REDUCED METRIBUZIN POLLUTION WITH PHOSPHATIDYLCHOLINE-CLAY FORMULATIONS

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ABSTRACT

BACKGROUND: Metribuzin is a widely used herbicide that has been identified as a groundwater contaminant. In this study, slow release formulations of metribuzin were designed by encapsulating the active ingredient in phosphatidylcholine (PC) vesicles and adsorbing the vesicles onto montmorillonite.

RESULTS: The maximum active ingredient content in the slow release formulations was 24.6% (w:w). Infrared spectroscopy results revealed that the hydrophobic interactions between metribuzin and the alkyl chains on PC were necessary for encapsulation. In addition, water bridges connecting the herbicide and the PC headgroup enhanced the solubility of metribuzin in PC. Adsorption experiments in soils were performed to evaluate the relationship between sorption and leaching. Funnel experiments in a sandy soil revealed that the herbicide was not irreversibly retained in the formulation matrix. In soil column experiments, PC-clay formulations enhanced herbicide accumulation and biological activity in the top soil layer relative to a commercial formulation. PC-clay formulations also reduced the dissipation of metribuzin by a factor of 1.6-2.5.

CONCLUSION: A reduction in the recommended dose of metribuzin can be achieved by employing PC-clay formulations, which reduces the environmental risk associated with herbicide applications. Moreover, PC and montmorillonite are non-toxic and do not negatively affect the environment.

Keywords: metribuzin, liposomes, slow release formulation, leaching, dissipation

Introduction

Surfactants are widely used in agricultural formulations of herbicides to improve the physical properties of the formulations. Surfactants act as a spreader, sticker, antifoamer, and compatibility agents. Thus, surfactants enhance the performance of the herbicide by facilitating penetration into the cuticle or by achieving a uniform soil application. Specifically, soil applications are improved by introducing water repellent surfactants into the formulation. [1,2]

Research on surfactants has been focused on slow release formulations of herbicides. In general, the immediate release of herbicides from conventional formulations results in the loss of the active ingredient by transfer and degradation processes, which decreases the effectiveness of herbicide. On the contrary, slow release formulations are aimed at reducing active ingredient losses and avoiding repetitive applications or larger dosages. Thus, slow release formulations reduce the risk of water and soil pollution while maintaining the desired effect.

A new approach in the design of slow release formulations has been recently developed. These novel formulations consist of an anionic herbicide in a cationic micelle or vesicle, which is formed in solution by the surfactant and adsorbed onto a negatively charged clay mineral. [3-6] Due to the toxicity of some cationic surfactants, current research is focused on the use of more environmentally friendly substances. [7,8] In the current study, the non-toxic surfactant PC was used in combination with montmorillonite for the preparation of slow release formulations of metribuzin. These formulants are EPA approved substances with low toxicological risk.

In a previous study, we optimized the adsorption of PC on montmorillonite for the preparation of slow release herbicide formulations. [9] This system was proved to be very effective at reducing the leaching of poorly water soluble herbicides such as atrazine and alachlor. [10] In this study, a similar approach was evaluated with metribuzin, a highly water soluble herbicide. We expected that metribuzin would be poorly encapsulated in PC vesicles due to its high water solubility. However, the presence of polar groups on the herbicide can interact with the PC headgroup, which enhances the solubility of the herbicide in the vesicles and increases the concentration of the active ingredient in the formulation.

Metribuzin (4-amino-6-*ter*-butyl-3-methylthio-1,2,4-triazin-5(4*H*)-one) is a herbicide used to control weeds in soybeans, potatoes, tomatoes and other crops. Due to its high water solubility (1050 mg L^{-1}), metribuzin has been detected in surface and ground waters. [11,12] Dissipation is rapid, and the half-life of metribuzin ranges from 11 to 46 days in laboratory and field studies. [13] Polymer-based [14,15] and clay-gel based formulations [16,17] have been designed to obtain slow release formulations of metribuzin. PC-clay formulations of metribuzin were prepared by inclusion of the active ingredient into PC vesicles and adsorbing the vesicles onto montmorillonite. Adsorbed and encapsulated metribuzin was characterised by infrared spectroscopy to determine the mechanisms of retention in the PC-clay matrix. Furthermore, the leaching and dissipation of PC-clay formulations were evaluated in soils with different physical-chemical properties, and the results were compared to those of commercial formulations. Previously, soil adsorption-desorption experiments were conducted to determine the factors that affect the behavior of metribuzin in soils and to relate the efficiency of the formulations to natural soil processes.

1. Materials and methods

1.1. Materials

Wyoming Na-montmorillonite (SWy-2) was obtained from the Source Clays Repository of The Clay Minerals Society (Columbia, MO) (cation exchange capacity 0.8 mmol/g). Phosphatidylcholine (SPC-3) (74% distearoyl-PC and 26% 1-palmitoyl-2stearoyl-PC) was supplied by Lipoid GmbH (Ludwigshafen, Germany). HPLC grade–methanol was purchased from Sigma-Aldrich (Sigma Chemical Co., St Louis, MO), and HPLC grade -acetonitrile was obtained from Teknokroma S.A. (Barcelona, Spain). Metribuzin was purchased from Sigma-Aldrich Co. The commercial formulation of metribuzin (Eclipse 70, 70% w:w) was supplied by Comercial Química Masso (Barcelona, Spain). Figure 1 shows the structural formulas of PC and metribuzin.

All soil samples were obtained from the soil surface (0-20 cm) and passed through a 2 mm sieve before use. The soils were classified as Aquic Haploxeralfs (P44 soil), Chromic Haploxererts (TM soil), Alfic Dystrict Eutrochrepts (LM soil) and Typic Xeropsamments (AR soil). Soils were analysed for their physical-chemical and microbiological properties (Table 1) according to the methods described by Undabeytia et al. [18] and Sopeña et al. [19]. A semiquantitative estimation of the clay mineralogy was performed using the methodology proposed by Stokke & Carson [20] (Table 2).

2.2 Preparation of herbicide-PC-clay formulations

PC formulations of metribuzin were prepared by dissolving the herbicide in a solution of 6 mM PC via sonication and further addition to montmorillonite. The added concentration of the herbicide was 19 mM whereas the clay concentrations were 1.6 and 5 g/L. After shaking for 24 h the suspensions were centrifuged at 20000 g for 10 min, and the supernatant was analysed for the remaining herbicide. Moreover, the pellet obtained from centrifugation was freeze-dried. A nomenclature for PC-clay formulations was introduced, where the first letters indicated the herbicide (MTZ), the first number denoted the initial herbicide concentration and the second number represented the clay concentration. The concentration of active ingredient in MTZ19/5 and MTZ19/1.6 were 16.6 and 24.8%, respectively (w: w).

2.3 Herbicide analysis

Metribuzin was analyzed by HPLC (Shimadzu Model 10A) equipped with a PDA detector. The reverse phase column was a 15 cm Kromasil 100 C18. The flow rate was 1.0 mL min^{-1}. The mobile phase was 60% acetonitrile and 40%: water. The wavelength was set at 230 nm, and the retention time of metribuzin was 7.5 min.

2.4. Fourier Transform Infrared spectroscopy

Fourier Transform Infrared (FTIR) spectra of the herbicide, PC-clay complex and herbicide formulations were recorded in KBr pellets (2 wt.% sample) using a Nicolet spectrometer (20SXB) with a DTGS detector, in the range of 4000-400 cm^{-1}. Resolution was of 2 cm^{-1}. 300 scans were accumulated for improving the signal to noise ratio in the spectra.

2.5. Adsorption-desorption of metribuzin

Metribuzin sorption experiments were performed in triplicate by mixing 10 g of the soils with 10 mL of herbicide solutions ranging up to 15 mg/L. Preliminary kinetic studies indicated that sorption reached equilibrium after 24 h; thus, the suspensions were shaken for 24 h at 20ºC. Subsequently, the suspensions were centrifuged and three successive desorption steps were performed by replacing half of the supernatant with water, re-equilibrating for 24 h and repeating the same procedure twice. The concentration of herbicide in all of the supernatants was analysed.

Sorption-desorption isotherms were fitted to the logarithmic form of the Freundlich equation:

$$\log C_s = \log K_f + n \log C_e$$

were C_s (µmol kg^{-1}) is the amount of adsorbed herbicide, C_e (µmol L^{-1}) is the equilibrium concentration of herbicide (µmol L^{-1}), and K_f and n are constants that characterise the relative sorption capacity and sorption intensity, respectively. The normalised distribution coefficient (K_{oc}) of organic carbon (OC) was calculated from the Kf values (K_{oc}=Kf/OC*100). The hysteresis coefficients (H) for the sorption-desorption isotherms were calculated according to the following equation:

$$H = n_a/n_d$$

where n_a and n_d are the Freundlich n constants obtained from the sorption and desorption isotherms, respectively.

2.6. Release of herbicide

The release of the herbicide from PC-clay and commercial formulations was conducted in triplicate by using a Büchner funnels. In this procedure 98.9 g of the AR soil was added to a Büchner funnel (9.5 cm internal diameter) that had a paper filter on the bottom. The soil layer was homogeneized to a 0.5 cm height. The soil surface was uniformly sprayed with the different herbicide formulations at a rate of 1 kg ha^{-1}. The soil layer in each funnel was irrigated 45 times with 15 mL, each washing corresponding to 2.12 mm rain at 20 minutes intervals. The volume eluted after each irrigation was collected, and the concentration of herbicide in the eluent was determined.

2.7. Soil columns experiments

Metacrylate tubes with a diameter of 3.0 cm were cut into 4- and 8-cm sections, and three units of 4 cm were glued together with a 8 cm unit at one end to construct a 20 cm column. The column was covered at the end opposite to the 8 cm unit with 1 mm nylon screen padded with a thin layer of glass wool (0.5 g) to hold the soil firmly in the column. AR (0.160 kg) or P44 (0.164 kg) soils were packed from the top of the column to create a 16 cm soil column that could be readily separated into 4 cm segments. The pore volume of AR and P44 was 38 and 44 mL, respectively.

Suspensions of commercial and PC-clay formulations (3 mL) were sprayed uniformly with a proper design syringe onto the soil surface at a rate of 1 kg *a.i.* ha^{-1}, and each soil column experiment was performed in triplicate. Distilled water equivalent to 70 mm of rain (50 mL) was added to the top of the column in two portions, and the soil was allowed to equilibrate for 24 h after each addition. The leachates were collected, and the concentration of the herbicide in the eluent was analysed.

Each soil column was separated into 4 segments. The amount of herbicide remaining in each segment was extracted in triplicate by shaking 5 g of soil with 15 mL of methanol for 24 h. The suspensions were centrifuged and the herbicide was analysed by HPLC.

A bioassay was used to calculate the residual activity of the herbicides throughout the first two upper rings of the soil column. Six beakers containing 15 g of soil of each segment for each formulation were planted with 7 seeds of *Setaria viridis* and were irrigated daily for 2 weeks. The bioactivity of the formulations was determined by measuring the reduction in fresh weight per shoot of *S. Viridis* with respect to a control.

2.8. Dissipation studies

200 g of each soil was incubated in triplicate at a rate of 1 kg a.i. ha^{-1} with the commercial formulation and MTZ19/1.6. Water was periodically added to the pots to achieve a moisture content that was 66% of field capacity, and the temperature was maintained at 20ºC. 5 g of soil was sampled from each pot at several time intervals, extracted with methanol and analysed by HPLC. The dissipation of herbicide in the soils was fitted to a first-order equation, and the time required for 50% dissipation (DT50) was calculated.

2.9. *Data analysis*

One-way ANOVA was used to analyse the differences in leaching and herbicidal activity between formulations and soil depths. The means were compared by conducting a Student´s t-test (α=0.05), in SAS software (SAS Institute, 2008).

2. Results and discussion

2.1. Characterization of the formulations

The formulations were examined through infrared spectroscopy to elucidate the interactions between metribuzin and the matrix, which may affect the release rate of the herbicide.

As shown in Fig. 2a, the infrared spectrum of PC adsorbed onto clay showed absorption bands at 1737 and 1643 cm^{-1} , which were attributed to the stretching of nonhydrogen- and hydrogen-bonded C=O groups, respectively. The band at 3437 cm^{-1} was assigned to the OH stretching vibration of water bound to the PC headgroup, whereas the band at 1245 cm^{-1} was assigned to the asymmetric stretching vibration of PO_2^-. Moreover, the band at 1473 cm^{-1} was attributed to CH_2 scissoring, and the bands at 2850 and 2920 cm^{-1} were attributed to symmetric and asymmetric CH_2 stretching vibrations, respectively.[10] The IR spectra of metribuzin adsorbed onto the PC-clay complex revealed new features (Fig. 2b). The intensity of both absorption bands at 2920 and 2850 cm^{-1} notably decreased whereas the absorption due to CH_2 scissoring was lowered by 6 cm^{-1} , which indicated that the herbicide interacts strongly with the hydrophobic chains of PC. In the spectra of adsorbed metribuzin, the band at 1676 cm^{-1} that corresponded to the carbonyl (amide) of the herbicide appeared as well as a small shoulder assigned to the NH_2 deformation band at 1632 cm^{-1}, which was shifted from its original value (1617 cm^{-1}) in pristine metribuzin (Fig. 2c). This shift indicated involvement of NH_2 groups in hydrogen bonding, so that the frequency for its resonance increased. The band at 1245 cm^{-1} in the PC-clay complex is very sensitive to the hydration of PC. [21] In the spectra of adsorbed metribuzin, a decrease in this band was observed, indicating that this functional group interacted with the herbicide (5 cm^{-1}). A similar phenomenon was also observed for the band at 3437 cm^{-1} in the PC-clay complex. Therefore, the incorporation of the herbicide perturbs the PC-clay system by replacing a fraction of water bound to the PC headgroup with metribuzin, which can form water bridges with the PC headgroup.

2.2. Adsorption-desorption of metribuzin

Metribuzin sorption on soils was greatest on LM soil, followed by TM, ≈P44 and AR (Fig. 3). The Koc values of the soils were not related to the general trend in metribuzin sorption (Table 3). Daniel et al. [22] conducted a multiple regression analysis and found that sorption of metribuzin was related to the organic carbon content of the soil. Due to its sandy texture, potential sorption sites of AR soil are exclusively in organic matter. Alternatively, the high degree of sorption and the low value of Koc in other soils (relative to the Koc of AR soil) indicated that the clay mineral fraction controls metribuzin sorption. This result is in agreement with previous studies, which have shown that the mineral phase of soil plays an important role in the sorption of triazine herbicides at high clay/organic carbon ratios. [23-25] Herbicides belonging to the triazine family are adsorbed onto clay minerals through hydrophobic interactions with the siloxane surface. These interactions are favourable in swelling minerals when the interlayer space is saturated with common soil elements such as K, whereas adsorbed Ca and Mg decrease herbicide-clay interactions. This is due to the lower hydration of the cation to which the herbicide is complexed [26] or to the higher partial dehydration of the herbicide molecules [25]; therefore increasing the contacts of the herbicide in the interlayer space with the two clay layers. Nevertheless, triazines are readily adsorbed on montmorillonites saturated with Ca. [27] On the contrary, the affinity of metribuzin is notably lower on kaolinite than on montmorillonite.[28] Thus, metribuzin sorption on P44, LM and TM soils is related to the content and type of clay mineral present in the soil (Table 2). This information is frequently omitted when correlating and predicting metribuzin sorption, and can explain discrepancies in these types of studies. For example, Harper [29] and Kah and Brown [30] determined that metribuzin adsorption was related to the pH and the concentration of organic matter, and clay. Barriuso & Calvet [31] concluded that the clay content was the best predictor for the sorption of metribuzin.

However, Peek and Appleby [32] did not found any correlation between the soil properties and metribuzin sorption.

The highest adsorption of metribuzin was observed on LM soil. This herbicide shows larger sorption kinetics and higher affinity for amorphous oxides of iron and aluminum than clay minerals, even when the latter are saturated with monovalent cations. [28] Therefore, the high content of iron and aluminum oxides in LM soil could be responsible for the high metribuzin sorption.

Metribuzin desorption isotherms obtained from the soils (not shown) showed significant hysteresis. The hysteresis coefficients and desorption of the soils are listed in Table 4. In general, the desorption percentage and hysteresis coefficient of each soil was similar and independent of the initial concentration. The desorption percentage was higher in AR and P44 soils, where values ranged from 54 to 65%. The hysteresis coefficients in these soils were close to one, indicating a quasi-reversible desorption. On the contrary, the desorption percentage was significantly lower in LM and TM soils, and high H values were observed. Moreover, a relationship between the H of metribuzin and the porosity of the soil could be traced. Soils with a higher porosity as reflected in the higher S_{BET} values, led to greater intramolecular diffusion of the herbicide. The increase in intramolecular diffusion, improved the contact between the herbicide and the soil matrix and reduced the rate of desorption.

2.3. Release studies: funnel experiments

Fig. 4 shows the elution curves of the commercial formulation as well as those of the PC-clay complexes in AR soil. The shape of the curve from the commercial formulation is highly symmetric, indicating little retention of the herbicide. AR soil displayed a low sorption capacity for metribuzin and limited hysteresis. PC-clay formulations yielded broader peaks with extended tails, and elution peaks were observed at a greater number of irrigations than that of the commercial, which suggested a slower and more gradual release of the active ingredient. In the elution profile of the commercial formulation, the cumulative amount of released metribuzin was 74.2 ± 8.0% after 6 irrigations, whereas 33.1 ± 0.5 and 20.0 ± 1.9% of metribuzin was released with MTZ19/1.6 and MTZ19/5, respectively. Thus, the amount of metribuzin released from the soil with MTZ19/1.6 and MTZ19/5 was reduced by 56 and 73%.

The herbicide was completely released from the commercial formulation after 23 irrigations, whereas formulations based on PC-clay complexes were fully removed after 39 irrigations. The total amount of metribuzin released from the soils was 100.1 ± 11.1 for the commercial formulation, 100.3 ± 14.4% for MTZ19/1.6 and 100.2 ± 7.2% for MTZ19/5, indicating that the herbicide was not irreversibly trapped inside the PC-clay matrix, which would decrease the effective amount of the active ingredient in the formulation.

2.4. Leaching studies: Soil column experiments

In the previous experiments, the funnels were excessively irrigated to determine the availability of the active ingredient in PC-clay formulations, and to compare the amount of herbicide released from the PC-clay matrix to that of the commercial formulation. The objective of soil column experiments was to follow the mobility and bioactivity along soil columns under conditions similar to those found in Mediterranean regions. Soil column experiments employed soils with a lower affinity for the herbicide (AR and P44 soils) and higher vulnerability for metribuzin loss.

As shown in Fig. 5, the leaching pattern of the herbicide was different in AR and P44 due to the sorption capacity of the soils. For the commercial formulation, the greatest accumulation of metribuzin was observed at a depth of 8-12 cm of AR soil, whereas the greatest accumulation was observed at 4-8 cm of P44.

When the commercial formulation was applied to AR soil (Fig. 5a), 19.5 ± 0.2 and 25.8 ± 1.0% of the herbicide was retained on the first two segments of the soil column, whereas 33.0 ± 4.5 and 32.4 ± 2.9% of metribuzin was retained upon application of MTZ19/5. Furthermore, when MTZ19/1.6 was applied to AR soil, 30.1 ± 1.7 and 40.2 ± 4.8% of the herbicide was adsorbed. Thus, PC-clay formulations decreased the amount of metribuzin leached from the soil profile by an average of 38% in the first 4 cm of the column, and 19% in the following 4 cm. On the other hand, the commercial formulation resulted in 38% more metribuzin adsorbed at a depth of 8-12 cm compared to PC-clay formulations.

The results of bioactivity assays revealed that inhibition was 6 times greater with the PC-clay formulations in the upper segment of the soil column. However, differences in the following layer were not readily apparent with the exception of MTZ19/1.6, which is consistent with the results of the soil column experiments (Fig. 6a).

In P44 soil (Fig. 5b), 45.3 ± 2.2% and 45.7 ± 0.2% of metribuzin was extracted from the first segment of the column when MTZ19/5 and MTZ19/1.6 were applied, respectively. On the other hand, the amount of metribuzin extracted from the upper layer of soil was 27.3 ± 6.1% for the commercial formulation; thus, PC-clay formulations displayed 40% less leaching compared to the commercial formulation. In the following segment, the difference in leaching potential was greater due to the larger accumulation of metribuzin with the commercial formulation and the lower accumulation of herbicide with the PC-clay formulations. In lower segments, the amount of adsorbed metribuzin was not significantly different between the formulations, which concurs with the results obtained from bioactivity assays (Fig. 6b). The assay results indicated that the inhibition percent with PC-clay formulations was approximately 1.6-fold greater than the inhibition percent of the commercial formulation in the first segment. In the following segment, there was no statistical difference in the herbicidal activity between the commercial and the PC-clay formulations.

2.5. *Dissipation studies.*

The aim of the dissipation study was to determine whether or not the PC-clay formulations could increase the residence time of the active ingredient in the soil and prolong herbicidal activity.

The dissipation of metribuzin on the four soils was fitted to a first-order kinetics model (R^2>0.94), and the half-life time (DT50) of the herbicide in each soil was calculated (Table 5). The results indicated that the dissipation of metribuzin was greater in AR soil, which possessed a sandy texture. The greatest DT50 value was observed in LM soil, followed by P44, TM and AR. However, the difference in the DT50 of P44 and TM was minor.

Metribuzin dissipation occurs by abiotic and microbial degradation and by incorporation of the herbicide into the soil matrix in a form that is unavailable to the microbial population. [33, 34] Henriksen et al. [33]observed a significant loss of the parent compound (up to 40% of the initial amount) in sterilised soils. Part of the abiotic loss is due to interaction between metribuzin and humic substances in the soil. Specifically, the amine group of the herbicide is eliminated upon reaction with alcohols and carboxylic groups of organic matter. [35] On the other hand, positive correlations were observed between metribuzin dissipation and soil properties such as pH, organic carbon content and bioactivity. [36, 37] These parameters are also correlated to each other, as an increase in the organic carbon content usually parallels soil bioactivity. Moreover, enzymatic activity is optimal at a pH between 7.0 and 8.5. Consequently, metribuzin dissipation in soils by abiotic and microbial processes is strongly dependent on the organic carbon content. [38] In Table 5, the observed trend in DT50 values does not follow the inverse order of the organic carbon content. Specifically, TM soil possessed the largest organic content, but metribuzin dissipation in TM soil was lower than that of AR, which possessed an organic matter content that was nearly one-half of the organic matter content of TM. The observed trend in DT50 among soils cannot be fully explained by microbiological parameters (Table 1). AR soil led to the greatest amount of dissipation, and possessed a relatively high concentration of microbial biomass carbon, whereas LM soil led to the least amount of dissipation and possessed a low level of microbial biomass carbon. However, despite identical microbial biomass content, dissipation kinetics in TM soil were slower than in AR soil. Some authors have suggested that the activity of the dehydrogenase enzyme is a better indicator of soil bioactivity. [19, 37] However, according to dehydrogenase activity, dissipation should be fast in TM soil and slow in AR and LM soils.

Juhler et al. [39] studied metribuzin dissipation in 24 soil profiles, and observed a positive correlation between the organic carbon content and herbicide, as noted previously. To identify factors that affect compound disappearance, a partial least square regression was applied to the experimental data. The results indicated that inorganic (amorphous Al, CEC, base saturation percent, pH) and microbiological soil properties were responsible for metribuzin dissipation. Thus, the DT50 values displayed in Table 5 cannot be fully explained by microbiological parameters. The concentration of amorphous Al oxides and the value of DT50 are inversely proportional; thus, the higher concentration of Al oxides in TM soil leads to slower dissipation kinetics than in AR soil, despite the fact that the microbial C content of both soils is similar or that the dehydrogenase activity is larger in TM soil. The slow dissipation kinetics on TM soil due to the sorption of the herbicide on high affinity sites located on amorphous Al oxides, where desorption is limited. Alternatively, LM soil displayed the lowest amount of dissipation due to its low bioactivity, high aluminum amorphous oxides content, and high *H* values, which limit the accessibility of the herbicide to microbial flora.

As shown in Table 5, dissipation was significantly lower with PC-clay formulation than the commercial formulation. In AR soil, the DT50 value of PC-clay formulation was 1.6 times greater than the DT50 of the commercial formulation, and larger differences were observed in other soils (2.2-2.5). Maqueda et al. [40] found that slow-release formulations of metribuzin based on sepiolite gels increased herbicide, increasing the microbial activity of the local environment surrounding the formulation. However, in *in vitro* experiments, these formulations provided a very slow release of the herbicide. The observed trend in DT50 values for PC-clay formulation was also observed with the commercial formulation, indicating that the differences in herbicide dissipation on the four soils was due to a slower release of the active ingredient from the formulation matrix, which does not alter the microbiological and/or chemical properties of the soils.

In general, slow release formulations should reduce leaching and maximise herbicidal activity in the top layer of the soil. PC-clay formulations of metribuzin showed a significant reduction in leaching and a higher bioactivity in the top soil layers, which are of the greatest interest for weed control. The reduction in leaching and increased bioefficacy was dependent on the physical-chemical properties of the soils. Moreover, these formulations were able to prolong the herbicidal activity of metribuzin by decreasing dissipation losses. Consequently, a reduction in the recommended dose of metribuzin can be achieved with the use of PC-clay formulations, which reduces the environmental risk associated with herbicide applications.

Moreover, the main auxiliary components of these formulations (PC and montmorillonite) are non-toxic and do not negatively affect the environment.

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Table 1. Properties of the studied soils.

	AR	P44	LM	TM
OM (g kg^{-1})	7.9	14.0	14.1	17.6
CEC (cmol$_c$ kg^{-1})	4.8	7.8	12.4	39.0
Carbonate (g kg^{-1})	69.0	<0.1	<0.1	241.0
pH	8.0	5.5	6.0	8.0
Sand (%)	87.6	49.8	16.7	2.7
Silt (%)	4.0	34.5	58.6	31.5
Clay (%)	8.4	15.7	24.7	65.9
Total Fe_2O_3 (g kg^{-1})	11.4	46.1	356.8	43.9
Amorphous Fe_2O_3 (g kg^{-1})	0.4	1.9	8.43	0.8
Total Al_2O_3 (g kg^{-1})	53.2	111.8	148.2	116.4
Amorphous Al_2O_3 (g kg^{-1})	0.2	1.0	8.5	3.5
Total MnO (g kg^{-1})	<0.1	0.6	21.9	0.7
Amorphous MnO (g kg^{-1})	<0.1	0.1	9.8	0.2
S_{BET} (m^2/g)	1.5	7.2	42.6	69.8
Microbial biomass C (g C kg^{-1} soil)	737 (±28)	470 (±24)	277 (±60)	686 (±37)
Dehydrogenase activity (mg 1,3,5-triphenyltetrazolium formazane kg^{-1} soil)	9.51 (±1.11)	22.76 (±4.43)	6.72 (±1.17)	32.08 (±1.87)

Table 2. Mineralogy of the clay fraction of each soil.[†]

Soil	S%	I%	K%	V%	Cl%
AR	-	25	52	-	-
P44	-	58	42	-	-
LM	-	45	30	25	-
TM	70	15	15	-	-

† S: smectite, I: illite, K: kaolinite, V: vermiculite, Cl: chlorite.

Table 3. Parameters of the Freundlich adsorption isotherms and Koc values of metribuzin adsorption.

	Kf (µmol^{1-n} L^{n} kg^{-1})	n	R^{2}	Koc (µmol^{1-n} L^{n} kg^{-1} g^{-1})
AR	0.563±0.039[†]	0.74±0.04	0.989	71.34±4.93
P44	0.506±0.013	0.88±0.02	0.996	36.14±0.93
LM	0.737±0.025	0.88±0.06	0.915	52.26±2.6
TM	0.607±0.021	0.90±0.03	0.993	34.54±1.19

†. Means +/- standard errors.

Table 4. Percentage of desorbed metribuzin (%D) and hysteresis coefficients (*H*) as a function of initial concentration. †

Metribuzin	AR		P44		LM		TM	
(μM)	%D	*H*	%D	*H*	%D	*H*	%D	*H*
14.1	61.34	1.17	57.30	2.33	22.02	4.12	39.13	2.93
	(±2.35)	(±0.04)	(±0.72)	(±1.13)	(±5.75)	(±0.39)	(±1.99)	(±0.28)
23.5	59.32	0.87	59.04	1.47	28.19	4.31	37.12	2.97
	(±2.86)	(±0.15)	(±1.85)	(±0.21)	(±3.30)	(±0.56)	(±5.62)	(±0.65)
32.9	68.58	1.08	55.04	1.78	28.11	4.44	38.52	2.38
	(±2.04)	(±0.04)	(±3.65)	(±0.39)	(±9.14)	(±0.19)	(±3.54)	(±1.05)
47.0	65.21	1.23	58.63	1.54	32.34	3.76	37.65	3.05
	(±5.60)	(±0.15)	(±0.81)	(±0.11)	(±10.33)	(±0.49)	(±4.37)	(±0.31)
70.5	64.85	1.03	54.70	1.69	30.89	3.73	39.48	3.22
	(±2.70)	(±0.05)	(±8.72)	(±0.12)	(±2.10)	(±0.49)	(±1.37)	(±0.91)

† Numbers in parentheses are the standard errors.

Table 5. DT50 (days) of metribuzin with the comercial formulation and MTZ19/5.

Soils	Commercial	MTZ19/5
LM	32.1±0.5[†]	71.3±1.2
TM	21.3±0.3	53.3±0.5
P44	25.8±0.2	57.8±0.6
AR	14.3±0.1	22.7±0.8

†. Means +/- standard errors.

Figure captions.

Figure 1. Structural formula of metribuzin and PC.

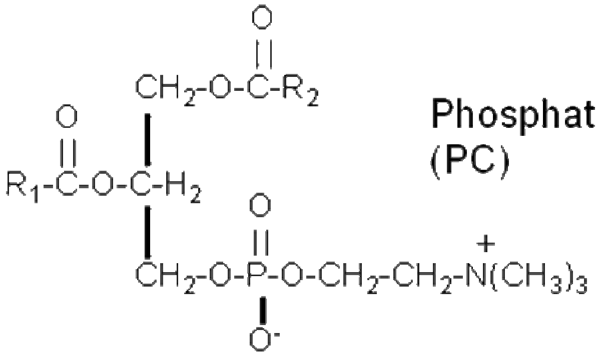
Figure 2. Infrared spectra of (a) the PC-clay complex (6 mM PC: 5 g L-1), (b) MTZ19/5 and (c) pristine metribuzin.

Figure 3. Adsorption isotherms of metribuzin in soils.

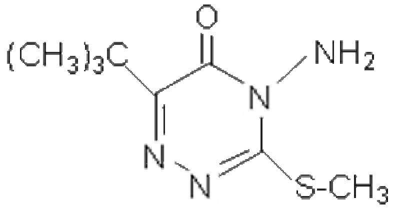
Figure 4. The amount of metribuzin released from commercial and PC-clay formulations.

Figure 5. Percents retained of metribuzin in soil column experiments employing AR (a) and P44 (b) soils. Means followed by the same letter indicate that the formulation, depth and soil type were not significantly different according to Student´s multiple range test at *P*=0.05. Vertical bars indicate the standard errors.

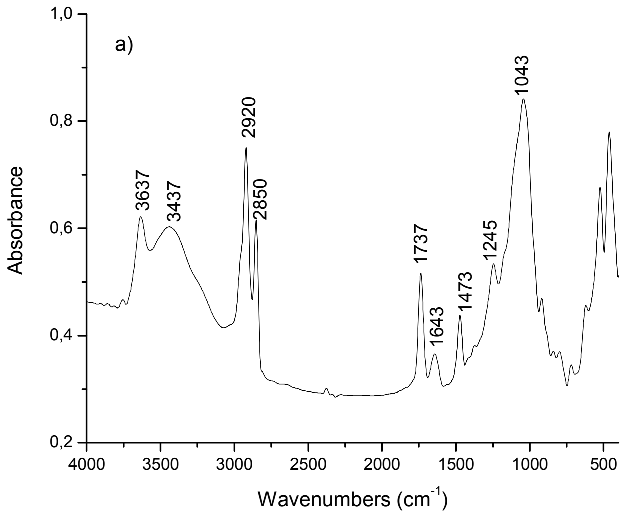
Figure 6. Herbicidal activity in the upper segments of AR (a) and P44 (b) soil columns with different metribuzin formulations. Means followed by the same letter indicate that the formulation depth and soil type were not significantly different according to Student´s multiple range test at *P*=0.05. Vertical bars indicate standard errors.

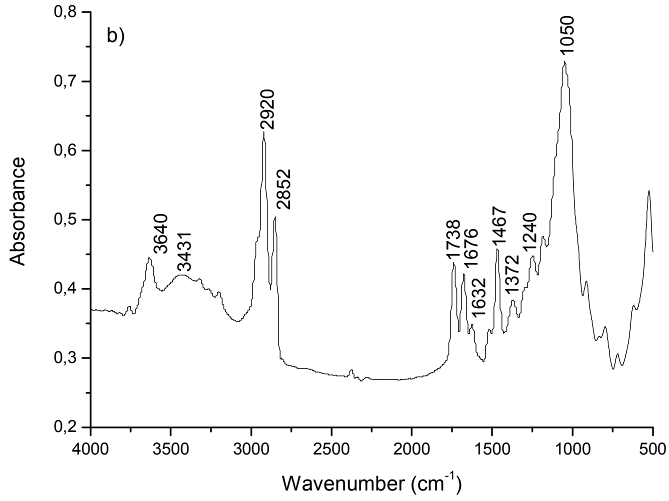


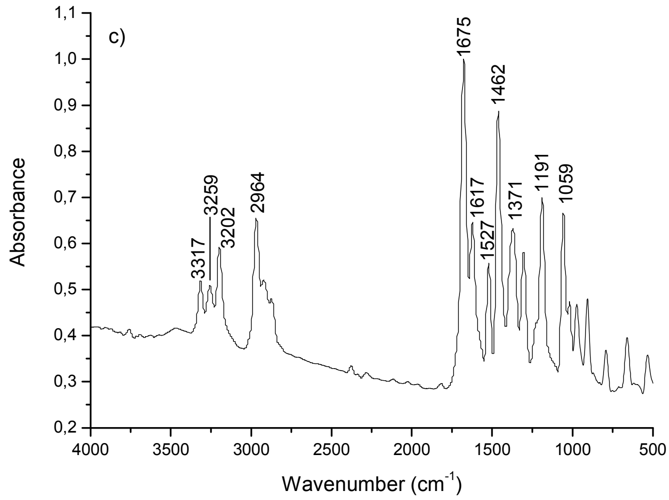
Phosphatidylcholine
(PC)



Metribuzin


a)
Absorbance
Wavenumbers (cm^{-1})
0,2
0,4
0,6
0,8
1,0
4000
3500
3000
2500
2000
1500
1000
500
3637
3437
2920
2850
1737
1643
1473
1245
1043


b)
Absorbance
Wavenumber (cm^{-1})
0,2
0,3
0,4
0,5
0,6
0,7
0,8
4000
3500
3000
2500
2000
1500
1000
500
3640
3431
2920
2852
1738
1676
1632
1467
1372
1240
1050


c)
Absorbance
Wavenumber (cm^{-1})
0,2
0,3
0,4
0,5
0,6
0,7
0,8
0,9
1,0
1,1
4000
3500
3000
2500
2000
1500
1000
500
3317
3259
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1617
1527
1462
1371
1191
1059

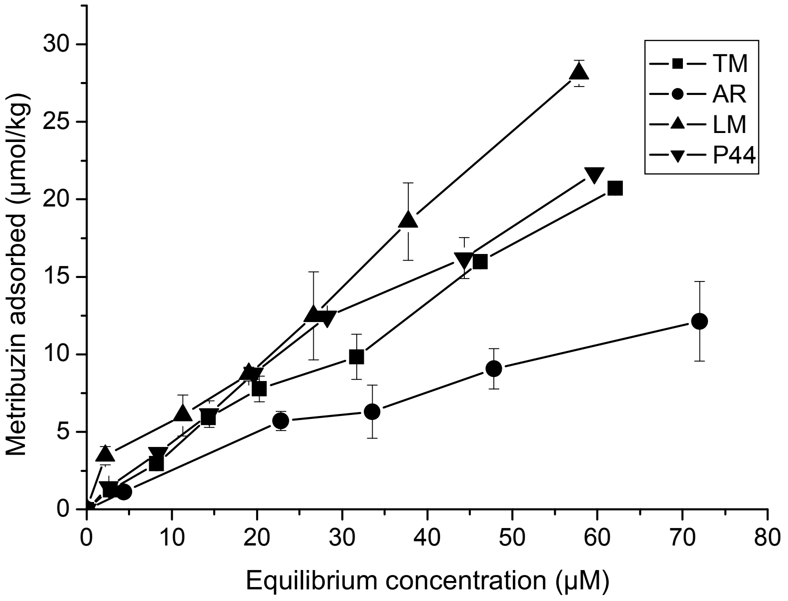
Metribuzin adsorbed (µmol/kg)
Equilibrium concentration (µM)
0
5
10
15
20
25
30
0
10
20
30
40
50
60
70
80
TM
AR
LM
P44

Figure 3

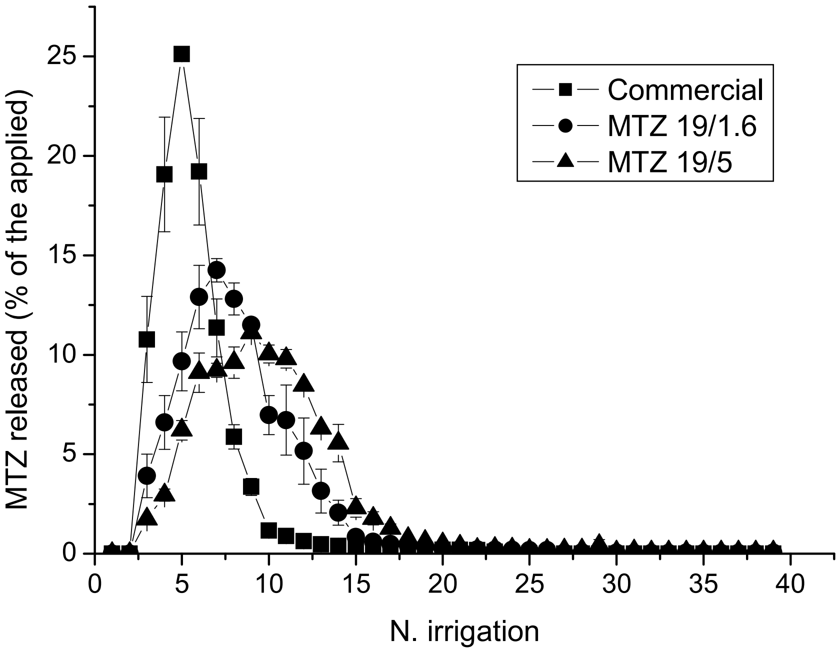
MTZ released (% of the applied)
0
5
10
15
20
25
N. irrigation
0
5
10
15
20
25
30
35
40
■ Commercial
● MTZ 19/1.6
▲ MTZ 19/5

Figure 4

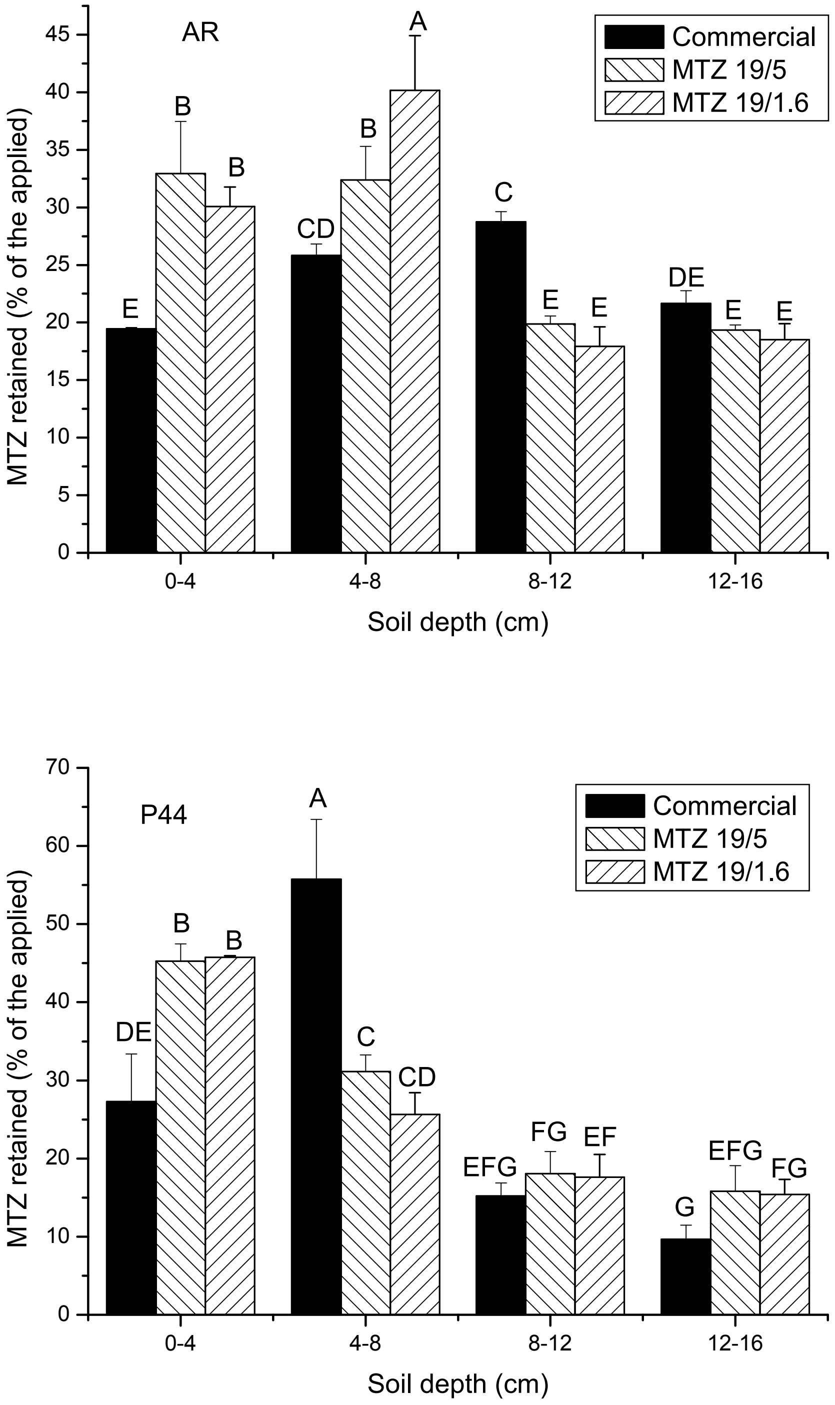
AR
Commercial
MTZ 19/5
MTZ 19/1.6
MTZ retained (% of the applied)
0
5
10
15
20
25
30
35
40
45
E
B
B
CD
B
A
C
E
E
DE
E
E
0-4
4-8
8-12
12-16
Soil depth (cm)
P44
Commercial
MTZ 19/5
MTZ 19/1.6
MTZ retained (% of the applied)
0
10
20
30
40
50
60
70
DE
B
B
A
C
CD
EFG
FG
EF
G
EFG
FG
0-4
4-8
8-12
12-16
Soil depth (cm)

Figure 5

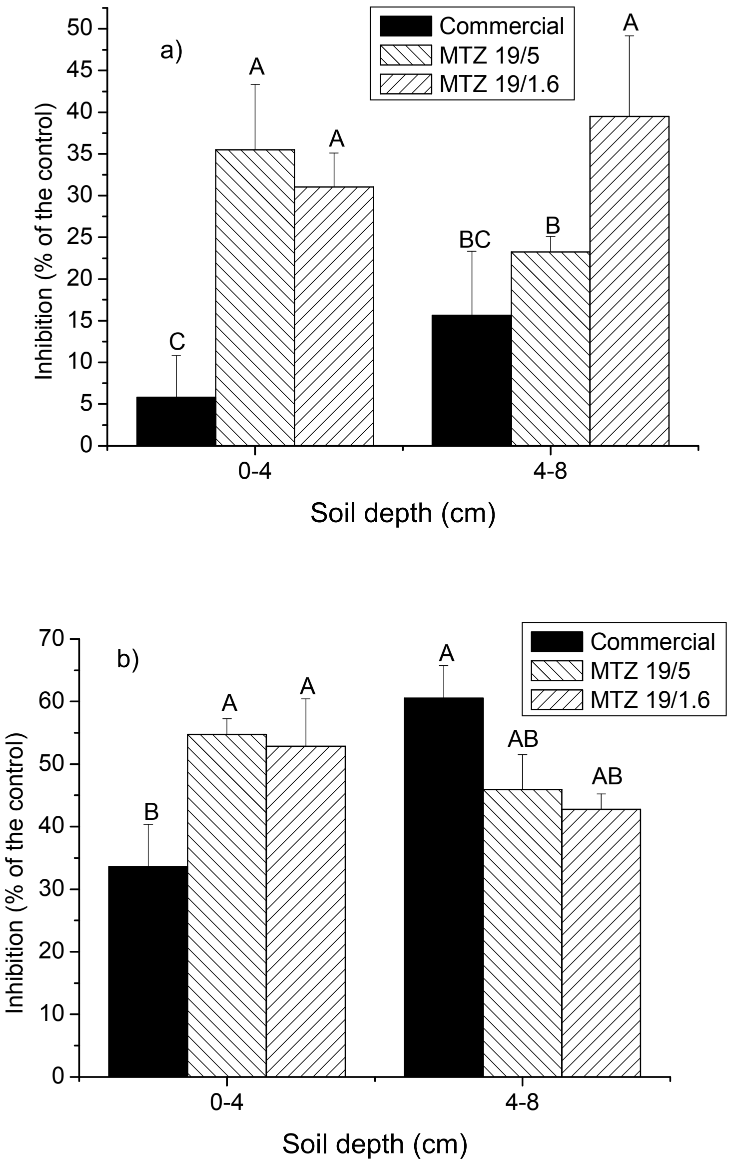
a)
Commercial
MTZ 19/5
MTZ 19/1.6
Inhibition (% of the control)
0
5
10
15
20
25
30
35
40
45
50
C
A
A
BC
B
A
0-4
4-8
Soil depth (cm)
b)
Commercial
MTZ 19/5
MTZ 19/1.6
Inhibition (% of the control)
0
10
20
30
40
50
60
70
B
A
A
A
AB
AB
0-4
4-8
Soil depth (cm)

Figure 6