

Book Chapter

Abiotic Degradation of Profoxydim Herbicide in Soils Amended with Alperujo Compost

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Abstract

The application of organic amendments can offer significant benefits for the soil when using pesticides. Profoxydim is a relevance herbicide for the weed control in rice crops. The objective of this work was to evaluate the chemical behavior in the degradation of profoxydim herbicide in two different soils (Seville and Galicia) and its interaction with two doses of alperujo compost as organic amendment.

In unamended soils, two different half-lives were obtained, ranging from 1.65 days in Seville to 8.75 days for Galicia. In both amended soils, degradation occurred more rapidly, and similar half-lives were achieved (0.55-0.69 days) regardless of soil or alperujo dose. Various kinetic models were examined, with DFOP kinetic model providing a good fitting and revealing a biphasic behavior of profoxydim in soil attributed to sorption and degradation processes. Five different degradation products were detected by means of HPLC-DAD in the soil. The main product exhibited higher persistence in soil compared to the profoxydim herbicide. This finding underscores the importance of conducting further studies to assess the potential environmental risks associated with this main degradation product.

Introduction

The use of pesticides has increased considerably in recent decades due to the notable growth of the world's population. This increase in population has led to an increased demand for food, making it necessary to improve crop productivity. According to OECD and FAO reports, by 2050 it will be necessary to increase production to 60% of the current level [1]. Plant protection products are active substances and/or chemical and/or biological preparations whose main objective is the prevention and/or control of harmful organisms that affect crops and reduce their production. The number of pesticides used in the world is around 3 million tons [1]. Due to this increase in the use of pesticides, several regulations have been put in place for the regulation and use of these compounds. Plant protection products are mainly

regulated by European Union Regulation (EC) No. 1107/2009. The main problems affecting agriculture are diseases, pests and weeds. Weeds adapt easily to the ecosystem and therefore cause a large decrease in crop yields as they compete for light, water and nutrients.

Since the emergence of herbicides around 1950, their use has substantially increased. Herbicides account for 45-50% of the pesticides used in the world. Herbicides are widely used in agriculture and industry because they provide effective control at low cost [2]. In recent decades, the use of herbicides has increased considerably compared to other pesticides (Figure 1). Despite the benefits of the use of these compounds, it is necessary to know their fate and behavior in the environment, including the study of their degradation products. One of the main risks of pesticides lies in their persistence in the environment, and in the possible phenomena of transport to surface and/or groundwater.

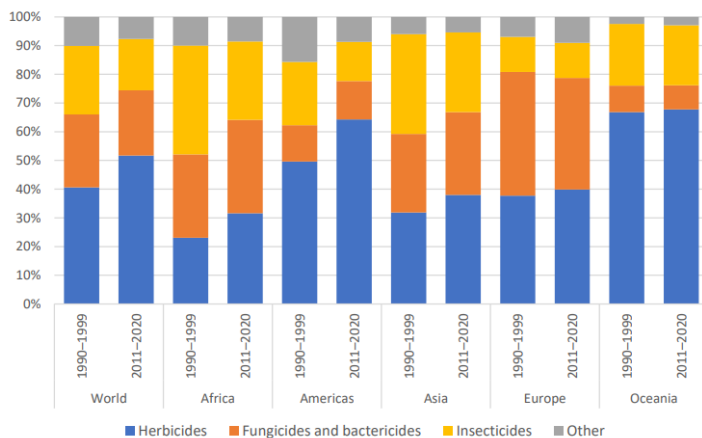


Figure 1: Use of pesticides in the world from 1990-2020. Data reported by FAOSTAT Analytical Briefs, no. 46. Rome.

When pesticides enter the environment, their fate in the environment are governed by many processes such as physical, chemical and biological. Currently, pesticides are expected to degrade rapidly in the environment and present high effective at

low doses. This is of great importance since it is known that only 0.1% of the pesticide introduced into the environment reaches pests, being the remaining 99% is a potential danger to the environment [3]. Once the pesticide is applied are subjected to sorption, transport and degradation processes. Chemical degradation is one of the main abiotic factors that can influence the behavior and stability of the herbicide in the environment. Some of the most common transformations are oxidations, hydrolysis and isomerizations.

These processes and the consequent high degradation of pesticides makes necessary to study the nature of the degradation products (DPs) generated, which could represent an important problem for the environment. DPs can be more persistent and present a higher toxicity and/or a higher mobility compared to their parent compounds [4,5].

To study abiotic processes, it is necessary to eliminate the microbial systems, but it is important to keep the chemical and physical characteristics of soil. Different sterilization methods have been investigated by several authors [6,7]. Tuominen et al. reported that soil sterilization with formaldehyde eliminate the microorganisms and kill emerging spores, and produces smaller changes in chemical and physical characteristics. Degradation studies in sterile soil allow to distinguish biological from chemical degradation of pesticides and to know the contribution of each type of degradation to the whole transformation process

It is known that the use of organic amendments exerts favourable properties to the soil for crop production improving soil fertility and nutrients due to their high organic matter. However, the addition of organic amendments can have an important impact on pesticide dissipation, affecting their sorption, degradation and transport processes. Alperujo compost is a common organic amendment in agriculture generated from olive oil industry. Different authors found that the use of alperujo reduce the degradation of pesticides, increasing their absorption due to the increase of organic matter. As a result, leaching of pesticides can be reduced, with a consequent reduction, in some cases, of water pollution [8,9].

On the other hand, there are studies that indicate a decrease in the pesticide bioavailability, which would decrease its effectiveness [10]. Thus, the behavior of the pesticides in soils with amendments is in many cases unknown and it will depend on different factors, such as the properties and origin of the amendment and the physical-chemical properties of the pesticide.

The cyclohexanedione oxime ether herbicides were discovered in the 1970s to control grass weeds. These family of herbicides inhibit lipid synthesis by inhibiting acetyl-coenzyme A carboxylase [11,12]. This group of herbicides are used for cereal and dicotyledonous crops as post-emergence herbicides, except profoxydim which used for the control of grass weeds in rice and tralkoxydim which is used for the control grass weeds in wheat and barley fields [13]. One of the advantages of these herbicides is the low concentration necessary to produce a lethal effect on weeds and their low toxicity for other non-target organisms. Regarding their behavior in the environment, the degradation rate of cyclohexanediones varying with the pH of aqueous solution obtaining faster degradation in acidic media [14]. Previous studies performed by our research group shown the rapid degradation of these compounds in different abiotic conditions [15,16]. Photolysis performed under simulated and natural sunlight in different environmental waters showed a rapid degradation of these herbicides. For example, clethodim, alloxydim and sethoxydim degrade completely in few hours generating degradation products that present a higher stability and persistence than the active substance [15,16].

Profoxydim ((1EZ)-N-[(2RS)-2-(4-chlorophenoxy)propoxy]butanimidoyl)-3-hydroxy-5-[(3RS)-thian-3-yl]cyclohex-2-en-1-one) is one of the limited herbicides approved for use in rice crops belonging to the last-generation cyclohexanedione oxime family. It is widely used for the postemergence control of rice grass weeds such as *Echinochloa crusgalli* and acts as an inhibitor of the acetyl coenzyme A carboxylase activity in the chloroplasts [17].

Rice crops are one of the most important food crops in the world, the global production of rice in 2022 was 520 million tonnes [1]. Rice growing techniques most common involve flooding processes, for this reason is important performed tracing the fate of pesticides in wetlands. Several authors shown numerous studies of contaminants in soil and drinking waters [18,19]. The main problem of rice cultivation is weeds, due to their competitiveness with the crop and their negative effects on yield.

Profoxydim has been shown to be readily degraded in the environment. A limited number of studies have investigated the persistence of this herbicide in paddy environments. Sanchez et al, (2006) [20] and Tsochatzis et al, (2013) [21], observed a rapid dissipation of profoxydim in field paddy systems with a half-life below 1 day indicating that profoxydim is nonpersistent in paddy fields. However, up to our knowledge, no studies have been performed under controlled conditions to know separately the contribution of abiotic and biotic processes. Furthermore, the study of degradation products is essential to know the environment behavior of this nonpersistent herbicide.

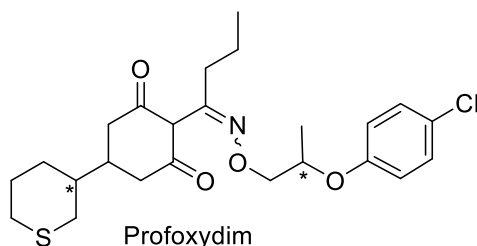


Figure 2: Chemical structure of profoxydim herbicide.

The objectives of this work were to study the abiotic degradation of the herbicide profoxydim in soil in the absence of light and microorganisms in paddy soil and to evaluate the effect exerted by the presence of alperujo compost in the degradation of the herbicide. Furthermore, degradation products were detected and monitored during the experiment by means of HPLC-DAD.

Materials & Methods

Chemicals and Reagents

The analytical standard of profoxydim (98.7% purity) was purchased from HPC Standards GmbH (Cunnersdorf, Deutschland). Acetonitrile (HPLC superGRAD grade) and methanol (HPLC grade) were acquired from Macron Fine Chemicals (Gliwice, Poland), and formaldehyde were purchased from Sigma–Aldrich (Steinheim, Germany). The water used for the LC mobile phase and the aqueous solutions were purified with a Millipore system (Milli-Q-50, 18 mΩ). The filters of regenerated cellulose 0.45 μm was purchased from Sigma–Aldrich (Steinheim, Germany)

Commercial QuEChERS method, a citrate-buffered version Method EN (4.0 g magnesium sulfate, 1.0 g sodium chloride, 0.5 g sodium citrate dibasic sesquihydrate, 1.0 g sodium citrate tribasic dihydrate) purchased from HPC Standards GmbH (Cunnersdorf, Germany).

Soils and Organic Amendment

Samples of paddy soils were collected from two different agricultural areas of Spain; Seville in the south (37°12'27.4" N 5°49'13.1" W) and Galicia in the northwest (43°14'50.2" N 8°16'22.4" W). Alperujo compost was used as organic amendment and was obtained from olive residues from an olive industry. The physico-chemical properties of soils and the compost are shown in Table 1. Mainly, Seville soil has a pH of 8.15 and contains 1.26% organic matter and Galicia soil has a pH of 6.04 and 3.73% organic matter and compost has a pH 8.17 and 28.63% of organic matter (Table 1). Soil samples and organic amendment were stored at 5 °C, air-dried overnight and passed through a 2 mm sieve before use.

Table 1: Physicochemical characterization of the two soils and compost employed in the experiments.

Origin of the soil	Seville	Galicia	Alperujo
pH (suspended 2:5 in water)	8.15	6.04	8.17
Organic matter oxidizable (%)	1.26	3.73	28.63
Clay (%) $D < 0.002$ mm	30.8	30.8	-
Silt (%) $0.002 < D < 0.05$ mm	14.0	44.0	-
Sand (%) $0.05 < 2$ mm	55.2	25.2	-
N_{total} (%)	0.10	0.23	2.30
P ($mg\ kg^{-1}$)	1.02	1.91	158.29
K ($mg\ kg^{-1}$)	253	329	12180
Mg ($mg\ kg^{-1}$)	225.5	100.3	585.5
Na ($mg\ kg^{-1}$)	85.9	21.2	505.8
Cu (Ext. EDTA) ($mg\ kg^{-1}$)	4.04	4.35	5.51
Fe (Ext. EDTA) ($mg\ kg^{-1}$)	46.76	15.27	101.31
Zn (Ext. EDTA) ($mg\ kg^{-1}$)	1.38	0.34	34.83

Sterilization of soil samples were performed following the method employed by Tuominen et al, [6] to kill emerging spores due to reactions with vital organic nitrogen compounds. Briefly, Soil samples were autoclaved in P-Selecta Model 12 (Barcelona, Spain) three times for 60 min at 121 °C for three consecutive days and 0.04% of formaldehyde was added to autoclaved samples.

Soil Degradation Experiments

Dissipation Studies

Soils experiments were performed in a climate chamber from Binder KBWF 240 (Tuttlingen, Germany) in the dark at 25 °C and 80% of relative humidity (Figure 3). All experiments were conducted in 50 mL polypropylene centrifuge tubes in triplicate.



Figure 3: Binder growth chamber.

40 centrifuged tubes of 50 mL were used for the experiment and 4.0 g of soil were added to each tube. The soils were fortified by adding 500 μ L of profoxydim field dose [14] that correspond to a concentration of 78 mg/L. To simulate rice paddy soil, that is a flooded crop, it was necessary to determinate the maximum water-holding capacity of soil. This parameter corresponds to the total quantity of water that can be absorbed by soil. This measure was performed saturating the soil with ultrapure water until the complete absorption. The amount of water retained in soil was determined by difference in weight between dry and wet soil, obtaining 70% for Seville and 65% for Galicia. The tubes were weighed regularly, and the loss of water by evaporation was compensated by the addition of ultrapure water. In the experiments conducted with amended soil, two concentrations of alperujo compost, 40% and 60%, were used. Previous screening studies were carried out in order to have a first data on the time necessary for profoxydim to degrade under these conditions. These experiments made it possible to determine the duration of the experiment at 25 days.

Analytical Procedure

For the extraction of profoxydim from amended and unamended soils, a previous QuEChERS method developed by our group [22] was used. Briefly, 8 mL of a mixture of 40% acetonitrile and 60% methanol were added to each 50 mL centrifuged tubes to extract profoxydim. These tubes were shaken vigorously for 1 min to homogenize the samples by using a multi-vortex BenchMixer XL Multi-Tube Vortexer (Benchmark Scientific, New Jersey, USA). Then, to obtain a good extraction, a citrate-buffered version EN (4.0 g magnesium sulfate, 1.0 g sodium chloride, 0.5 g sodium citrate dibasic sesquihydrate, 1.0 g sodium citrate tribasic dihydrate) were added (Figure 4). This commercial QuEChERS is usually to extract pesticides from fruits and vegetables [23,24].

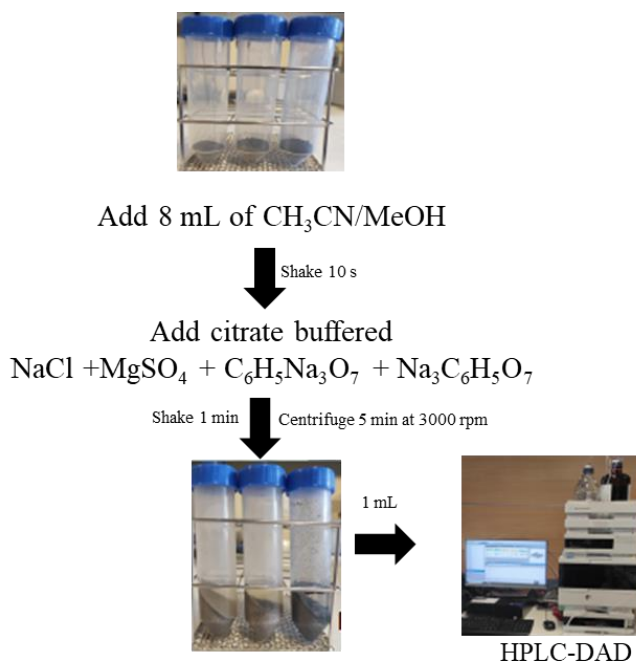


Figure 4: Scheme of QuEChERS extraction method of profoxydim in soil.

Finally, the samples were centrifuged at 3000 rpm and 4 °C for 5 min by Eppendorf Centrifuge 5810 (Eppendorf Iberica, Hamburg, Germany), and the supernatant was filter by regenerated cellulose 0.45 µm and analyzed by HPLC-DAD.

An HPLC system (Agilent Technologies 1100 series model; Agilent Technologies, Palo Alto, US) coupled to a diode array detector (DAD) were used to follow the degradation of profoxydim. The mobile phase was a mixture of ultrapure water acidified (0.1% formic acid) (A) and acetonitrile (B). Analytical column was a C18 Waters Atlantis column (3 µm particle size, 4.6 mm x 150 mm) (Waters, Dublin, Ireland) with an isocratic method using 90% of acetonitrile and the flow rate was 1 mL min⁻¹. The profoxydim retention time under these conditions was 6.2 min. Three replicates were carried out for each degradation experiment and the numerical results presented correspond to the mathematical average of these three independent analyses.

Evolution of Degradation Products

To study the degradation products formed during the dissipation of profoxydim, a gradient method was developed. The evolution of byproducts was performed by an HPLC system (series 1100; Agilent Technologies, Palo Alto, CA, USA) coupled to a mass spectrometer with a gradient method was developed to detect and follow their evolution. The percentages of B in the mobile phase were as follows: 0-4 min, 23%; 4-17 min, 23-62% and 17-20 min, 62-90%. The other parameters, flow rate and injection were kept constant (1 mL min⁻¹; 20 µL).

Data Analysis

The herbicide dissipation kinetics were fitted to different kinetic models: a single first order kinetic model (SFO), a first order multicompartamental model (FOMC), a double first order parallel model (DFOP) or the Gustafson and Holden (HS) model. For the choice of the best kinetic model, the recommendations of the FOCUS working group were followed [25]. The kinetic parameters were estimated using Cake Software. The error of the

χ^2 test was used to compare the goodness of fit of the models tested.

Single First Order Model (SFO)

This model assumes that the number of pesticide molecules is small relative to that of degrading microorganisms and their enzymes. The rate of the decrease in pesticide concentration is at any time directly proportional to the remaining herbicide concentration in the system:

$$C(t) = C_0 \times \exp(-kt) \quad (1)$$

where C (%) is the concentration of herbicide at time t (days), C_0 is the initial concentration (100%), and k (days⁻¹) the dissipation rate constant. The half-life times, $t_{1/2}$, named from now on DT_{50} , are calculated as $\ln 2/k$.

First-Order Multi Compartment Model (FOMC)

In this model, the soil is considered as a heterogeneous medium formed of unconnected sub-compartments, each with a different first-order dissipation rate constant. The integrated form of this model is

$$C = \frac{C_0}{\left(\frac{t}{\beta} + 1\right)^\alpha} \quad (2)$$

In this model, α is a shape parameter, determined by the coefficient of variation of rate constant values, and β a location parameter. Herbicide transformation in the FOMC model occurs more rapidly at higher α and lower β values. Regulatory endpoints can be calculated by the following equation:

$$DT_{50} = \beta \left(2^{\frac{1}{\alpha}} - 1 \right) \quad (2A)$$

$$DT_{90} = \beta \left(10^{\frac{1}{\alpha}} - 1 \right) \quad (2B)$$

Hockey Stick Model (HS)

The hockey-stick model (HS) consists of two sequential first-order curves. The main assumptions in this model are that concentration of the compound initially declines according to first-order kinetics with a rate constant k_1 . At a certain point in time (referred to as the breakpoint), the rate constant changes to a different value k_2 . Although the hockey-stick model is continuous with time, the derivative with time of the total amount is not continuous. For typical biphasic patterns, the rate constant k_1 is usually larger than k_2 . The overall decline is calculated by the equations given below. The DT_{50} value for the overall decline of compound concentrations can only be calculated from k_1 if the DT_{50} is reached before the breakpoint denoted by t_b . The half-life value calculated from k_2 refers to the slow later stage of decline only and will be longer than the DT_{50} . For this model, the equation is a simple first-order differential equation as given below:

$$\frac{dc}{dt} = -k_1 C \text{ for } t \leq t_b$$

its integrated form

$$C = C_0 \exp(-k_1 t)$$

and

$$\frac{dc}{dt} = -k_2 C \text{ for } t > t_b$$

its integrated form

$$C = C_0 \exp(-k_1 t_b) \exp(-k_2(t - t_b)) \quad (3)$$

where C is the compound concentration at time (days) after application, C_0 is the initial concentration, k_1 is the rate constant (days^{-1}) until $t=t_b$, while k_2 is the rate constant (days^{-1}) from the time when $t=t_b$. The time at which rate constant changes from k_1 to k_2 is denoted by t_b (break point). Regulatory endpoints can be calculated by the following equation:

$$DT_x = \frac{\ln \frac{100}{100-x}}{k_1} \quad \text{if } DT_x \leq t_b$$

$$DT_x = t_b + \frac{\left[\ln \frac{100}{100-x} - k_1 t_b \right]}{k_2} \quad \text{if } DT_x > t_b$$

Double First Order in Parallel (DFOP)

The Double first order in parallel (DFOP) is a model which uses the sum of two first order equations. The integrated form of the bi-exponential model is a sum of two exponential equations with four parameters. Because of its exponentials, an autonomous constituting differential equation does not exist. The integrated form of this model is presented in equation (4):

$$C = C_0(g e^{-k_1 t} + (1 - g)e^{-k_2 t}) \quad (4)$$

where C is the compound concentration at time after application, C_0 is the initial concentration, k_1 and k_2 are the rate constant under each exponential equation, g is the fraction of degradation occurring under rate constant k_1 . There is no analytical equation to calculate degradation endpoints and these must be derived by an iterative procedure.

We conducted different Kruskal-Wallis tests to evaluate the effects of organic amendments on the dissipation of profoxydim. Statistical analysis was carried out using Statgraphics v.19 software (Statgraphics Technologies, Inc, USA).

Results and Discussion

Persistence of Profoxydim in Sterile Soils

Figure 5 shows the degradation of profoxydim in Seville and Galicia soils during 25 days. Error bars of replicates represent a good repeatability and good precision of the method performance. The degradation in Seville was greater than in Galicia. At the end of experiment, in Seville profoxydim was almost completely degraded, while in Galicia 30% remained undegraded.

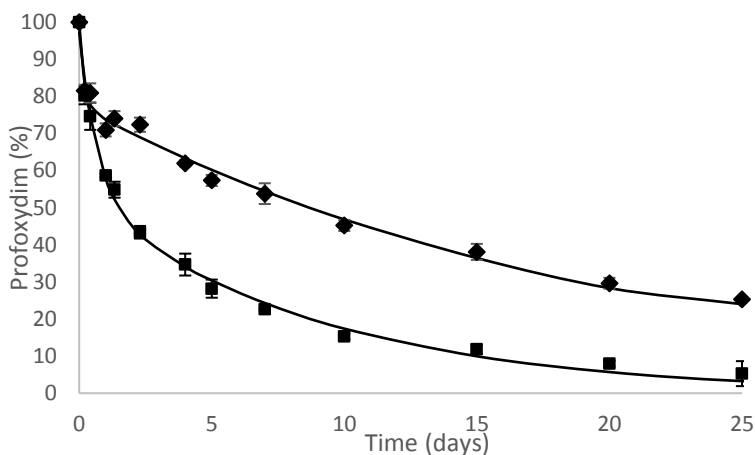


Figure 5: Degradation kinetic of profoxydim in Seville and Galicia soils. Each point is the mean of three replicates and the standard deviation. Experimental datasets are fitted to a DFOP kinetic model.

Following FOCUS recommendations [25], we tested the following kinetic models: SFO, FOMC, DFOP and HS for this experiment. In Seville, SFO kinetic model was rejected due to were obtained a high % error of the χ^2 test and a visual assessment did not show a good fit at the end of experiment (Table 2). Once the SFO was rejected, we tested FOMC that correspond to an intermediate between first-order and biphasic behavior. In this case we observe a better visual fitting which led to testing biphasic models as DFOP and HS. The best fit was obtained for DFOP model due to present a good visual assessment and low error of χ^2 test and half-life obtained for Seville was 1.65 days. (Table 2).

A similar situation occurred on Galicia soil: the SFO kinetic model did not achieve a good fit, observing biphasic behavior. Comparing the results obtained with biphasic models, the best model was DFOP, with good visual fit and low error. In this case, half-life obtained was 8.75 days a significant increase compared to SS (Table 3).

Table 2: Principal kinetic parameters of kinetic models for amended and nonamended Seville soil. SS (Seville soil), SS-40/SS-60 (Seville soil amended with 40/60% of alperujo).

Sample	Kinetic model	k (days)	χ^2 (%)	DT ₅₀ (days)	DT ₉₀ (days)
SS	SFO	0.22 ± 0.03	14.5	3.04	10.1
	FOMC	-	6.05	1.8	19.7
	DFOP	k ₁ =1.43 ± 0.27	4.7	1.65	15.2
		k ₂ = 0.11 ± 0.01			
	HS	k ₁ =0.46 ± 0.05	6.6	1.52	15.5
		k ₂ = 0.11 ± 0.01			
SS-40	SFO	0.66 ± 0.14	21	1.05	3.47
	FOMC	-	12.7	0.598	7.67
	DFOP	k ₁ =1.55 ± 0.93	9.1	0.69	8.59
		k ₂ = 0.12 ± 0.02			
	HS	k ₁ =0.80 ± 0.12	14.6	0.86	9.56
		k ₂ = 0.09 ± 0.19			
SS-60	SFO	0.3001 ± 0.05317	27	2.31	7.67
	FOMC	-	12.3	0.47	12.1
	DFOP	k ₁ =8.52 ± 3.29	6.27	0.55	8.13
		k ₂ = 0.21 ± 0.01			
	HS	k ₁ =1.66 ± 0.21	11	0.42	9.09
		k ₂ = 0.16 ± 0.04			

Table 3: Principal kinetic parameters of kinetic models for amended and nonamended Galicia soil. GS (Galicia soil), GS-40/GS-60 (Galicia soil amended with 40/60% of alperujo).

Sample	Kinetic model	k (days)	χ^2 (%)	DT ₅₀ (days)	DT ₉₀ (days)
GS	SFO	0.062 ± 0.002	7.46	11.6	38.6
	FOMC	-	6.04	9.83	344
	DFOP	k ₁ =5.82 ± 1.95	2.87	8.75	40.8
		k ₂ = 0.051 ± 0.003			
	HS	k ₁ =0.5422 ± 0.1276	3.67	9.2	41.8
		k ₂ = 0.049 ± 0.004			
GS-40	SFO	0.44 ± 0.11	21.9	1.55	5.16
	FOMC	-	6.5	0.65	16.5
	DFOP	k ₁ =2.49 ± 0.55	6.03	0.61	11.1
		k ₂ = 0.13 ± 0.02			
	HS	k ₁ =0.77 ± 0.11	11.2	0.89	13.6
		k ₂ = 0.09 ± 0.04			
GS-60	SFO	0.26 ± 0.03	21.8	2.61	8.66
	FOMC	-	10.7	0.70	16.2
	DFOP	k ₁ =3.37 ± 1.04	7.76	0.61	10.6
		k ₂ = 0.14 ± 0.02			
	HS	k ₁ =1.28 ± 0.18	9.61	0.54	11.2
		k ₂ = 0.13 ± 0.02			

We found significant differences between both soils (p-value=0.0005), as shown by the half-life, from 1.65 to 8.75 days, for Seville and Galicia, respectively. This could be attributed to the organic matter content and low pH of Galicia soil. GS has pH of 6.04 and organic matter of 3.73%, while SS has a higher pH 8.15 and lower organic matter 1.26%. It's known that cyclohexanedione herbicide presented pH dependence with acidic values, lower than 4 [14,26]. In our case, both soils have pH values above 6, so we suppose that this parameter did not

affect degradation in this experiment since degradation in Galicia should be faster than Seville soil.

Different authors reported that the high organic matter could increase the sorption of herbicide in soil [27,28]. This occurs in Galicia, where organic matter is quite high (3.73%), and its degradation slows down after 5 days when sorption can play a major role. This low persistence observed under these conditions generates the need to study the degradation products formed during degradation.

Also, different works observed the influence of some metals on the degradation of pesticides, as iron cations [29,30]. These studies shown a faster degradation of pesticides in presence of iron, regardless the chemical form. These results are in line with our experiments, since Seville soil has a higher content of iron compared to Galicia soil with a concentration of 46.76 mg kg⁻¹ (Seville) vs 15.22 mg kg⁻¹ (Galicia). This could be an explanation for the rapid degradation in the Seville soil. Further studies would be interested to be carried out to corroborate this hypothesis.

Effect of Alperujo in Degradation in Soil

In the literature, alperujo compost is one of the most common organic amendments used in agriculture [31-33]. To observe the effect of alperujo under laboratory conditions two high concentrations were tested 40 and 60% of alperujo (SS-40, SS-60, GS-40, GS-60). Table 2 shows the parameters of different kinetic models for experiments in SS, for both concentrations of alperujo we can observe values of χ^2 test above 20% for this reason the biphasic behavior was analyzed. The DFOP obtained the best fit in both doses of alperujo obtaining similar values of half-life 0.69 days for 40% and 0.55 days for 60% of alperujo.

In GS, also the DFOP model shown the best results of the fitting, supporting the biphasic model for this amended soil, obtaining a half-life of 0.61 days for both doses of alperujo.

Thus, as occur with unamended soils, the best fit for soils with alperujo were obtained with the DFOP kinetic model in both soils (Figure 6). Considering that degradation by microorganisms is neglected in sterile soils, the processes that may be involved are sorption and degradation. Thus, this biphasic behavior may be the result and can be explained by different chemical and sorption processes.

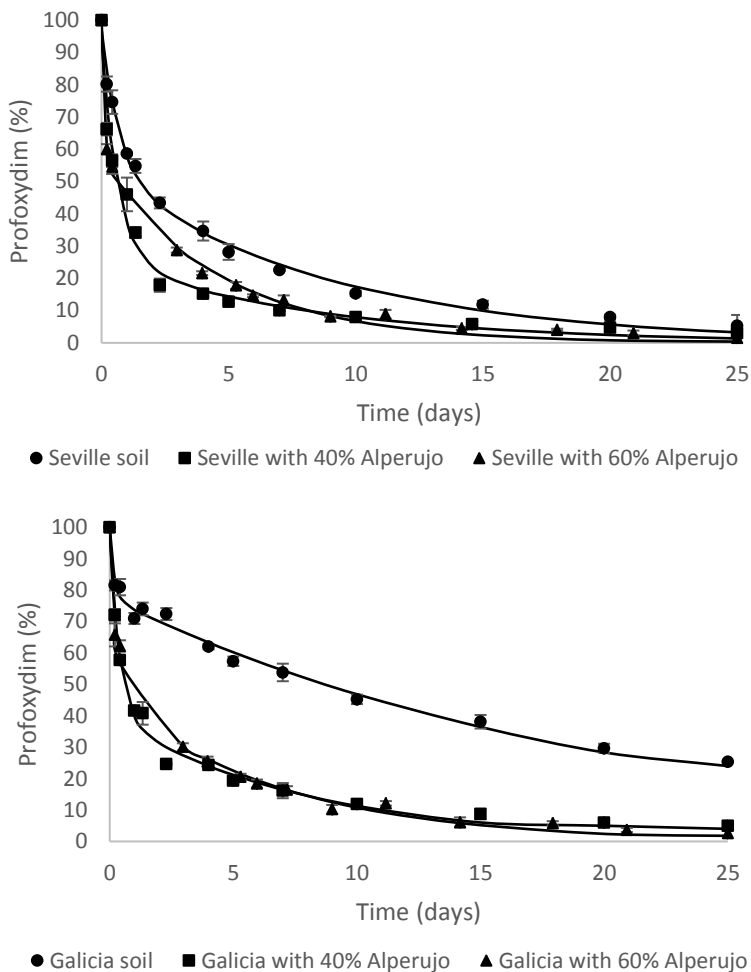


Figure 6: Degradation of profoxydim in Seville and Galicia soils unamended. SS-40/60, GS-40/60, Seville and Galicia soils amended with 40 and 60% of alperujo, respectively. Each point is the mean of three replicates and the standard deviation. Experimental datasets are fitted to a DFOP kinetic model.

Regarding the results in Seville, we can observe that amended and unamended soil obtained similar kinetic parameter with half-life from 1.6 for unamended soil to 0.55 and 0.69 for both concentrations of alperujo. No significant differences were found comparing these experiments in SS (p-value= 0.528). The increase in the rate of degradation is more noticeable at DT90 where it decreases from 15.2 days (unamended soil) to 8.59 and 8.13 days (alperujo amended soil) (Table 2). Our results differ from those of other authors who observed that the presence of alperujo produced a decrease in the degradation rate due to a higher sorption because of the increase in organic matter. [32,33].

On the other hand, the data obtained for GS showed visual differences in the kinetic curve (Figure 6). We can observe that in amended soil the degradation of profoxydim increase considerably. This increase in degradation is reflected in the half-lives with a significant decrease from 8.75 days in unamended soil to 0.61 days with amended soil at both doses. Significant differences were found comparing these experiments in GS (p-value= 0.006). Furthermore, this change in degradation is also observed in the DT 90 values, from 40.8 days for GS to 11.1 and 10.6 days in amended soils (Table 3).

As was previously the case in SS, the higher organic matter content of alperujo could not be responsible for the increased degradation related to the increase in microorganisms, as usually explained, as the soils were sterile. However, organic matter can have in its structure different functional groups (ketone, aldehyde, alcohol...) that can produce chemical reactions with profoxydim and degrade the molecule. On the other hand, one characteristic of alperujo is the high content of assimilable iron with a concentration $101.31 \text{ mg kg}^{-1}$. This value of iron content is very important compared to 15.51 mg kg^{-1} of the unamended soil. Thus, this metal could also favour the rapid degradation of profoxydim in line with other previous studies in pesticides [29,30]. It would be interesting to investigate further to find out the explanation for this behavior.

Evolution and Kinetic Modelling of Degradation Products

Since the degradation of profoxydim is so rapid, the study of degradation products is of great interest. A gradient method was developed by HPLC-DAD to detect the degradation products since degradation products present different physico-chemical properties than profoxydim. All by-products are more polar than the active substance, which increases solubility and thus the possibility of leaching to groundwater. Therefore, it is interesting to know more about their persistence and behavior in soil. Figure 7A, represents one of the experiments in Seville soil, the number of degradation products detected in both soils and amended soils were the same and similar amount of each one. HPLC chromatograms of profoxydim degradation shown five different byproducts, all of which were more polar than the parent compound.

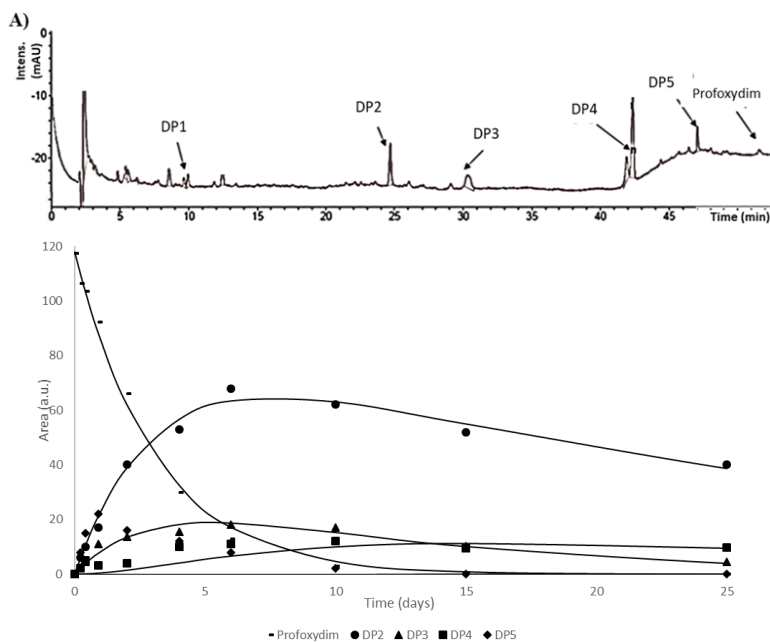


Figure 7: A) Typical HPLC-DAD chromatogram of profoxydim degradation in sterile Seville soil and its degradation products (DPs). B) Evolution of DPs formed during the degradation in soil of profoxydim.

The formation of these products shows a continuous increase as profoxydim degrades. The kinetic model for degradation products was adjusted to estimate the degradation parameters (Figure 7B) of the DPs, considering first order kinetic. This adjustment was not possible to monitor the DP1 due to the low amount formed. The degradation products DP3, DP4 and DP5 were minor and their concentrations were low during all the process of degradation. They reached their maximum concentration in the first 10 days and then the concentration slowly decreased.

The main degradation product, DP2, showed high stability, as after 25 days it had not completely degraded. Sanchez et al. [20] also obtained a more persistent degradation product than profoxydim in agreement with our main product DP2, which could be the same compound. Considering the FAO pesticide disposal, the herbicide profoxydim was classified as readily degradable, while the major by-product (DP2) has a longer degradation time and could be considered as fairly degradable.

Conclusions

The herbicide profoxydim is rapidly degraded in sterile soils under laboratory conditions due to chemical processes. The degradation of this herbicide in the studied soils presented a biphasic behavior, obtaining good fit with the DFOP kinetic model. The use of alperujo compost as organic amendment increased the degradation rate, reducing the persistence in soil of the herbicide. Degradation products formed are the same in unamended and amended soil, resulting the main byproduct more polar and persistent than the parent. In future, we will perform studies to identify degradation products and to assess the possible risk of the most stable DPs.

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