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Application of Diflufenican Herbicide on Soils Amended with Different Organic Wastes

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1. Introduction

Pesticides are important tools in agriculture that help to minimize economic losses caused by weeds, insects and pathogens and have greatly improved food production (Farenhorst, 2006; Song et al., 2008; Romero et al., 2010). The ideal outcome of pesticide use occurs when a pesticide accomplishes the purposes for which it was applied and then rapidly breaks down into harmless components, such as carbon dioxide and water. However, extensive use of pesticides in conventional agricultural practices has increased dramatically over time and the potential negative effects on human health and the environment are now of concern (Renaud et al., 2004; Majumdar & Singh, 2007; Song et al., 2008).

According to El-Nahhal (2003), achieving proper weed control needs some incorporation usually via rainfall or irrigation and movement of the active ingredients through the soil profile. However, movement of the active ingredients may be associated with three problems: (1) dilution of herbicide in the zone of weed seed germination and accumulation in the root zone of the crop which may cause crop injury, (2) reduction of herbicidal activity due to distribution of the active ingredient below the root zone of the target weeds and (3) possible contamination of ground water. Thus only a small fraction of the applied herbicide contributes to the desired activity. These problems may result in repeated applications and/or increasing the applied rate, increasing cost and potential for ecological damage.

In Europe, pesticides are considered hazardous substances in accordance with current legislation regarding water (Directives 2000/60/EC, 2006/11/EC). Because of this, public concern over the residues of pesticides in environment, food and related commodities has increased over the last decades (Majumdar & Singh, 2007).

The presence of pesticides in groundwater has grown considerably in the last few years (Cox et al., 1997, Renaud et al., 2004; Carabias Martínez et al., 2000; Gomides Freitas et al., 2008). The relatively high water solubility and not readily degradable of these chemicals makes them potentially mobile in soil and surface and ground waters vulnerable to contamination (Carrizosa et al., 2001; El-Nahhal, 2003). For this reason it is of maximum interest that the development of agricultural strategies be directed to the decrease in pesticide movement.

However, pesticides pollution is not only limited to groundwater. Several studies on widely-used pesticides such as benomyl, chlorothalonil, dinocap, chlorpyrifos, metsulfuron-methyl, 2,4- D, glyphosate, MCPA, etc. have already shown that pesticide application leads to changes in soil nutrient levels and alterations to soil microbial activity, diversity and/or genetic structure (Edwards, 1989; Chen et al., 2001; Pandey & Singh, 2004; Zabaloy & Gómez, 2008; Černohlávková et al., 2009). Consequently, disturbances of microbial communities ensuring several key ecological processes in soil such as organic matter degradation and nutrient cycling, could harmfully altered soil fertility and sustainable agricultural productivity. Therefore, population size, enzymatic activity and biodiversity of certain systematic and physiological groups of microorganisms may serve as bioindicators of changes taking place in the soil following herbicide application.

Many soil enzymes can be used as potential indicators of soil quality for sustainable management because they are sensitive to ecological stress and land management practices (Tejada et al., 2009; 2010). Enzymes may react to changes in soil management more quickly than other variables and therefore may be useful as early indicators of biological changes (Bandick & Dick, 1999; Masciandaro et al., 2004).

Dehydrogenase activity has been proposed as a measure of overall microbial activity (Masciandaro et al., 2001), since it is an intracellular enzyme related to oxidative phosphorylation processes (Trevors, 1984). García et al. (1997) found that dehydrogenase activity is a good index of the soil microbial biomass in semiarid Mediterranean areas.

Respect to the hydrolases enzymes, urease is involved in the hydrolysis of urea to carbon dioxide and ammonia, which can be assimilated by microbes and plants (Kizilkaya & Bayrakli, 2005). β-glucosidase catalyzes the hydrolysis of β-D-glucopyranoside, an important source of energy for soil microorganisms (Turner et al., 2002; Kizilkaya & Bayrakli, 2005). Phosphatase is the enzyme involved in the hydrolysis of organic phosphorus to different forms of inorganic phosphorus (Amador et al., 1997; Kizilkaya & Bayrakli, 2005). Arylsulphatase is the enzyme involved in the mineralization of ester sulphate in soils (Pascual et al., 1998; Kizilkaya & Bayrakli, 2005).

There have been many investigations into the mechanisms by which herbicides are adsorbed by soil components of soil, particularly the clay minerals and organic matter. Sorption of herbicides is determined by the complex interrelationship between the physiochemical properties of the adsorbate, the nature of soil constituents and the experimental and environmental conditions under which sorption occurs (Ying & Williams, 2000).

An increasing number of studies conducted at the field scale, with lysimeters or in the laboratory have shown that organic matter is the main soil component contributing to the sorption of herbicides (Renaud et al., 2004) and sorption is one of the main processes that reducing the mobility of these chemicals in soils, the addition of exogenous organic matter to soil land has been suggested as a possible method to reduce herbicide movement (Sheng et al., 2001; Ben-Hur et al., 2003). Application of organic carbon to soils in the form of compost, sludge, efluent and crop residues is a common practice, because of its low costs and recycling of nutrients (Majumdar & Singh, 2007; Song et al., 2008). The use of organic wastes to enrich soils of low organic matter content and to aid as nutrient input can modify surfaces of soils and subsurface materials promoting sorption of herbicides and retarding their movement as a secondary effect.

However, the influence of organic matter on soil properties and sorption process depends upon the type, amount and dominant components of the added organic materials (Tejada et

al., 2007, 2008). This aspect is of great interest, since it supposes an important advance in the behavior of the herbicides in the soil after addition of different sources of organic matter.

Organic amendment can also affect the soil microbiota and soil enzymes production and therefore, biodegradation of pesticides by enhancing microbial activity and thus biodegradation, although the increase in sorption with the organic amendment can also protect the pesticides from biodegradation (Cox et al., 1997). Thus, organic wastes affecting both soil properties and microbial populations, generating a more complex herbicide behaviour in the soil system (Romero et al., 2010).

The objectives of this study were (1) to evaluate the sorption and mobility of the diflufenican herbicide in a soil amended with two organic wastes, and (2) to study the influence of these organic wastes on the diflufenican herbicide polluted soil, by analyzing their effects on soil enzymatic activities.

2. Materials and methods

2.1 Soils, organic wastes and herbicide characteristics

The soils used in this experiment were a Xerollic Calciorthid and a Typic Xerofluvent (Soil Survey Staff, 1987). The main characteristics of both soils are shown in Table 1.

Soil pH was determined in distilled water with a glass electrode (soil:H_2O ratio 1:2.5). Soil texture was determined by the Robinson's pipette method (SSEW, 1982). Soil total N was determined by the Kjeldhal method (MAPA, 1986). Soil total C was determined by the method of Yeomans & Bremner (1988). Cation exchange capacity was determined with 1 M ammonium chloride solution in ethanol/water (60:40, v/v) at pH 8.2 (Tucker, 1954

	Xerollic Calciorthid	Typic Xerofluvent
pH	7.7 ± 0.2	7.6 ± 0.1
Clay (g kg^{-1})	382 ± 15	180 ± 12
Silt (g kg^{-1})	332 ± 27	131 ± 15
Sand (g kg^{-1})	286 ± 39	689 ± 21
Textural class	Clay loam	Sandy loam
Total N (g kg^{-1})	0.83 ± 0.04	0.41 ± 0.03
Total C (g kg^{-1})	6.4 ± 0.5	2.6 ± 0.7
C/N ratio	7.7 ± 2.1	6.3 ± 1.2
Cation exchange capacity (cmol$_c$ kg^{-1})	11.3 ± 1.1	4.1 ± 0.6

Table 1. Main features of the experimental soils (mean ± standard error). Data are the means of four samples

The organic wastes applied were the organic fraction of a municipal solid waste (MSW) and cow manure (CM). The general properties of the organic wastes are shown in Table 2. Organic matter was determined by dry combustion, according to the official methods of the Spanish Ministry of Agriculture (MAPA, 1986). Humic and fulvic acids-like were extracted with 0.1 M sodium pyrophosphate and 0.1 M sodium hydroxide at pH 13 (Kononova, 1966). The supernatant was acidified to pH 2 with HCl and allowed to stand for 24 h at room temperature. To separate humic acids-like from fulvic acids-like, the solution was centrifuged and the precipitate containing humic acids-like was dissolved with sodium hydroxide (Yeomans & Bremner, 1988). After the removal of humic acids-like, the acidic

filtrate containing the dissolved fulvic acid-like fraction was passed through a column of XAD-8 resin. The adsorbed fulvic was then recovered by elution with 0.1 M NaOH, desalted using Amberlyst 15-cation-exchange resin, and finally freeze-dried. The carbon content of humic and fulvic acids-like were determined by the method described. Total N was determined by the Kjeldhal method (MAPA, 1986). After nitric and perchloric acid digestion, total Ca, Mg, Fe, Cu, Mn, Zn, Cd, Pb, Ni and Cr concentrations were determined by atomic absorption spectrometer and K was determined by atomic emission spectrometer, according to MAPA methods (1986).

	MSW	CM
pH (H_2O)	6.2 ± 0.3	8.3 ± 0.2
Organic matter (g kg^{-1})	469 ± 15	764 ± 29
Humic acid-C (mg kg^{-1})	1030 ± 17	461 ± 13
Fulvic acid-C (mg kg^{-1})	711 ± 10	631 ± 24
Total N (g kg^{-1})	17.3 ± 1.3	29.2 ± 2.1
Fe (mg kg^{-1})	815 ± 38	407 ± 28
Cu (mg kg^{-1})	82.6 ± 9.8	24.2 ± 1.8
Mn (mg kg^{-1})	75.6 ± 8.1	14.1 ± 1.2
Zn (mg kg^{-1})	134 ± 13	10.3 ± 1.6
Cd (mg kg^{-1})	1.1 ± 0.3	0.28 ± 0.09
Pb (mg kg^{-1})	82.4 ± 3.6	5.3 ± 0.8
Ni (mg kg^{-1})	13.6 ± 1.5	2.4 ± 0.6
Cr (mg kg^{-1})	19.4 ± 1.7	0.29 ± 0.04

Table 2. Organic wastes chemical characteristics (mean ± standard error). Data are the means of four samples

Table 3 shows the acidic functional group contents of humic acids isolated from both organic wastes. 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 was calculated by addition (Ritchie & Perdue, 2003).

The herbicide used in this experiment was the diflufenican (Brodal®, Bayer CropScience). Diflufenican (2',4' - dichloro-2 - (α,α,α -trifluoro-m-toly-loxy) nicotinanilide, $C_{16}H_{11}F_5N_2O_2$) is a pre- and early post-emergence herbicide used for the selective control of broad leaf and grass weeds in winter cereals, with lower solubility in water than glyphosate and variable half-life in the environment (15 to 30 weeks) (Ashton et al., 1994). Brodal® physical and chemical properties are shown in Table 4.

	Total acidity	COOH	Phenolic-OH
	(mol kg^{-1})		
MSW	4.29 ± 0.04	3.19 ± 0.03	1.10 ± 0.03
CM	2.81 ± 0.02	2.00 ± 0.03	0.80 ± 0.01

Table 3. Acidic functional group contents (mean ± standard error) of humic acids isolated from MSW and CM. Data are the means of four samples

Ingredients:	Diflufenican	500 g l^{-1}
	Propane-1,2-diol	50 g l^{-1}
Appearance:		A viscous, light brown liquid suspension
Odour:		Characteristic slightly sweet
pH:		7.5 to 8.5 (undiluted)
Vapour pressure:		4.25 x 10^{-3} mPa (diflufenican) at 25 ° C
Vapour density:		Not available
Boiling point:		Greater than 100°C
Freezing/melting point:		Not available
Solubility:		Miscible with water
Density:		1.175 g ml^{-1} at 20° C
Flash Point:		> 100° C
Flammability (explosive) limits:		Not available
Auto-ignition temperature:		Not available
Partition coefficient (octanol/water):		Diflufenican: Log P_{OW}= 4.2

Table 4. Physical and chemical properties of Brodal®. The date are taken from Bayer CropScience

2.2 Experimental layout
2.2.1 Field experiments
For the field experiments and for each soil, the experimental layout was a randomized complete block design with four replications with a total amount of 24 plots, with each plot measuring 4 m × 3 m. Six treatments were used:

1. C, control soil (non organic amendment and without the application of herbicide)
2. C+H, plots non organic amendment and with application of herbicide
3. C+CM, plots organic amendment with CM and without the application of herbicide
4. C+MSW, plots organic amendment with MSW and without the application of herbicide
5. C+CM+H, plots organic amendment with CM and with the application of herbicide
6. C+MSW+H, plots organic amendment with MSW and with the application of herbicide

The CM was applied at a rate of 5 t ha^{-1} (fresh matter), whereas MSW was applied at a rate of 8.1 t ha^{-1} (fresh matter), in order to apply the same amount of organic matter to soil. The herbicide dose used was 200 ml ha^{-1}). The dose used was 200 ml ha^{-1}. Organic wastes were applied to the soil 1 month before applying the herbicide. Organic wastes were mechanically mixed with the soil, allowing the organic wastes to reach up to 25 cm from the soil surface

At 3, 15, 45, 90, 120, 150, 180, 210 and 250 days after applying the herbicide to soil, diflufenican was measured. To extract diflufenican we used the method described by Tejada (2009). Five grams of soil was shaken with 20 ml of 90:10 v/v methanol:H$_2$O for 1 h at 25 ± 1° C. After allowing the soil to settle, pesticide concentrations were determined by HPLC using a column Merck C18 ultrasphere (250 x 4.6 mm x 5 μm and 80 Å). The pesticide was eluted with a mobile phase of acetonitrile:H$_2$O:orthophosphoric acid of 85:15:0.25. The flow rate and wavelength were 1 ml min^{-1} and 220 nm. The temperature was constant at 25 °C using a Model 234 Column Oven Heater. The injected volume was 50 μl.

Also, the activity levels of four soil enzymes were measured at 3, 15, 45, 90, 120, 150, 180, 210 and 250 days after applying the herbicide to soil. Dehydrogenase activity was measured by

reduction of 2-*p*-iodo-3-nitrophenyl 5-phenyl tetrazolium chloride to iodonitrophenyl formazan (García et al., 1993). Urease activity was determined by the buffered method of Kandeler & Gerver (1988) using urea as substrate. β-Glucosidase activity was determined using *p*-nitrophenyl-β-D-glucopyranoside as substrate (Masciandaro et al., 1994). Phosphatase activity was measured using *p*-nitrophenyl phosphate as substrate (Tabatabai & Bremner, 1969).

2.2.2 Laboratory experiments

For the laboratory experiments and for each soil, sorption studies and leaching experiments in methacrylate columns were made.

For sorption studies and for each experimental soil, the treatments used were:

1.   C, non-organic amended control soil (10 g of soil)
2.   C+CM, soil amended with CW at rate of 10% (10 g of soil + 1 g of CW)
3.   C+MSW, soil amended with MSW at a rate of 16.3% (10 g of soil + 1.63 g of CW)

Diflufenican sorption was determined according to Cabrera et al. (2009) criteria. Triplicate samples (5 g) of the unamended and organic amended soil (C, C+CM and C+MSW) were treated with 10 ml of diflufenican (50%:50%, v/v) solution (initial concentrations, Ci, ranging from to 50 μM in 0.01 CaCl$_2$). Previously, it was determined that equilibrium was reached in less than 24 h, and that no measurable degradation occurred during this period. Equilibrium concentrations (Ce) in the supernatants were determined by HPLC. Sorption isotherms were fitted to Freundlich equation (Cs=K$_f$ x Ce$^{1/n_f}$) and sorption coefficients K$_f$ and $1/n_f$ were calculated.

The diflufenican analysis were performed following the methodology previously described.

The system to determine diflufenican leaching in soils, consisted of 30-cm long X 3.1 cm i.d. methacrylate columns filled with 20 cm of soil. Glass wool plus 10 g of sea was placed on the bottom of the columns to prevent losses of soil and contamination of leachates with soil particles. The columns were hand-packed with 180 g of soil without and with organic wastes (10 g of MSW and 10.9 g of CM) with the intention of applied the same amount of organic matter and 10 g of sea sand was placed on the soil surface.

The columns were saturated with water, allowed to drain for 24 h, and then the amount of diflufenican corresponding to an application rate of 2 kg ha^{-1} was applied to the top of the columns. Daily, the columns were leached with 200 mm d^{-1} of distilled water and the leachates were collected and filtered. The diflufenican concentration was analyzed by HPLC at 1, 3, 7, 10, 15, 20 and 30 days after the application of herbicide to soil. The leaching experiment was conducted in triplicate.

2.3 Statistical analysis

Data were submitted to two-way ANOVA with treatment and sampling time as factors using the Statgraphics Plus 2.1 software package. The means were separated by the Tukey's test, considering a significance level of $P<0.05$ throughout the study. For the ANOVA, triplicate data were used for each treatment and every incubation day.

3. Results

3.1 Field experiments

During the experimental period and for both soils, the diflufenican soil contents applied as Brodal® presented higher values in clay loam texture soil than for sandy loam texture soil

(Figure 1). The slow diflufenican degradation makes think that the degradation of the diflufenican is not carried out in short time, indicating that this degradation happens superior at one time to the 250 days. For both soils, the diflufenican contents were decreasing progressively in soils amended with organic matter. However, this decrease depended on soil type and organic matter type applied. At the end of the experimental period and compared with the unamended soil, the diflufenican content significantly decreased 25.5% and 41.2% in the amended with CM and MSW, respectively, whereas for the sandy loam texture soil the diflufenican content significantly decreased 32.5% and 50.2% in the amended with CM and MSW, respectively.

Table 5 shows the evolution of soil dehydrogenase activity during the experimental period. The results indicated that at the end of the experiment and compared with the control soil, the dehydrogenase activity significantly decreased in the non-organic amended polluted soils (59.4% for the clay loam texture soil and 53.8% for the sandy loam texture soil), reflecting the adverse effects of the herbicide on this intracellular activity. Also and compared to the control soil, in non-polluted soil the application of organic matter increased significantly the dehydrogenase activity. However, this increase depended of the soil type and organic matter applied to the soil. For the Xerollic Calciorthid soil and at the end of the incubation period, the dehydrogenase activity increased 92.9% and 80% in soil amended with MSW and CM, respectively, whereas for the Typic Xerofluvent soil, the dehydrogenase activity increased 93.5% and 82.4% in soil amended with MSW and CM, respectively. The application of herbicide in organic-amended soils decreased dehydrogenase activity. However, this decrease was lower than for the non-amended herbicide polluted soil. At the end of the experiment and for the clay loam texture soil, the dehydrogenase activity significantly decreased 23.9% for the C+MSW+H treatment (compared to the C+MSW treatment), followed by 24.7% for the C+CM+H treatment (compared to the C+CM treatment), whereas for the sandy loam texture soil, the dehydrogenase significantly decreased 18.3% for the C+MSW+H treatment (compared to the C+MSW treatment), followed by 20.4% for the C+CM+H treatment (compared to the C+CM treatment).

Similar to the dehydrogenase activity, at the end of the experimental period the urease activity decreased in the non-organic polluted soils compared to the non-organic unpolluted soils (32.5% for the Xerollic Calciorthid soil and 28.6% for the Typic Xerofluvent soil) (Table 6). At the end of the incubation period, the application of organic matter to unpolluted soils significantly increased the soil urease activity. Again, this increase was higher for MSW-amended soils than for CM-amended soils. Also, the application of herbicide in organic-amended soils decreased urease activity. For the Xerollic Calciorthid soil, the urease activity decreased 15.4% for the C+MSW+H treatment (compared to the C+MSW treatment), followed by 21.9% for the C+CM+H treatment (compared to the C+CM treatment), whereas for the Typic Xerofluvent soil, the urease activity decreased 15% for the C+MSW+H treatment (compared to the C+MSW treatment), followed by 19% for the C+CM+H treatment (compared to the C+CM treatment).

The evolution of β-glucosidase and phosphatase activities was very similar to the enzymes described (Tables 7 and 8). The application of diflufenican in organic-amended soils decreased both enzymatic activities. Again, this decrease was higher in the sandy loam texture soil than the clay loam texture soil. Also, the application of organic matter to soils contaminated with the herbicide increased both enzyme activities compared with the non-organic amended polluted soils.
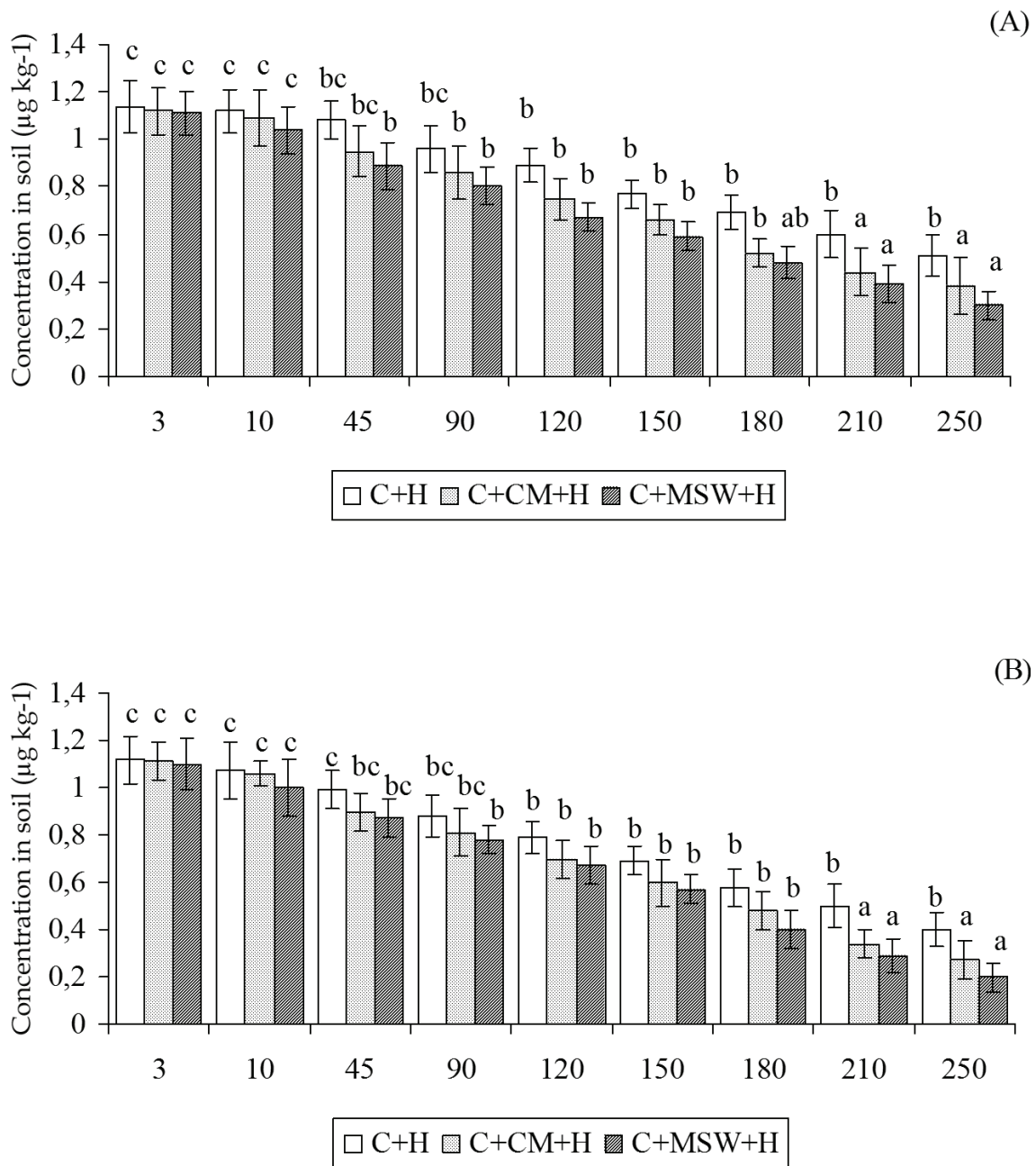
Fig. 1. Evolution of diflufenican (mean ± standard errors) in Xerollic Calciorthid (A) and Typic Xerofluvent (B) during the experimental for all experimental treatments
Column followed by the same letter(s) are not significantly different (*p*<0.05)

Table 5. Evolution of dehydrogenase activity (μINTF g^{-1} h^{-1}) (mean ± standard errors) in Xerollic Calciorthid and Typic Xerofluvent during the experimental for all experimental treatments. INTF: 2-*p*-iodo-3-nitrophenyl formazan

†Different letters following the numbers indicate a significant difference at *P*<0.05

	3	15	45	90	120	150	
			Xerollic Calciorthid				
C	2.5b† ± 0.4	2.4b ± 0.6	2.3b ± 0.3	2.1b ± 0.3	2.1b ± 0.4	2.0b ± 0.5	1.9
C+H	2.2b ± 0.7	1.9b ± 0.3	1.7b ± 0.4	1.4a ± 0.5	1.1a ± 0.4	0.92a ± 0.15	0.88
C+CM	2.8b ± 1.0	3.2bc ± 0.9	4.0bc ± 1.2	4.7c ± 1.1	5.4c ± 1.4	6.1c ± 1.7	7.0
C+MSW	2.9b ± 0.8	3.3bc ± 1.1	4.3bc ± 1.0	5.0c ± 1.2	5.9c ± 1.1	6.5c ± 1.6	7.6
C+CM+H	2.2b ± 0.5	2.6b ± 0.8	3.3bc ± 1.1	3.7bc ± 1.3	4.1bc ± 1.6	4.6c ± 1.0	5.3
C+MSW+H	2.3b ± 0.6	2.8b ± 1.0	3.7bc ± 1.0	4.2bc ± 0.9	4.9c ± 1.5	5.5c ± 1.3	6.1
			Typic Xerofluvent				
C	2.0b† ± 0.5	2.0b ± 0.6	1.8b ± 0.8	1.7b ± 0.6	1.6b ± 0.3	1.6b ± 0.4	1.5
C+H	1.7b ± 0.3	1.5b ± 0.5	1.3b ± 0.2	1.1a ± 0.2	0.96a ± 0.11	0.89a ± 0.14	0.75
C+CM	2.3b ± 0.4	2.6b ± 1.0	3.5bc ± 1.3	4.0bc ± 1.1	4.6c ± 1.2	5.3c ± 1.0	5.9
C+MSW	2.4b ± 0.3	2.9b ± 0.9	3.9bc ± 1.2	4.8c ± 1.3	5.9c ± 1.0	6.9c ± 1.3	7.8
C+CM+H	2.1b ± 0.7	2.3b ± 0.6	2.9b ± 1.5	3.4bc ± 1.1	4.0bc ± 1.0	4.4bc ± 1.2	4.9
C+MSW+H	2.2b ± 0.5	2.5b ± 0.7	3.3bc ± 1.0	3.8bc ± 0.8	4.3bc ± 0.9	5.1c ± 1.1	5.6

Table 6. Evolution of urease activity (μmol NH_4^+ g^{-1} h^{-1}) (mean ± standard errors) in Xerollic Calciorthid and Typic Xerofluvent during the experimental for all experimental treatments
†Different letters following the numbers indicate a significant difference at $P<0.05$

Xerollic Calciorthid						
	3	15	45	90	120	150	
C	1.6† ± 0.3	1.5b ± 0.2	1.2ab ± 0.3	1.2ab ± 0.2	1.1ab ± 0.3	1.0ab ± 0.2	0.91a
C+H	1.5b ± 0.6	1.3b ± 0.5	1.1ab ± 0.2	0.95ab ± 0.19	0.88a ± 0.12	0.79a ± 0.18	0.68a
C+CM	1.7b ± 0.4	1.8b ± 0.4	2.3b ± 0.6	2.5bc ± 0.9	2.6bc ± 1.0	2.8b ± 0.6	2.9b
C+MSW	1.7b ± 0.2	1.8b ± 0.7	2.5bc ± 0.5	2.7bc ± 0.8	3.0bc ± 0.7	3.2c ± 0.7	3.4c
C+CM+H	1.7b ± 0.5	1.7b ± 0.5	2.0b ± 0.4	2.1b ± 0.3	2.1b ± 0.6	2.2bc ± 0.7	2.3b
C+MSW+H	1.7b ± 0.4	1.6b ± 0.3	2.2b ± 0.6	2.4bc ± 0.5	2.5bc ± 0.5	2.7bc ± 0.4	2.9b

Typic Xerofluvent						
C	1.3b† ± 0.2	1.2b ± 0.3	0.98ab ± 0.12	0.88ab ± 0.15	0.76ab ± 0.11	0.68a ± 0.10	0.59a
C+H	1.2b ± 0.3	1.0b ± 0.1	0.90ab ± 0.15	0.82ab ± 0.16	0.74ab ± 0.17	0.62a ± 0.10	0.50a
C+CM	1.4b ± 0.3	1.5b ± 0.2	1.6b ± 0.4	1.7b ± 0.4	1.8b ± 0.5	1.9b ± 0.5	2.0b
C+MSW	1.4b ± 0.2	1.6b ± 0.4	1.8b ± 0.4	2.2bc ± 0.8	2.5bc ± 0.4	2.9bc ± 0.7	3.2c
C+CM+H	1.3b ± 0.2	1.3b ± 0.2	1.4b ± 0.3	1.4b ± 0.3	1.3b ± 0.2	1.5b ± 0.2	1.6b
C+MSW+H	1.4b ± 0.3	1.6b ± 0.3	1.9b ± 0.5	2.2bc ± 0.9	2.5bc ± 0.6	2.7bc ± 0.8	2.9b

Table 7. Evolution of β-glucosidase activity (μmol PNP g⁻¹ h⁻¹) (mean ± standard errors) in Xerollic Calciorthid and Typic Xerofluvent during the experimental for all experimental treatments. PNP: p-nitrophenol

†Different letters following the numbers indicate a significant difference at $P<0.05$

	3	15	45	90	120	150	
				Xerollic Calciorthid			
C	5.6b† ± 1.9	5.6b ± 1.8	5.5b ± 1.3	5.4b ± 1.1	5.3b ± 1.4	5.1b ± 1.2	4.9
C+H	5.2b ± 1.5	5.0b ± 1.1	4.5ab ± 0.9	4.0ab ± 1.1	3.3a ± 0.8	2.9a ± 1.0	2.5
C+CM	6.7b ± 1.8	8.0b ± 1.6	10.8c ± 1.1	12.5c ± 1.4	14.6c ± 1.5	16.6c ± 1.7	18.1
C+MSW	6.8b ± 2.0	8.2b ± 2.0	11.9c ± 1.6	13.8c ± 1.7	15.4c ± 1.6	17.8c ± 1.6	20.2
C+CM+H	6.5b ± 1.5	6.9b ± 1.6	8.8b ± 1.0	9.7bc ± 1.5	11.8c ± 1.1	14.1c ± 1.4	15.6
C+MSW+H	6.6b ± 1.8	7.5b ± 2.1	10.0bc ± 1.8	12.1c ± 1.2	14.6c ± 1.3	15.7c ± 1.8	17.6
				Typic Xerofluvent			
C	3.8bc† ± 1.1	3.7bc ± 1.3	3.4b ± 0.9	3.3b ± 1.0	3.2b ± 1.1	3.0b ± 0.6	2.9
C+H	3.6bc ± 1.3	3.5b ± 1.1	3.1b ± 0.8	2.7b ± 0.8	2.4b ± 0.7	2.1a ± 0.4	1.7
C+CM	4.0bc ± 1.6	4.5c ± 1.5	6.7c ± 1.5	8.0c ± 1.4	9.2c ± 1.5	10.3cd ± 1.9	11.5
C+MSW	4.1bc ± 1.2	4.8c ± 1.6	7.2c ± 1.3	8.9c ± 2.0	10.1cd ± 1.9	11.5d ± 1.6	13.0
C+CM+H	3.9bc ± 1.0	4.2c ± 1.2	5.4c ± 1.1	6.2c ± 1.6	7.5c ± 1.6	8.6c ± 1.1	9.7c
C+MSW+H	3.9bc ± 1.3	4.5c ± 1.3	5.9c ± 1.9	7.0c ± 1.5	8.2c ± 1.8	9.9cd ± 1.3	11.0

Table 8. Evolution of phosphatase activity (μmol PNP g^{-1} h^{-1}) (mean ± standard errors) in Xerollic Calciorthid and Typic Xerofluvent during the experimental for all experimental treatments. PNP: p-nitrophenol

†Different letters following the numbers indicate a significant difference at $P < 0.05$

	3	15	45	90	120	150	
				Xerollic Calciorthid			
C	21.8b† ± 2.3	21.7b ± 2.0	21.2b ± 2.3	20.8b ± 2.2	20.5b ± 2.6	20.1b ± 2.4	19.7
C+H	21.0b ± 1.9	19.8b ± 1.6	17.8a ± 1.6	16.7a ± 1.5	15.5a ± 1.6	14.7a ± 1.4	13.6
C+CM	22.2b ± 2.0	23.1b ± 1.7	26.2bc ± 2.1	27.9c ± 2.2	29.1c ± 3.0	30.2c ± 2.6	31.4
C+MSW	22.4b ± 2.4	23.7b ± 2.5	27.0bc ± 1.8	28.4c ± 2.3	30.2c ± 1.7	31.8c ± 2.6	33.0
C+CM+H	21.7b ± 2.0	22.0b ± 1.8	22.9bc ± 2.4	24.0bc ± 2.4	25.0bc ± 2.8	25.8bc ± 1.9	26.5
C+MSW+H	21.7b ± 2.2	22.3b ± 2.4	24.4bc ± 2.2	25.4bc ± 2.1	26.1bc ± 1.4	27.5c ± 2.0	28.8
				Typic Xerofluvent			
C	18.7b† ± 1.2	18.5b ± 1.3	18.2b ± 1.4	17.9b ± 1.1	17.5b ± 1.9	16.6b ± 1.4	16.1
C+H	18.2b ± 1.6	17.1b ± 1.5	15.1b ± 1.1	14.0a ± 1.4	13.2a ± 1.0	12.0a ± 1.8	11.1
C+CM	19.2b ± 1.3	19.9b ± 1.1	22.5bc ± 1.4	23.7c ± 2.0	24.9c ± 2.1	26.6c ± 1.4	27.8
C+MSW	19.4b ± 1.5	20.3b ± 1.8	23.6c ± 2.3	24.9c ± 1.8	26.5c ± 2.0	28.9c ± 1.7	30.6
C+CM+H	19.1b ± 1.7	19.5b ± 1.6	21.1b ± 2.2	22.0bc ± 1.2	22.7bc ± 2.0	23.6c ± 2.2	24.6
C+MSW+H	19.1b ± 1.8	19.8b ± 1.8	21.8b ± 1.8	22.9bc ± 1.6	24.2c ± 1.2	25.0c ± 1.9	26.6

3.2 Laboratory experiments

For both soils, sorption isotherms of diflufenican on C, C+MSW, and C+CM treatments are shown in Figure 2. The results indicated that sorption of diflufenican on the clay loam texture soil was higher than the sandy loam texture soil. Also and for each experimental soil, the sorption of diflufenican on organic amended soils increased compared to non-organic amended soil. For each organic amended soil, the herbicide sorption with MSW was higher than with CM.
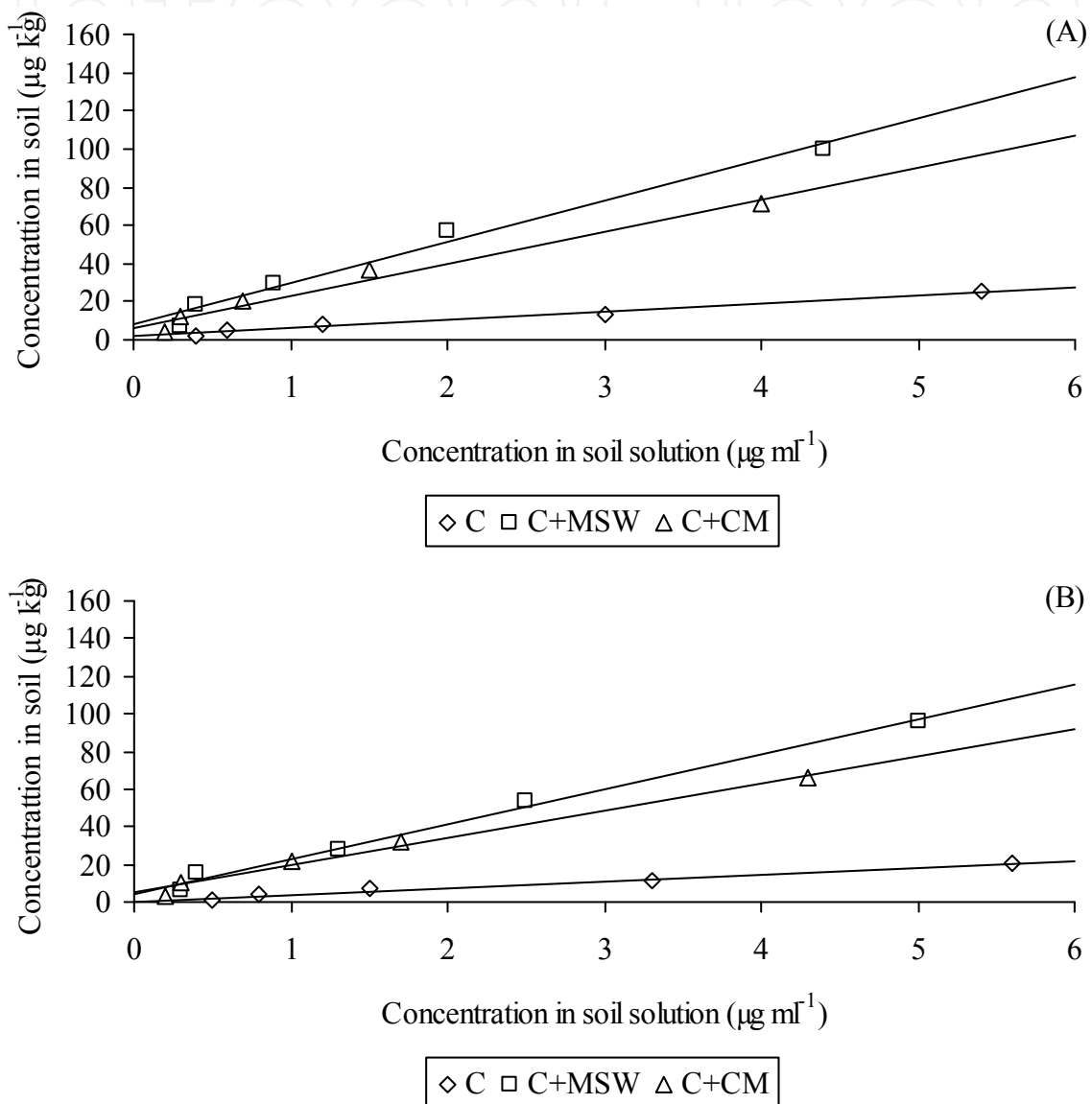

Fig. 2. Diflufenican sorption isotherms for Xerollic Calciorthid soil (A) and Typic Xerofluvent (B) soil. Symbols are experimental data points, whereas lines are the Freundlick-fit sorption isotherms

Sorption isotherms were fit to the Freundlich equation and sorption coefficients K_f and $1/n_f$ were calculated (Table 9). For non-organic amended soils, the K_f values were highest for Xerollic Calciorthid soil than for the Typic Xerofluvent soil. For both experimental soils, the results indicated that K_f values significantly increased in organic amended soils than for

non-organic amended soils. However, diflufenican sorption was higher in soils amended with MSW than with CM. Also, the $1/n_f$ coefficients significantly decreased in organic amended soils than for non-organic amended soil. For organic amended soils, the $1/n_f$ coefficient was higher in the soil amended with MSW than for CM.

	Xerollic Calciorthid		
	K_f	$1/n_f$	R^2
C	7.94a[†] ± 1.2	0.89b ± 0.08	0.982
C+M	28.45b ± 2.3	0.77a ± 0.11	0.974
C+MSW	34.72b ± 2.8	0.81a ± 0.07	0.981
	Typic Xerofluvent		
C	5.76a[†] ± 0.96	0.93b ± 0.05	0.978
C+M	24.69b ± 2.8	0.81a ± 0.09	0.984
C+MSW	30.15b ± 2.7	0.87a ± 0.06	0.993

Table 9. Freundlich sorption coefficients K_f and $1/n_f$ (mean ± standard errors) for diflufenican in unamended and organic amended soils.
[†]Different letters following the numbers indicate a significant difference at *P<0.05*

Figure 3 shows the cumulative diflufenican leachates applied to unamended and organic-amended soil columns. At the end of the experiment, the diflufenican leachates were higher in the Xerollic Calciorthid soil than in the Typic Xerofluvent soil. Diflufenican leachates significantly decreased in organic-amended soils. However, this decrease depended of the organic matter type applied to the soil. For the Xerollic Calciorthid soil, the maximum concentratin of diflufenican in leachates was reduced from 7.4 µM for the unamended soil, to 4.1 µM or 4.9 µM for the MSW or CM-amended soil, whereas for the the Typic Xerofluvent soil the maximum concentratin of diflufenican in leachates was reduced from 8.9 µM for the unamended soil, to 5.8 µM or 6.2 µM for the MSW or CM-amended soil For both soils, no significant differences between the herbicide in leachates for organic-amended soils exits.

4. Discussion

Firstly, our results suggested that the herbicide time persistence depended of the soil texture type. The highest contents of diflufenican occurred in the clay loam texture soil probably due to adsorption of the herbicide with the clay, and therefore, the herbicide leachates were lowest in the clay loam texture soil than in sandy loam texture soil. These results are in agreement with those obtained by Singh et al., (2002), Renaud et al. (2004), Yen et al. (2003) and Flores et al. (2009), who suggested that when in low soil organic matter content, texture plays a fundamental role in the adsorption process of pesticides in soil. Also, these results suggest that the diflufenican persistence time was higher in the clay loam texture soil than in sandy loam texture soil. These results are in agreement with those obtained by Rouchaud et al. (1998) and Flores et al. (2009), who suggested that in soils with low organic matter content the herbicide adsorption, persistence and mobility depends mainly on soil texture.

Also, our results indicated that the diflufenican herbicide caused a toxic effect on soil enzymatic activity. These results are in agreement with those obtained by Tejada (2009), which observed the toxic effect of this herbicide in microcosm studies. Therefore, the study of soil enzymatic activities are an essential tool for knowing the state of soil pollution (Gianfreda et al., 2005; Tejada et al., 2007, 2008, Tejada, 2009). Since the diflufenican content
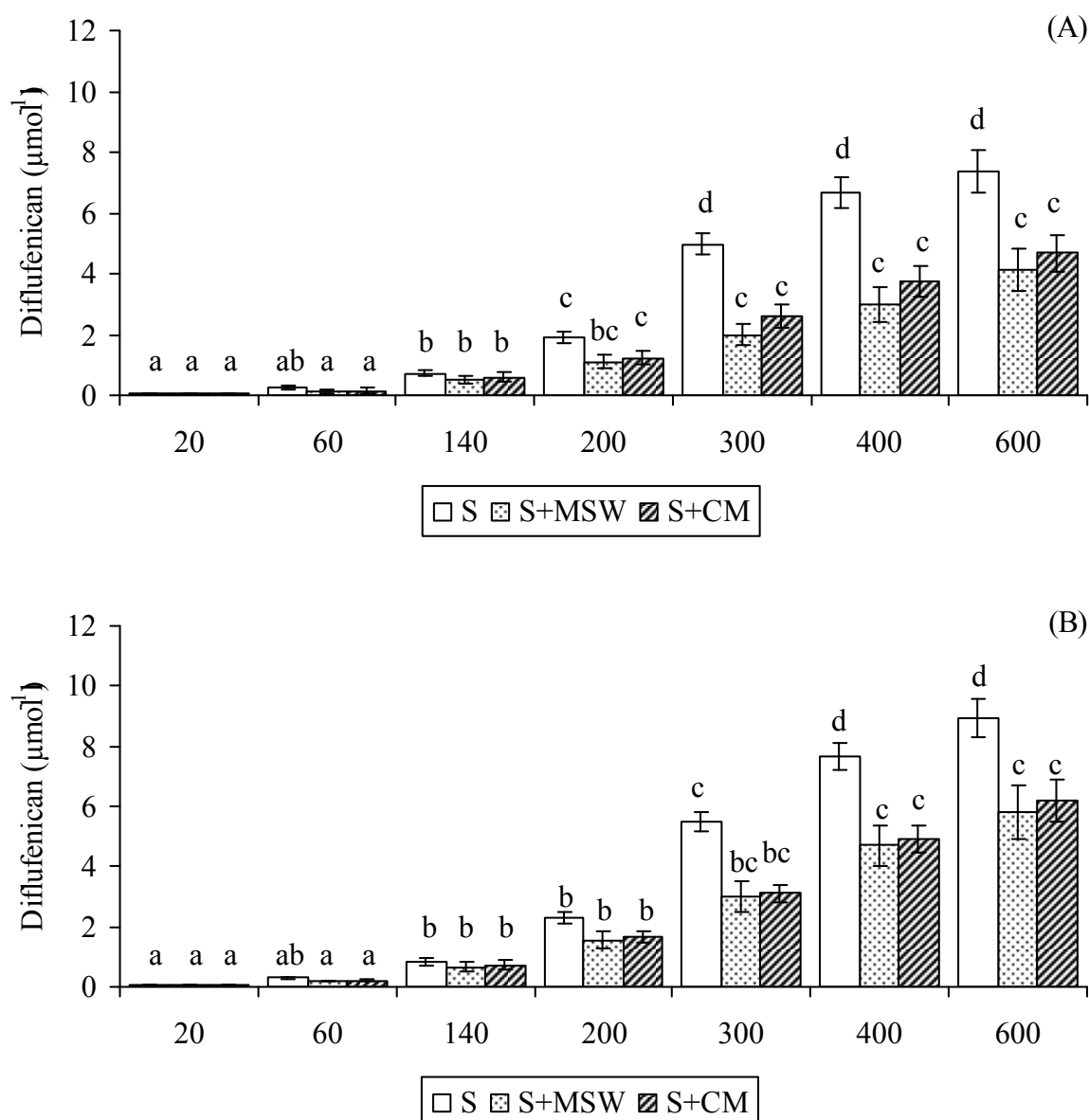
Fig. 3. Cumulative diflufenican leachates (mean ± standard errors) in unamended and organic amended treatments for Xerollic Calciorthid soil (A) and Typic Xerofluvent (B) soil. Column followed by the same letter(s) are not significantly different ($p < 0.05$)

was higher the clay loam texture soil than in sandy loam texture soil, the inhibition of soil enzymatic activities in soil was higher in the Calciorthid Xerollic soil than in the Typic Xerofluvent soil.

The application of organic matter in unpolluted soils significantly increased enzymatic activities (Ros et al., 2003; Ferreras et al., 2006, Tejada et al., 2010). Soil microorganisms degrade organic matter through the production of a variety of extracellular enzymes, which explains the increase in enzymatic activities observed after the application of vermicomposts to the soil. However, this increase in soil enzyme activity was higher in the MSW than in the CM-amended soil. According to Tejada et al. (2010), the organic matter with higher fulvic than humic-acids contents is degraded more rapidly in the soil, promoting more positively the biological properties.

The application of the herbicide in MSW and CM-amended soils caused a decrease in the soil enzymatic activity inhibition. The diflufenican sorption isotherms and Freundlich sorption coefficients obtained in this study, suggested that organic matter play a fundamental role in the sorption of the herbicide in agricultural soils, probably as a result of the humic substances containing several major functional groups, such as carboxyl, phenolic, alcohol and carbonyl (Sluzny et al., 1999; Datta et al., 2001) According to Rouchaud et al. (1998) and Flores et al. (2009), when soils have a high content of organic matter, herbicide adsorption, persistence and mobility in soil depends mainly on the organic matter, whereas soil texture playing a more secondary role. However, our results also suggested that the chemical composition of the organic matter influenced in the diflufenican sorption.

Several studies of metal complexation with organic matter indicated that the sorption of heavy metals increased when the humic acid-like content increased in the organic matter, compared to the fulvic acid content, probably due to the humic acid-like possess a higher number of carboxylic groups than fulvic acid (Tejada et al., 2007, 2008).

The diflufenican sorption isotherms and Freundlich sorption coefficients indicated higher herbicide sorption in MSW than for CM-amended soils. Therefore, and similar to the heavy metals complexation, the sorption of herbicide increased with the humic acid content in the organic waste applied to the soil. The higher sorption probably caused a higher decrease of herbicide in the soil solution, and therefore, lowest availability of diflufenican availability for the soil microorganisms. This fact probably is the responsible of the increase in the soil enzymatic activities.

Also, the adsorption of the herbicide in organic-amended soils produced a decrease of the leachates herbicide. Since the diflufenican adsorption in soils amended with organic matter rich in humic acids was higher, herbicide losses were lower in soils amended with MSW than with CM.

5. Conclusions

It can be concluded that the diflufenican adsorption, persistence and mobility depends mainly on soil texture. Since the herbicide adsorption was higher in in the clay loam texture soil than in sandy loam texture soil, the leaching losses were highest in sandy loam texture soil than in clay loam texture soil. Also, the diflufenican herbicide caused a negative effect on soil enzymatic activities. The application of organic matter to the soil is a good environmental practice because decrease the diflufenican concentration in leachates and decreased the enzymatic activities inhibition. However, the beneficial effect depended of the organic matter chemical composition. The herbicide decrease was higher in MSW than for CM-amended soils. These results indicated that the addition of organic materials with a higher humic acid concentration may be considered a good strategy for decreased the diflufenican environmental pollution.

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Herbicides and Environment

Edited by Dr Andreas Kortekamp

Herbicides are much more than just weed killers. They may exhibit beneficial or adverse effects on other organisms. Given their toxicological, environmental but also agricultural relevance, herbicides are an interesting field of activity not only for scientists working in the field of agriculture. It seems that the investigation of herbicide-induced effects on weeds, crop plants, ecosystems, microorganisms, and higher organism requires a multidisciplinary approach. Some important aspects regarding the multisided impacts of herbicides on the living world are highlighted in this book. I am sure that the readers will find a lot of helpful information, even if they are only slightly interested in the topic.

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