



# Flazasulfuron behavior in a soil amended with different organic wastes



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## ABSTRACT

We studied the flazasulfuron behavior in a soil amended with three organic wastes (municipal solid waste, MSW, poultry manure, PM, and cow manure, CM) and their influence both on its mobility and on the soil's biochemical properties (soil humus-enzymatic complexes, soil microbial biomass-C and soil ATP). The organic wastes were applied to soil on February 2nd 2014. On June 6th 2014, flazasulfuron was applied. Adsorption and leaching experiments were also performed in soils with herbicide and without and with organic wastes. Flazasulfuron did not cause changes in soil biochemical properties. Soil biochemical properties were highest in MSW-amended soil, followed by PM and CM. With the MSW amendment, flazasulfuron sorption increased by a factor of 6.7 while for PM and CM, the factor increased 5.8 and 5, respectively. The application of organic matter decreased the soil's herbicide concentration, possibly due to flazasulfuron sorption by organic matter. The maximum concentration of flazasulfuron in leachates was reduced from 8.9  $\mu\text{M}$  for the unamended soil, to 3.5, 5.4, 6.9  $\mu\text{M}$  for the MSW, PM and CM-amended soils, respectively. Therefore, because decrease the flazasulfuron concentration in leachates, the application to the soil of organic matter with higher humic acid contents is a good environmental practice.

## 1. Introduction

The use of herbicides for weed control is a very common practice in intensive agriculture. However, the continued use of herbicides leads to increase levels of residues of such chemicals in soils and water, with possible negative consequences for public and ecological health (Sørensen et al., 2006; Hiller et al., 2012; López-Piñero et al., 2013).

In recent decades, acceptance of the use of herbicides belonging to the sulfonylurea family has become widespread among farmers and scientists concerned with the environment. This wide acceptance is mainly due to sulfonylurea's ability to provide weed control at doses within the range of 10–40 g grams per ha. Other qualities include: very low acute, chronic animal toxicity (the LD50 in rats are generally > 5000 mg kg<sup>-1</sup>), a good crop selectivity, and a broad-spectrum weed control (Beyer et al., 1988; Brown, 1990). Furthermore, Fenoll et al. (2013) indicated that because of their favorable environmental properties and low acute mammalian toxicology compared with most other herbicides, sulfonylureas usually provide a large margin of safety with regards to ecological and ecotoxicological effects.

Flazasulfuron (*N*-[4,6-dimethoxypyrimidin-2-yl]-3-(3-trifluoromethyl-2-pyridylsulfonyl)urea] is an herbicide belonging to the sulfonylurea family which is widely used in the olive oil crop to control wide

range of grasses.

Both the use of herbicides and the application of organic amendments to agricultural soil is a common farming practice. The application of organic matter to agricultural soil aimed at improving the soil's physical, chemical and biological properties (Aranda et al., 2015; Li and Han, 2016; Parihar et al., 2016). Moreover, the chemical composition of the organic matter applied to the soil greatly influences the dynamics of the herbicides in soil. In this respect, there is extensive literature indicating that the organic matter applied can adsorb herbicides, thus decreasing their mobility and reducing the possibility of aquifer contamination (Albarrán et al., 2004; López-Piñero et al., 2013).

Sorption/desorption is one of the key processes that affect the behavior of agrochemicals in the soil-water environment. They can, therefore, help to understand how to predict the mobility and availability of pesticides in soils (Iglesias et al., 2010). In addition, it also of great environmental importance to know whether soil-applied herbicides might have a toxic effect on soil microorganisms. There are a large number of scientific studies indicating that many herbicides have toxic effects on soil microorganisms (Tejada, 2009; Tejada et al., 2010a,b; Gómez et al., 2014; Franco-Andreu et al., 2016). Therefore, knowledge of this effect, together with the herbicide's soil mobility, can provide a complete idea of the environmental effect of these chemical com-

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pounds.

Many herbicides are degraded by soil microorganisms. For it, the microorganisms excrete different enzymes into the medium that are responsible for degrading these chemical compounds. However, free enzymes normally have a short-lived activity because they can be rapidly denatured, degraded, or inhibited (Bastida et al., 2008). Enzymes that bind clay and/or humic substances are more resistant to degradation than free enzymes and can persist in soil. Tejada and Benítez (2011) suggested that because immobilized enzymes may act as stable catalysts for the detection of potential substrates. Enzymes immobilized on humus molecules play an important role in soil microbial ecology. Masciandaro et al. (2012) also suggested that their importance arises from the fact they can represent a reservoir of biochemical energy and nutrients. Therefore, studying humus-enzyme complexes could be useful for understanding the herbicide's toxicity on soil microorganisms.

However, despite the widespread use of flazasulfuron, both its behavior in soil and its effects on soil biochemical properties after the application of different sources of organic matter is not known. Therefore, the objective of this study was to investigate the behavior of flazasulfuron in a soil amended with three organic wastes and their influence on its mobility and on the soil's biochemical properties.

## 2. Material and methods

### 2.1. Characteristics of soil, organic wastes and herbicide

A semiarid soil (0–25 cm surface layer) was collected from an agricultural area located in Córdoba (Spain). The soil is classified as Xerollic Calciorthid (Soil Survey Staff, 1987). Three sources of organic matter were used: a municipal solid waste (MSW), a poultry manure (PM), and a cow manure (CM). Organic wastes were composted under aerobic conditions in a trapezoidal pile (4 m long, 2 m wide and the base and 1 m high) containing approximately 5000 kg each pile and for each organic waste. The piles were turned every two weeks and water was regularly added to maintain appropriate moisture level. For MSW, the composting process lasted approximately 6 months, whereas for PM and CM this process lasted about 4 months.

The main soil and organic characteristics are shown in Table 1. The methodology used in the determination of the physical and chemical parameters in soil and organic wastes is described in Gómez et al. (2014).

The acidic functional group contents of humic acids isolated from organic wastes are shown in Table 1. The carboxyl group content was estimated by direct potentiometric titration at pH 8, the phenolic hydroxyl group content was estimated as two times the change in charge between pH 8 and pH 10, and the total acidity as calculated by addition (Ritchie and Perdue, 2003).

The molecular mass distribution of protein in the organic wastes was also determined. The proteins were extracted at pH = 9, temperature = 55 °C and time = 3 h. The protein distribution was determined by size-exclusion chromatography using an ÄKTA-purifier (GE Healthcare), using a Superdex Peptide™ 10/300GL column (Table 1). Samples were centrifuged at 12,000g for 30 min at 4 °C to remove insoluble molecules, and the supernatant was passed through a 0.2 µm filter and loaded into a 0.1 ml loop connected to an ÄKTA-purifier system. The column was equilibrated, and eluted with 0.25 M Tris–HCl buffer (pH 7.0) in isocratic mode, at a flow-rate of 0.5 ml/min, and proteins/peptides were detected at 280 and 215 nm with a GE Healthcare UV900 module coupled to the elution column.

The herbicide used in this experiment was flazasulfuron. The commercial formulation Terafit (flazasulfuron 25% p/p) was purchased from Syngenta (Spain). The rate applied to the soil was 0.2 kg ha<sup>-1</sup> (recommended application rate).

**Table 1**

Main physico-chemical features of the experimental soil, organic wastes, isolated acidic functional group contents of humic acids and molecular weight proteins distribution from MSW, PM and CM (mean ± standard error). Data are the means of three samples.

	Soil	MSW	PM	CM
pH (H <sub>2</sub> O)	7.8 ± 0.2	6.6 ± 0.3	8.3 ± 0.2	8.0 ± 0.2
Sand (g kg <sup>-1</sup> )	420 ± 27			
Silt (g kg <sup>-1</sup> )	247 ± 13			
Clay (g kg <sup>-1</sup> )	333 ± 19			
Organic matter (g kg <sup>-1</sup> )	1.2 ± 0.2	466 ± 23	622 ± 24	776 ± 21
Humic acid-C (mg kg <sup>-1</sup> )	16.5 ± 3.1	1007 ± 17	681 ± 17	466 ± 11
COOH (mol kg <sup>-1</sup> )	Nd	3.8 ± 0.3	3.2 ± 0.1	3.0 ± 0.1
Phenolic OH (mol kg <sup>-1</sup> )	Nd	1.6 ± 0.2	1.2 ± 0.2	1.0 ± 0.1
Total acidity (mol kg <sup>-1</sup> )	Nd	5.4 ± 0.3	4.4 ± 0.2	4.0 ± 0.1
Fulvic acid-C (mg kg <sup>-1</sup> )	9.0 ± 2.0	882 ± 20	761 ± 25	610 ± 16
Kjeldahl N (g kg <sup>-1</sup> )	0.5 ± 0.1	14.1 ± 2.2	28.3 ± 2.9	21.1 ± 1.6
<b>Protein molecular weight (Daltons)</b>				
> 10000	Nd	43.6 ± 4.8	46.9 ± 3.9	48.2 ± 4.5
10000–5000	Nd	24.9 ± 3.0	27.8 ± 2.7	28.4 ± 3.1
5000–1000	Nd	11.5 ± 2.2	9.4 ± 2.9	10.6 ± 2.7
1000–300	Nd	9.2 ± 2.6	8.3 ± 2.4	7.0 ± 2.4
< 300	Nd	10.8 ± 1.7	7.6 ± 1.1	5.8 ± 1.3

Nd: Not determined.

### 2.2. Experimental layout

The experimental layout was a randomized, complete block design with eight treatments and three replicates per treatment. The plot size was 7 × 3 m. The organic wastes were surface broadcast on February 2nd 2014 and incorporated to a 25-cm depth by chisel ploughing and disc harrowing. Flazasulfuron herbicide was applied four months after the application of organic wastes to the soil (June 6th 2014). The treatments were the following:

- C, control soil, non-organic amended and without flazasulfuron
- F, soil with flazasulfuron and non-organically amended
- MSW, soil without flazasulfuron and amended with 10 t ha<sup>-1</sup> MSW (dry matter) (4710 kg OM ha<sup>-1</sup>)
- PM, soil without flazasulfuron and amended with 7.8 t ha<sup>-1</sup> PM (dry matter) (4710 kg OM ha<sup>-1</sup>)
- CM, soil without flazasulfuron and amended with 6 t ha<sup>-1</sup> CM (dry matter) (4710 kg OM ha<sup>-1</sup>)
- F + MSW, soil with flazasulfuron and amended with 10 t ha<sup>-1</sup> MSW (dry matter) (4710 kg OM ha<sup>-1</sup>)
- F + PM, soil with flazasulfuron and amended with 7.8 t ha<sup>-1</sup> PM (dry matter) (4710 kg OM ha<sup>-1</sup>)
- F + CM, soil with flazasulfuron and amended with 6 t ha<sup>-1</sup> CM (dry matter) (4710 kg OM ha<sup>-1</sup>)

### 2.3. Soil biochemical analysis

On days 5, 10, 20, 40, 80 and 120 days after the application of the herbicide to the soil and for each treatment, soil humus-enzymatic complexes were also determined. The extraction was carried out with 0.1 mol l<sup>-1</sup> sodium pyrophosphate (pH 7.0). The extraction was performed in cold after 5 h previous sonication of a 1/10 soil/pyrophosphate mixture. The extract was separated by centrifugation at 10 000 rpm for 30 min and filtered through 0.22-mm Millipore membrane filters (microbiological filtration). Finally an extract aliquot was dialyzed against distilled water in cut molecular weight membranes of 14000 units (Visking® dialysis tube, Serva, Germany). The extraction was performed in triplicate. In the extract, the urease activity was determined by the buffered method of Kandeler and Gerber (1988), using urea as substrate. The β-glucosidase activity was determined

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