	208	11
Min. [µg/L]	0.6	0.2	0.1
Max. [µg/L]	12856	5386	169

	Atrazine	2,4 D
n	15	15
Mean [µg/L]	0.15	0.48
Min. [µg/L]	0.02	0.04
Max. [µg/L]	1.03	2.24

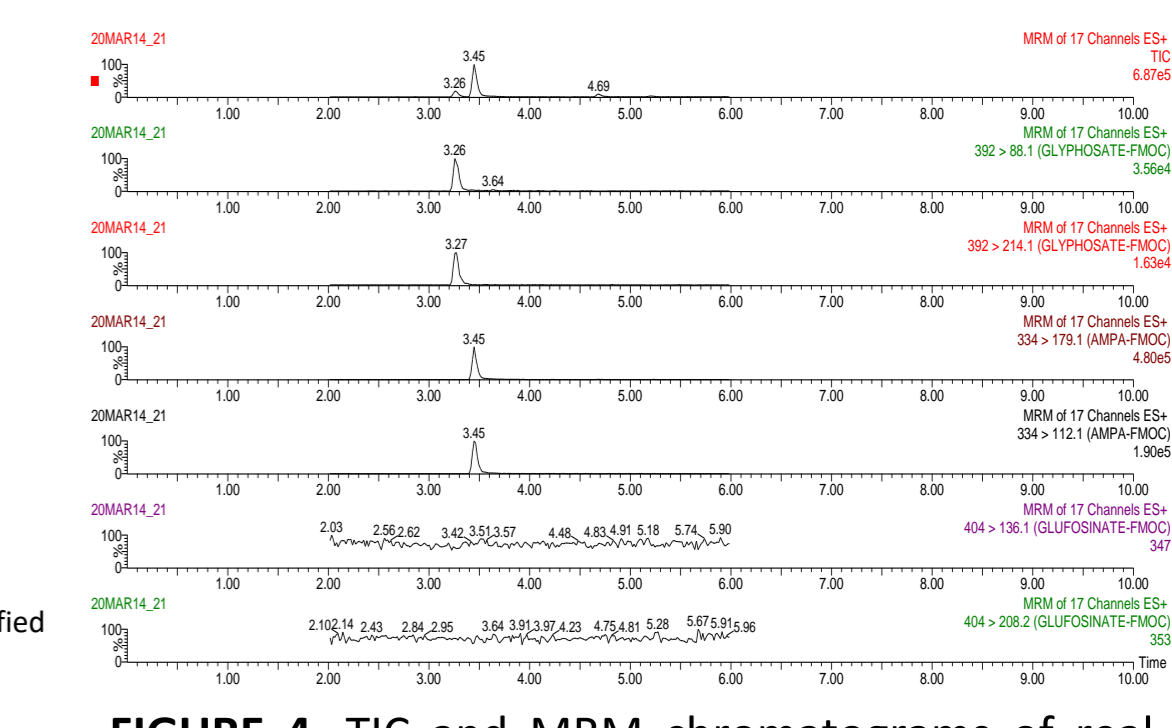
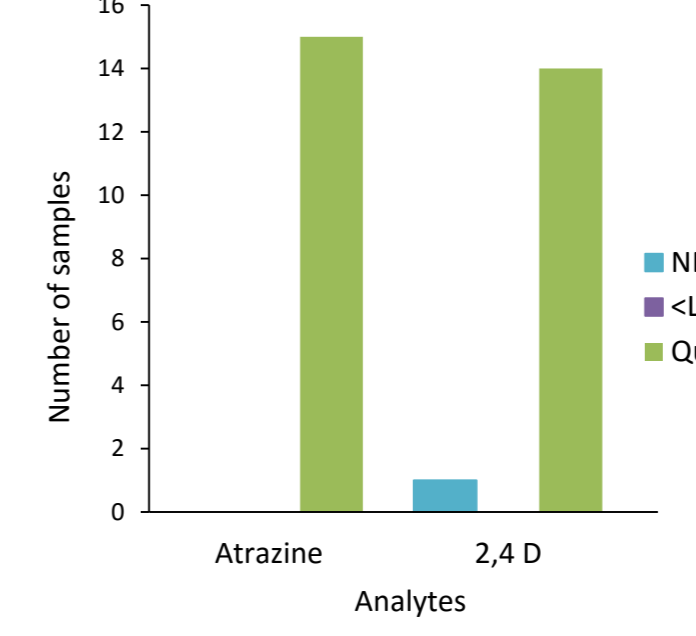
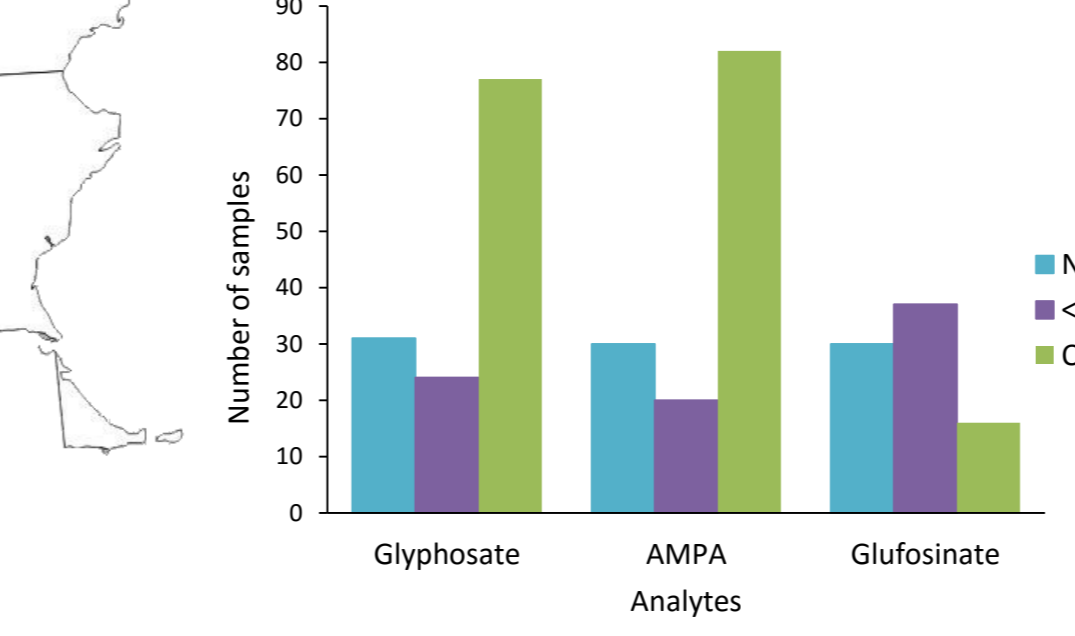


FIGURE 4. TIC and MRM chromatograms of real sample of surface water (µg/L): glyphosate (8.7), AMPA (73.7) and glufosinate (ND).

##### • Entre Ríos

Samples collected in water courses of the whole Province within a monitoring program led by INTA Experimental Station Paraná (E.Ríos). Other separated surface samples from the Entre Ríos basin were included.

	Glyphosate	AMPA	Glufosinate
n	95	95	96
Mean [µg/L]	891	43	-
Min. [µg/L]	0.6	0.4	-
Max. [µg/L]	19618	1202	-

	Atrazine	2,4 D
n	25	30
Mean [µg/L]	3.8	125
Min. [µg/L]	0.2	0.8
Max. [µg/L]	15	873

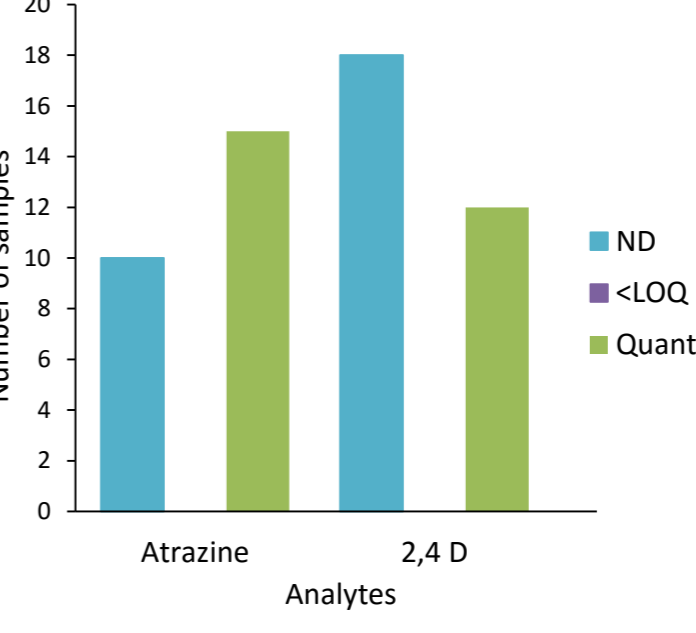
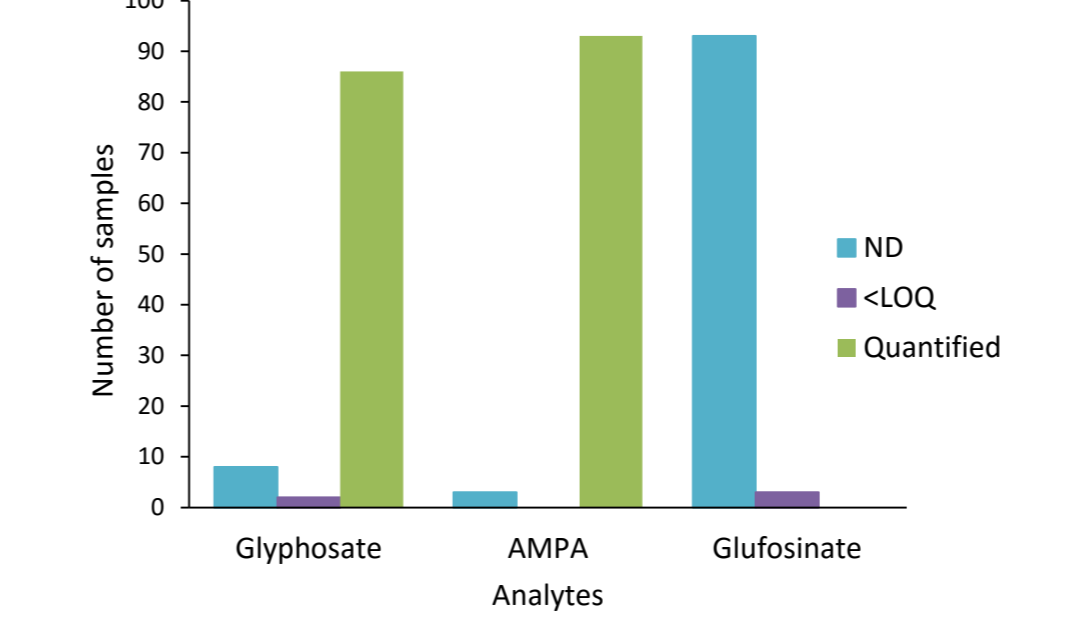


FIGURE 5. «Tajamar» (artificial pond) at Experimental Station INTA Paraná - Entre Ríos.

#### GROUNDWATER

##### Santa Fe

Samples were collected in milk farms from the central region of Santa Fe Province (Castellanos, Las Colonias) in the frame of Research Project MINCYT PICT N° 2011.

	Glyphosate	AMPA	Glufosinate
n	134	134	134
Mean [µg/L]	2.4	0.6	-
Min. [µg/L]	0.6	0.2	-
Max. [µg/L]	11.3	6.5	-

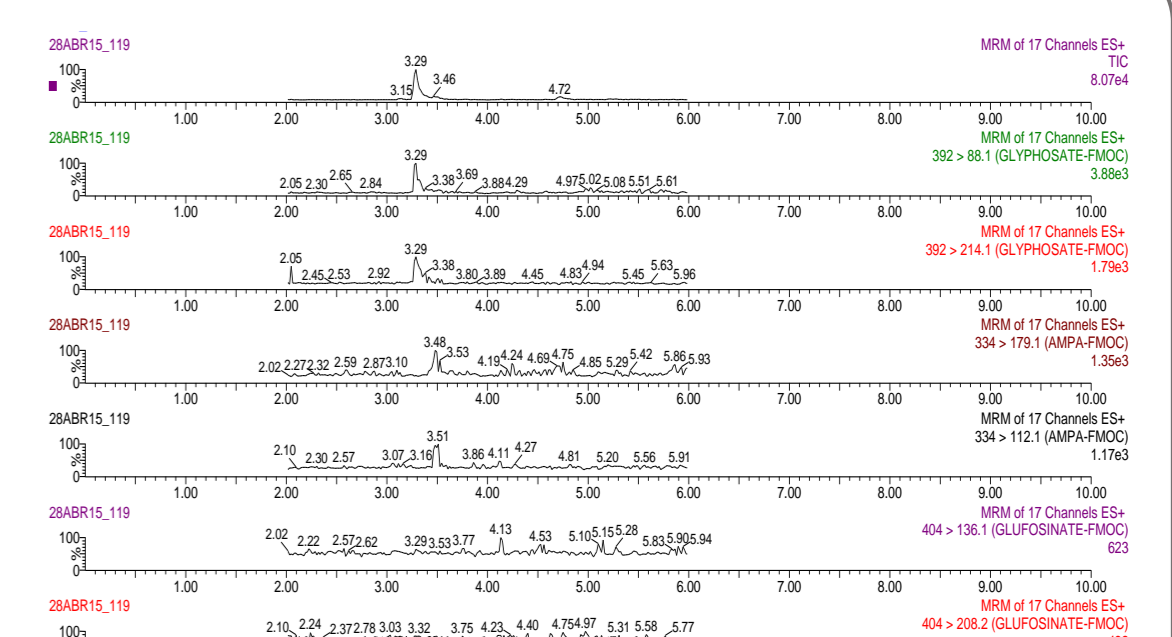
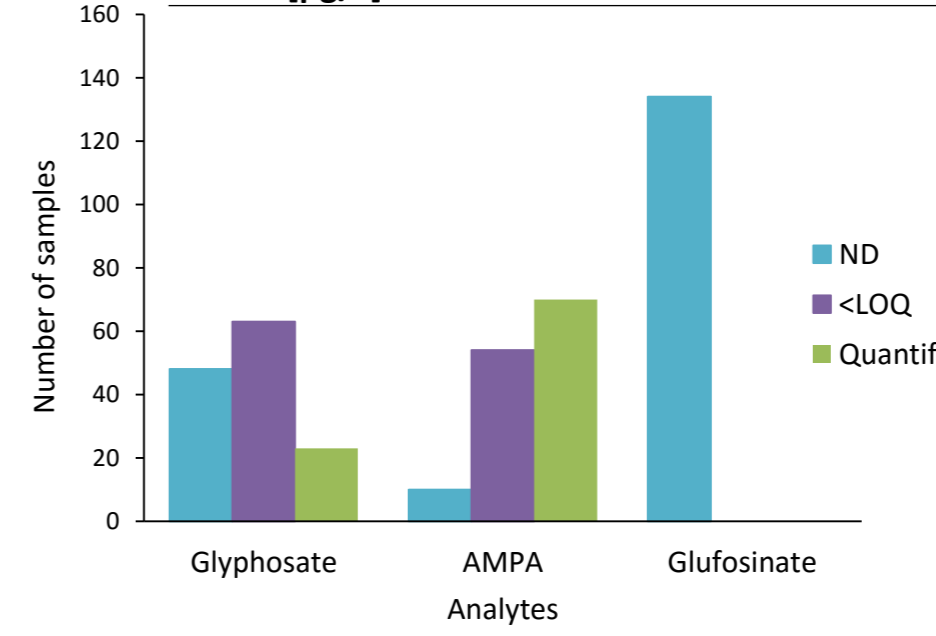


FIGURE 6. TIC and MRM chromatograms of real sample of groundwater (µg/L): glyphosate (1.1), AMPA (0.3) and glufosinate (ND).

## CONCLUSIONS

- The whole methodology fits adequately with current purposes on environmental evaluation.
- In general low concentration levels were detected complying with standard regulatory values. Higher levels were detected mainly concerning punctual contamination sources.
- The underground water samples studied showed very low incidence regarding herbicides contamination.
- Findings in surface waters, turns of first importance to follow exhaustive monitoring, under rigorous control plans, and the expansion of prevention through proliferation of sustainable practices.

## REFERENCES AND ACKNOWLEDGMENTS

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