Diuron, hexazinone, and sulfometuron-methyl applied alone and in mixture in soils: Distribution of extractable residue, bound residue, biodegradation, and mineralization

Fabricia Cristina dos Reis [a,b], Kamila Cabral Mielke [c], Kassio Ferreira Mendes [c,*], Rodrigo Nogueira de Sousa [d], Maísa Helena Heluany [e], Valdemar Luiz Tornisielo [f], Ricardo Victoria Filho [g]

[a] *Center of Nuclear Energy in Agriculture, University of São Paulo (CENA/USP), SP, Brazil*
[b] *Agricultural Assistant, Secretariat of Agriculture and Supply, Campinas, SP, Brazil*
[c] *Department of Agronomy, Federal University of Viçosa, Viçosa, MG, Brazil*
[d] *Department of Soil Science, Agriculture College "Luiz de Queiroz", University of São Paulo, Piracicaba, SP, Brazil*
[e] *Technical at Multiuser Laboratory for Biological Studies, Federal University of Santa Catarina, Florianópolis, SC, Brazil*
[f] *Ecotoxicology Laboratory, Center of Nuclear Energy in Agriculture, University of São Paulo, Piracicaba, SP, Brazil*
[g] *Department of Crop Science, Agriculture College "Luiz de Queiroz", University of São Paulo, Piracicaba, SP, Brazil*

A R T I C L E I N F O

A B S T R A C T

Biodegradation studies of herbicides applied to the soil alone and in a mixture are required since herbicides are often used in combinations to control weeds. When herbicides are applied in mixtures, interactions may affect their environmental fate. Thus, the objective of this study was to evaluate the distribution of extractable residue, bound residue, biodegradation, and mineralization of diuron, hexazinone, and sulfometuron-methyl when applied alone and in a mixture in two agricultural soils. Biometric flasks filled with two types of soil (clay and sandy) collected from an area cultivated with sugarcane and treated with ^{14}C-radiolabeled solutions of the herbicides were incubated for 70 d. More ^{14}C–CO$_2$ was released when sulfometuron-methyl and hexazinone were applied in a mixture compared to when applied alone. Being used in a combination did not affect the mineralization of diuron. The soil texture directly influenced the mineralization, bound residue, and extractable residue of the three herbicides. The percentage of extractable residue decreased over time for all herbicides. Hexazinone and sulfometuron-methyl had the highest residue extracted on sandy soil when applied alone. Diuron showed the highest percentage of bound residue. The degradation of the three herbicides was higher in the clay soil regardless of the mode of application, which is related to the higher potential of the bacterial community in the clay soil to mineralize the herbicides.

1. Introduction

Sugarcane is the most important cultivated crop in São Paulo, Brazil, and pesticides are constantly applied during its cultivation [1]. Herbicides are the most used pesticides in sugarcane, and their over-application can impact the biological processes in soil and

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non-target organisms [2]. For these reasons, studies evaluating soil pesticide behavior are required.

Soil microorganisms are capable of herbicide degradation, affecting their persistence [3]. Microorganisms that degrade herbicides can use the molecules as a substrate, transforming them into biomass and energy for survival (catabolism and metabolism), or change the chemical structure without using the herbicide as an energy source (co-metabolism) [4]. Complete biodegradation or mineralization involves the oxidation of intermediate compounds (metabolites) into simpler molecules such as H_2O, CO_2, and NH_4 [5]. In the mineralization process of environmental fate assessment studies using ^{14}C-herbicide, the $^{14}C–CO_2$ is quantified by oxidation of the radiolabeled C to describe the extent of complete mineralization of the herbicide [6].

Several commercial mixtures of herbicides are recommended for weed control during sugarcane cultivation. The commercial mixture diuron + hexazinone + sulfometuron-methyl is recommended for morning glory and grasses control (*Urochloa decumbens*, *U. plantaginea*, *Panicum maximum,* and *Digitaria* spp.). Application is recommended pre-emergence of sugarcane during the dry season [7]. However, there is a high potential risk of contamination of Brazilian soils by diuron, hexazinone, and sulfometuron-methyl [8]. Structural formulas and physicochemical properties of the three herbicides are shown in Table 1.
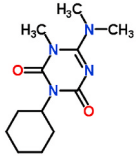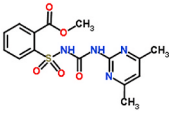
Diuron [3-(3,4-dichlorophenyl)-1,1-dimethylurea] is a neutral herbicide that belongs to the substituted urea compounds and exhibits low solubility in water (Table 1). Organic matter (OM), pH and clay content, as well as the base saturation of the soil, are the attributes with the most significant influence on the sorption and desorption of diuron [10]. Degradation by microorganisms is the primary means of diuron dissipation in soil [11]. Nevertheless, Dores et al. [12] noted a dissipation half-life time (DT_{50}) of 15 d in tropical soil where the herbicide had already been applied. Similarly, Rouchaud et al. [13] observed a DT_{50} of diuron of 37 d in an area where the herbicide had been applied for over 12 yr. At a site without previous application, the DT_{50} was 81 d, so the herbicide dissipation rate was lower.

Hexazinone [3-cyclohexyl-6-dimethylamino-1-methyl-1,3,5-triazine-2,4 ($1H,3H$)-dione] exhibits high solubility in water (S_w) (33,000 mg L^{-1}) and has a sorption coefficient normalized by organic carbon (K_{oc}) of 54 mL g^{-1} (Table 1). This herbicide is a weak base (dissociation constant, pK_b = 2.2), and when the soil pH is higher than its pK_b, the molecule form is neutral, consequently, the hexazinone sorption in the soil is low [5]. Volatilization of hexazinone is negligible, and it exhibits some susceptibility to photo-degradation [14]. It is degraded by soil microorganisms [11], and its DT_{50} is 90 d [14]. In a laboratory study with ^{14}C-hexazinone, the percentage of mineralization ($^{14}C–CO_2$ released) was dependent on the dose used [15]. Hexazinone applied at a dose of 4 ppm had 75% of the total mineralized at 80 d after application, while the 20 ppm dose had 45% mineralized.

Sulfometuron-methyl {methyl-2-[(4,6–dimethylpyrimidin–2–yl)carbamoylsulfamoyl] benzoate} exhibits intermediate solubility in water and is moderately persistent (Table 1). The degradation rate depends on the pH, as well as the water content of the soil [16]. In a study conducted at five sites in the USA, the initial DT_{50} of sulfometuron-methyl ranged from 12 to 25 d [17]. Sulfometuron-methyl belongs to the sulfonylurea chemical group, is a weak acid herbicide, and its pK_a is 5.2. The octanol/water partition coefficient (log K_{ow}) is 1.04 at pH 5, -0.46 to −0.51 at pH 7, and -1.87 at pH 9, which is deemed low [14].

Studies show results of the mixture of diuron, hexazinone, and sulfometuron-methyl to sorption, leaching, and dissipation in soil [18,19]; however, there is little information about possible changes in biodegradation and mineralization, when these herbicides are applied in the mixture. Some studies have shown the behavior of these herbicides alone or in a mixture with the different active ingredients in soil [12,16,20–22]. Inoue et al. [23] evaluated diuron + hexazinone + sulfometuron-methyl persistence using bioassays. In sandy soils, the residual lifetime (RL_{50}) of the mixture was 52 d, regardless of the dose applied. In clay soils, the RL_{50} was 100 and 80

Table 1
Structural formulas and physicochemical properties of three herbicides.

Attributes	Diuron	Hexazinone	Sulfometuron-methyl
Structural formula			
Molecular formula	$C_9H_{10}Cl_2N_2O$	$C_{12}H_{20}N_4O_2$	$C_{15}H_{16}N_4O_5S$
IUPAC name	3-(3,4-Dichlorophenyl)-1,1-dimethylurea	3-Cyclohexyl-6-dimethylamino-1-methyl-1,3,5-triazine-2,4(1H,3H)-dione	Methyl 2-(4,6-dimethylpyrimidin-2-ylcarbamoylsulfamoyl)benzoate
Chemical group	Urea	Triazinone	Sulphonylureas
Molecular weight (g mol^{-1})	233.09	252.31	364.38
Water solubility at 20 °C (mg L^{-1})	5.2 (low)	33,000 (high)	244 (moderate)
Log K_{ow}	2.87	1.17	−0.51
pK_a/pK_b at 25 °C	not applicable	pK_b = 11.8 (weak base)	pK_a = 5.2 (weak acid)
Vapor pressure at 25 °C (mPa)	1.15×10^{-03} (low volatility)	0.03 (low volatility)	7.3×10^{-11} (low volatility)
DT50 soil (d)	229 (persistent)	105 (persistent)	78.5 (moderately persistent)
Koc (L kg^{-1})	680 (slightly mobile)	54 (mobile)	85 (moderately mobile)
GUS leaching potential index	2.65 (transition state)	4.43 (high leachability)	3.92 (high leachability)

Source: Adapted from PPDB [9].

d for higher and lower doses, respectively. Nonetheless, the contribution of each molecule was not evaluated.

The use of mixtures can affect pesticide persistence. Swarcewicz and Gregorczyk [24] reported that mancozeb and thiamethoxam affected pendimethalin degradation when applied as a mixture. In another study, the isoproturon degradation rate was inhibited by the presence of chlorothalonil. The authors concluded that, among other factors, chlorothalonil could suppress the non-target microorganism activity responsible for isoproturon degradation [25].

In practice, herbicides and other pesticides are applied simultaneously or following the application of other agrochemicals, resulting in mixtures of residues in the soil. An estimate of global soil contamination by pesticide mixtures showed that 70% of global agricultural soils have multiple pesticide residues [26]. Simultaneous exposure of soil microbiota to several pesticides can lead to synergistic adverse effects that may deviate from the additive toxicity of a single pesticide [27]. Many interactions are possible when herbicides are applied in mixtures, including effects on the size and composition of the microorganism population, impact on specific enzymes, and the rate of degradation of organic compounds [28]. Given the above, the objective of this study was to evaluate the distribution of ^{14}C-extractable residues, bound residues, biodegradation, and mineralization of diuron, hexazinone, and sulfometuron-methyl when applied alone and in mixtures in two agricultural soils cultivated with sugarcane.

2. Material and methods

2.1. Sample collection

The soil was sampled from the surface layer (0–0.10 m) of areas cultivated with sugarcane after removing the vegetation layer. The soils sampled were classified as either Entisols (*Neossolo Quartzarenico órtico típico - NQ*, sandy soil) or Oxisols (*Latossolo Vermelho eutrófico – LVe*, clay soil). Soils were collected at three points, homogenized for all replicates and used in the diuron, hexazinone, and sulfometuron-methyl biodegradation studies. After sampling, the soils were sieved (2 mm-mesh) and stored at 4±2 °C for further analysis. The results of the physicochemical properties are shown in Table 2.

2.2. Herbicides

Radiolabeled diuron (Phenyl-^{14}C (U)) and sulfometuron-methyl (^{14}C-pyrimidine-2) (DUPONT, Wilmington, DE, USA) showed 97% and 99% radiochemical purity, respectively, and specific activity of 3.83 MBq mg^{-1} and 1.12 MBq mg^{-1}. Radiolabeled hexazinone (triazine-ring-6-^{14}C) (International Isotopes Inc., Idaho Falls, USA) had 97.99% radiochemical purity and specific activity of 3.14 MBq mg^{-1}. The purity of the non-radiolabeled herbicides was 98.7, 99.5, and 99.7% for diuron, hexazinone, and sulfometuron-methyl, respectively.

2.3. Distribution of ^{14}C-extractable residue, bound residue, and mineralization

The diuron, hexazinone, and sulfometuron-methyl biodegradation studies were performed in the Ecotoxicology Laboratory of the Center of Nuclear Energy in Agriculture, University of São Paulo, Piracicaba, São Paulo, Brazil. The methodology used was from the "Aerobic and Anaerobic Transformation in Soil" guideline [6,30].

50 g of soil (dry basis) were weighed inside 250 mL biometric flasks in duplicate. Then the soil water content was adjusted to 75% of

Table 2
Physicochemical attributes of soils (0–10 cm of depth) collected in sugarcane cultivated area. Piracicaba, SP, Brazil.

Attributes	Units	Sandy soil (NQ)	Clay soil (LVe)
pH (H$_2$O)	–	7.2 ± 0.26*	6.5 ± 0.10
pH (KCl)	–	5.8 ± 0.20	5.1 ± 0.03
OM	g kg^{-1}	12.7 ± 0.88	35.7 ± 1.18
P	mg kg^{-1}	34.7 ± 0.96	31.7 ± 1.86
K	mmolc kg^{-1}	0.6 ± 0.13	9.0 ± 0.20
Ca	mmolc kg^{-1}	17.3 ± 1.03	35.3 ± 0.40
Mg	mmolc kg^{-1}	5.3 ± 0.33	13.3 ± 1.33
H+Al	mmolc kg^{-1}	15.3 ± 0.33	45.0 ± 1.00
BS	mmolc kg^{-1}	23.3 ± 0.23	57.7 ± 1.84
CEC	mmolc kg^{-1}	38.6 ± 1.58	102.7 ± 1.44
V	%	60.3 ± 1.36	55.3 ± 0.84
Sand	g kg^{-1}	907.0 ± 0.18	238.0 ± 0.66
Silt	g kg^{-1}	22.0 ± 1.51	131.7 ± 1.67
Clay	g kg^{-1}	71.0 ± 1.51	630.0 ± 1.02
Textural class	–	Sandy	Clay

*Averages of the three soil collection points ± standard deviations (±SD) of means (*n* = 3). pH potential hydrogen, *K* potassium; *Ca* calcium, *Mg* magnesium; *CEC* cation exchange capacity; *P* phosphor; *OM* organic matter; *H + Al* potential acidity; *BS* base saturation. *NQ Neossolo Quartzarênico órtico típico* (Entisol), *LVe Latossolo Vermelho eutrófico* (Oxisol). According to Soil Taxonomy and Brazilian Soil Science Society [29].
Source: Soil Science Department Laboratory at ESALQ, USP, Piracicaba, São Paulo, Brazil.

field capacity, and measured during the whole experiment. The biometric flasks with the soils were pre-incubated in a dark room at 20 ± 2 °C for 10 d before herbicide treatment to promote microbial activity.

The biodegradation evaluation was performed separately for each herbicide. In the treatments they were evaluated alone, the herbicide in question was radiolabeled. For the treatments where involving mixtures, only the evaluated herbicide was radiolabeled, and the others were analytical standards of the herbicides (non-radiolabeled).

The concentration of non-radiolabeled herbicides in the solution was calculated according to the highest recommended dose of the diuron, hexazinone, and sulfometuron-methyl formulated in a mixture (1387, 391, and 33 g a.i. ha^{-1}), which correspond to soil concentrations of 0.0578, 0.0163, and 0.0014 mg kg^{-1}, respectively. The calculation included the following parameters: the soil depth (0.10 m), soil density (1200 kg m^{-3}), and the amount of soil (50 g – dry mass) in the biometric flasks. The solutions were prepared by dissolving the analytical standards in acetone, and then the solution containing the radiolabeled herbicide was added. The final solution concentration was 310, 108, and 82 µg mL^{-1} when diuron, hexazinone, and sulfometuron-methyl were radiolabeled, respectively. Two hundred microliters of the solutions were applied so that the soils of the flasks were treated with 17.9, 16.2, and 17.1 kBq of diuron, hexazinone, and sulfometuron-methyl, respectively. After application, the soils were homogenized with a spatula. The flasks were sealed with a rubber cap and a soda-lime filter containing cork to ensure that the collected CO_2 came only from soil respiration. Biometric flasks were incubated in a dark room at 20 $\pm$ 2 °C for 70 d.

Herbicide mineralization was evaluated at 7, 14, 21, 28, 35, 42, 49, 56, 63, and 70 d after treatment in triplicate. 10 mL of 2 M NaOH solution was added to the biometric flasks to monitor the release of ^{14}C–CO_2. At the evaluation times, two aliquots (1 mL) were taken in triplicate and transferred to flasks containing 10 mL of the scintillation solution. The ^{14}C–CO_2 fixed was determined by Liquid Scintillation Spectrometry (LSS) by 15 min with a Tri-Carb 2910 TR LSS counter (PerkinElmer, Waltham, MA, USA). The remaining NaOH solution was discarded, and a fresh solution was added. This process was repeated weekly at the evaluation times.

Extraction of the herbicides remaining in soils was performed at 0, 7, 14, 28, 42, 56, and 70 d. Samples were destructive, and all soil was transferred from the biometric flasks (50 g) to centrifuge tubes. In the diuron and hexazinone extraction, methanol (100 mL) was added to the soil samples, and the tubes were stirred for 1 h (200 rpm). Then, the tubes were centrifuged for 15 min (3000 rpm), and the supernatant was collected. This procedure was repeated twice. However, in the second and third stages, 80 and 70 mL of methanol were added, respectively. Two aliquots (1 mL) were collected from the total of the three extractions performed (250 mL methanol), transferred to flasks containing 10 mL of the scintillation solution, and the initial concentration of ^{14}C-herbicides was determined by LSS. For sulfometuron-methyl extraction, the soil samples were combined with 100 mL of a solution containing the solvents methylene chloride, methanol, and 2 M $(NH_4)_2CO_3$ (3:4:1). The suspension was stirred for 1 h, then centrifuged for 10 min (4000 rpm), and then the supernatant was collected. Another 70 mL of the solvent solution was added and the procedure was repeated. The final extracts were reduced to the volume of approximately 60 mL in a rotary evaporator (45 °C), and the pH was adjusted (pH 5) using glacial acetic acid. The extracts were transferred to a separation funnel, and the herbicide present in the solution was extracted with methylene chloride. This procedure was performed four times. The methylene chloride solution was reduced by a rotary evaporator under the same conditions as above. The generated aqueous phase was reduced to verify the presence of radioactivity and, once no radioactivity was detected, it was discarded. Two aliquots (1 mL) of concentrated extract were collected and transferred to flasks containing 10 mL of the scintillation solution, and the initial concentration of ^{14}C-herbicides was determined by LSS.

The presence of undegraded herbicide (extractable residue) in the soil was analyzed using Thin Layer Chromatography (TLC). For this, the herbicide extracts were then concentrated on a rotary evaporator (45 °C). 100 µL of the concentrated extracts and the herbicide standards were applied to silica gel TLC plates (60 F254 100 Aluminum, Merck, Darmstadt, Germany), previously activated at 250 °C for 2 h. The plates were placed in a TLC chamber containing 100 mL of acetonitrile solution for hexazinone and hexane-acetone (6:4, v/v) for diuron. For sulfometuron-methyl, the elution system was prepared with acetonitrile, ethyl acetate, and formic acid (150/50/1.5, v/v/v). After solvent elution, the plate was withdrawn from the TLC chamber and remained exposed until the solvent completely evaporated. The hexazinone samples were analyzed in a TLC automatic analyzer. The analysis of sulfometuron-methyl and diuron was performed using TLC plates (60F254, EMD Millipore). The TLC plates were kept in contact with the phosphorescent film for at least 24 h. After this period, the plates were visualized by autoradiography with X-ray film. The ^{14}C distribution of each herbicide was then determined using a Cyclone Plus Phosphor Imager equipped with Super Resolution Phosphor Screens (Model C431200, PerkinElmer Inc., Shelton, CT, USA).

After the herbicide extraction process, the soil (50 g) was used to determine the bound residue of herbicides in the soil. These samples were oven-dried overnight at 105 °C, weighed, ground, and homogenized in a mechanical mill (Marconi MA330, Piracicaba, SP, Brazil). Subsamples of each soil were weighed in triplicate (0.2 g, dry basis) and oxidized in an OX500 biological oxidizer (R.J. Harvey Instrument Corporation, Tappan, NY, USA) to determine the radioactivity amount of ^{14}C-herbicide mineralized to ^{14}C–CO_2. The ^{14}C-herbicides concentration was determined using an LSS.

The recovered radioactivity mass balance was verified by the sum of the percentage of ^{14}C–CO_2 released, the herbicides extracted (extractable residue) by the solvents, and the soil sample oxidation (non-extractable or bound residue). The results ranged between 90 and 110%, as shown in the "Aerobic and Anaerobic Transformation in Soil" guide [30].

2.4. Biodegradation model to determine DT$_{50}$

Based on the results obtained with TLC of the extracted residue, we were able to define the DT$_{50}$ of the three herbicides in the two studied soils to quantify the resulting metabolites from the degradation of the herbicides during incubation. The data on the amount of ^{14}C-herbicides were suitable for the Michaelis-Menten kinetics [31,32] adapted for hyperbolic decay with 2 parameters: C(t) = C_{max}xDT$_{50}$/(DT$_{50}$ + t), where C is the herbicide concentration in incubation time (% of initially applied herbicide); C_{max} is the highest

concentration of extractable herbicide on day 0 (% of initially applied herbicide); DT_{50} (d) is defined as the time required for 50% of the applied herbicide to be degraded, and t is time. This model has the assumption that the bound residues are inert and will not be degraded.

2.5. Statistical analysis

The experimental arrangement was completely randomized, in duplicate. Evaluation of ^{14}C–CO_2 release was performed in triplicate, and the statistical analysis was conducted separately for each herbicide. Box-Cox transformations were used to analyze the data [33]. After data transformation, according to the recommended lambda, the analysis of variance (ANOVA) was performed. Data analysis was performed using R software [34]. All results were considered significant when $p < 0.05$. Data on extractable and non-extractable radioactivity and herbicide degradation were evaluated in a descriptive way using figures.

3. Results

3.1. Herbicides mineralization

The ^{14}C–CO_2 released from diuron ranged from approximately 7%–11% (% total applied) in sandy and clay soils, respectively, after 70 d (Fig. 1). The hexazinone and sulfometuron-methyl applied in a mixture with diuron did not affect the percentage of ^{14}C–CO_2 released from diuron at 70 d, compared to the application of diuron alone, regardless of the soil (Fig. 1).

The percentage of ^{14}C–CO_2 of hexazinone in a mixture with diuron and sulfometuron-methyl was 35% (Fig. 2D), while applied alone was 28% in clay soil after at 70 d (Fig. 2C). After a lag phase between 7 and 21 d, the ^{14}C–CO_2 release increased. In sandy soil, hexazinone in a mixture with diuron and sulfometuron-methyl had a percentage of ^{14}C–CO_2 evolved by 22% (Figs. 2B) and 18% when applied alone (Fig. 2A).

The maximum percentage of ^{14}C–CO_2 released from sulfometuron-methyl mineralization applied alone was 5.9% in clay soil (Fig. 3C) and 0.88% in sandy soil (Fig. 3A), compared to the total applied after 70 d of incubation. On the other hand, sulfometuron-methyl used in a mixture showed slightly higher mineralization (0.33%) (Fig. 3D) than treatment with the alone herbicide in the clay
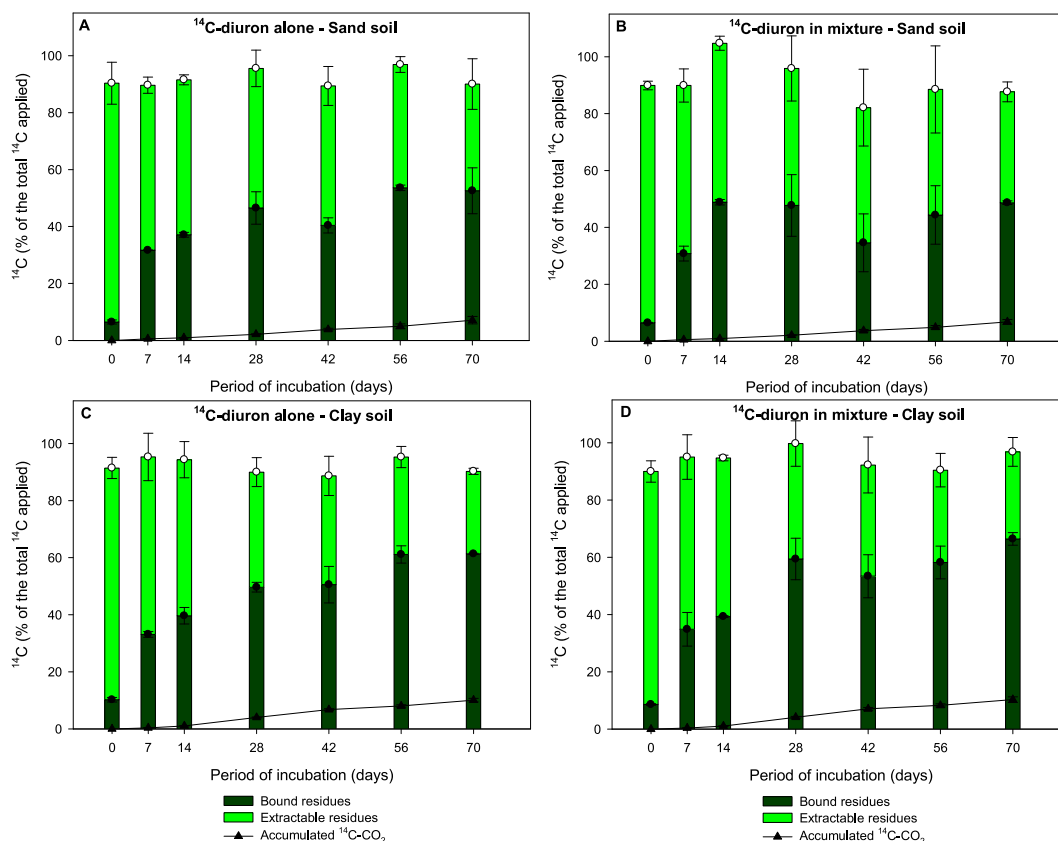
Fig. 1. Distribution of ^{14}C-diuron applied alone (A and C) and in a mixture (B and D) with hexazinone and sulfometuron-methyl in sand (A and B) and clay soils (C and D) among extractions with solvent, mineralized ^{14}C–CO_2, and bound residues (%) as a function of incubation time (70 d) in soil samples. Vertical bars represent standard deviations ($\pm$SD) of means ($n = 2$).
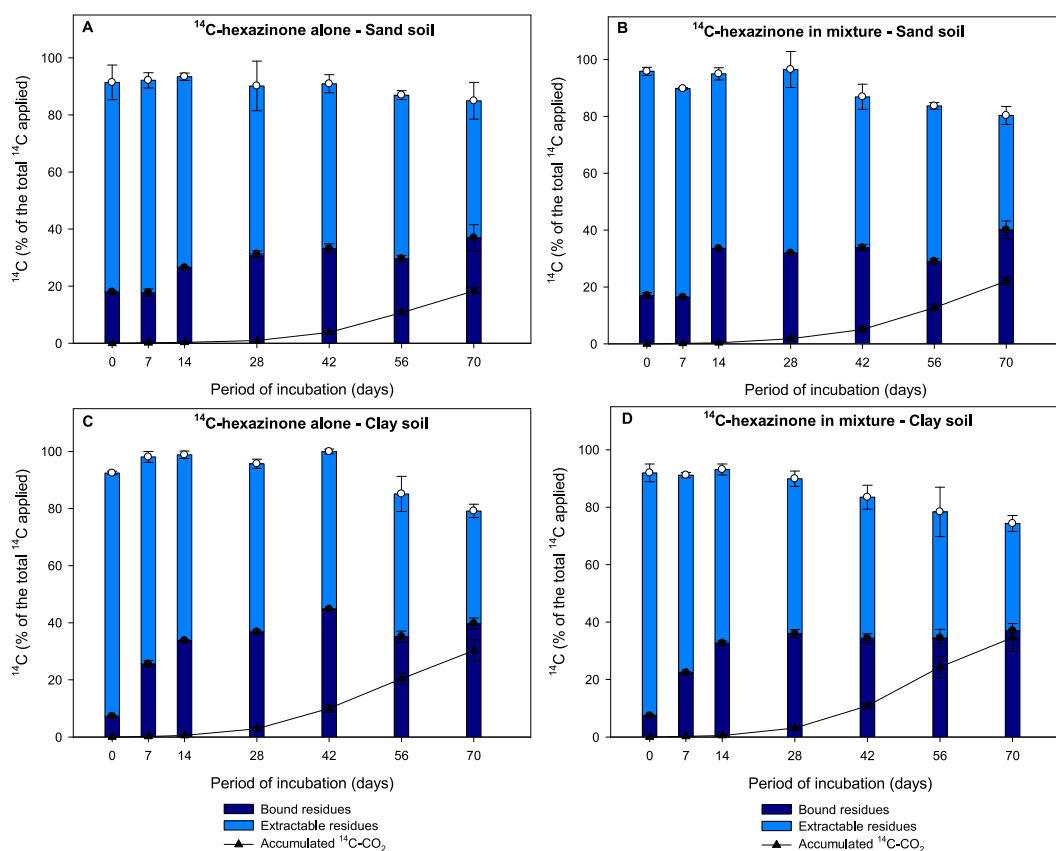
Fig. 2. Distribution of ^{14}C-hexazinone applied alone (A and C) and in a mixture (B and D) with diuron and sulfometuron-methyl in sand (A and B) and clay soils (C and D) among extractions with solvent, mineralized ^{14}C–CO$_2$, and bound residues (%) as a function of incubation time (70 d) in soil samples. Vertical bars represent standard deviations (±SD) of means ($n = 2$).

soil (Fig. 3C).

### 3.2. Herbicides extractable and bound residues

The average radioactivity obtained from the treatments in which herbicides were applied in mixtures or alone was calculated for each soil to evaluate the extractable and bound residues. The percentage of radioactivity extracted decreased with time (0–70 d) for all herbicides (Figs. 1–3). The percentage of the extractable residue of diuron was higher (~38%) in the sandy soil than in the clay soil (~30%) regardless of how it was applied (mixture or alone) (Fig. 1A and B). Hexazinone used alone showed 48% of extractable residue and this amount decreased to 40%, when applied in a mixture with diuron and sulfometuron-methyl on sandy soil (Fig. 2A and B). However, for the clay soil, the extractable residue was not different for the application forms of the herbicide (Fig. 2C and D). The extracted residue of sulfometuron-methyl was higher (70%) when applied alone than in a mixture with hexazinone and diuron (61%) on the sandy soil (Fig. 3A and 1B). For the clay soil, the extracted residue was similar regardless of the form of application (Fig. 3C and D).

The herbicides show no differences in the formation of bound residue when applied in a mixture or alone. However, differences were observed for the soil type analyzed (Figs. 1–3). The percentage bound residue of diuron was approximately 50 and 60% of the total applied in sandy and clay soils, respectively (70 d) (Fig. 1). Hexazinone bound residue was about 40% in both soils at 70 d (Fig. 2). The percentage bound residue of sulfometuron-methyl was approximately 30 and 50% in sandy (Fig. 3A and B) and clay soil (Fig. 3C and D), respectively (Fig. 3).

3.3. Herbicides biodegradation

The proposed model showed a good fit of the curves with R^2 ranging from 0.84 to 0.95 ($p < 0.01$). The behavior of the degradation curves of diuron (Fig. 4A) and sulfometuron-methyl (Fig. 4B) were similar, being possible to observe the decay over the evaluated period (70 d). Diuron, regardless of the form of application, in clayey soil showed the most pronounced curve decay. At 40 d, approximately 53% of diuron was degraded to the amount of initial total extractable residues (C(0) = 81.19%). On the other hand, in sandy soil, the behavior of the diuron degradation curve was less pronounced, with ~73% degradation to the initial extractable
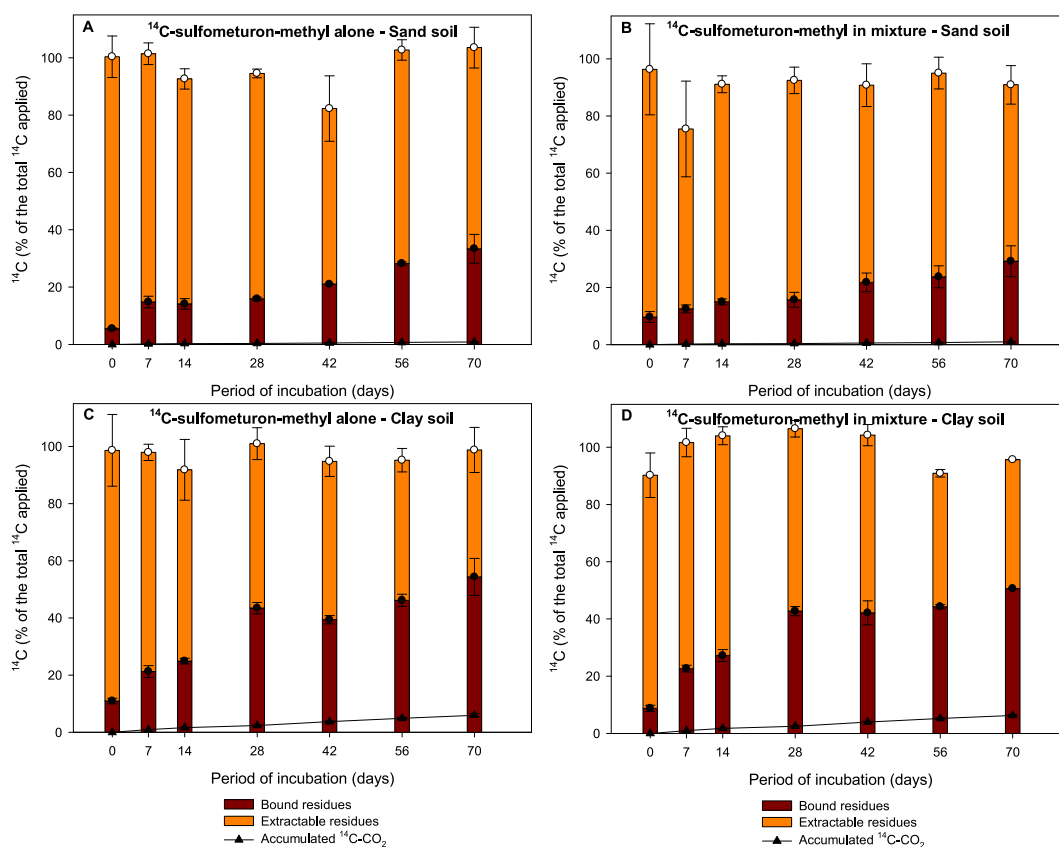
Fig. 3. Distribution of ^{14}C-sulfometuron-methyl applied alone (A and C) and in mixture (B and D) with hexazinone and diuron in sand (A and B) and clay soils (C and D) among extractions with solvent, mineralized ^{14}C–CO$_2$, and bound residues (%) as a function of incubation time (70 d) in soil samples. Vertical bars represent standard deviations ($\pm$SD) of means ($n = 2$).

residues (C(0) = 83.85%) at 40 d (Fig. 4A). For sulfometuron-methyl at 40 d, the degradation was 43% (C(0) = 87.67%) and 67% (C(0) = 94.86%) for the clay and sandy soils, respectively (Fig. 4C). The decay of the hexazinone degradation curve was steep for the clay soil and showed a low decline for the sandy soil (Fig. 4B). At 40 d, the degradation of hexazinone was ~69% (C(0) = 85.13%), while in sandy soil, it was 91% relative to the amount of extractable residue (Fig. 4B).

The DT$_{50}$ was greater than 70 d on the sandy soil for all three herbicides regardless of the form of application (Fig. 4). Among the three herbicides, only the DT$_{50}$ of hexazinone has also been above the study incubation time in clay soil (Fig. 4B). The degradation time of the diuron applied alone to the clay soil was slower (DT$_{50}$ = 58 d) than used in a mixture with hexazinone and sulfometuron-methyl (DT$_{50}$ = 54 d) (Fig. 4A). Sulfometuron-methyl had a DT$_{50}$ from 42 to 37 d when used alone and in a mixture in clay soil (Fig. 4C).

4. Discussion

The application of the herbicides alone or in a mixture in the soil affected the mineralization of the compounds. In the treatment with diuron application, there were no changes in mineralization and bound residue. However, the DT$_{50}$ value was slightly higher (4 d) when diuron was applied alone compared to mixture with hexazinone and sulfometuron-methyl. Diuron is a neutral herbicide with a higher affinity to the soil solid phase. When added to a mixture with hexazinone and sulfometuron-methyl, both have a lower affinity to the soil solid phase. Diuron can compete by sorptive soil sites and is little influenced by combination. In addition, diuron has the presence of an aromatic ring and low water solubility, which intensifies the competition for hydrophobic regions such as OM [35]. A greater difference was observed in the study with the mixture of diuron, hexazinone, and sulfometuron-methyl in Oxisol [19]. The authors observed that the mixture of diuron, hexazinone, and sulfometuron-methyl showed a reduction in DT$_{50}$ ranging from 101 to 66 d, being related to the higher sorption of diuron when applied alone.

The diuron behavior is strongly influenced by the soil physicochemical properties, as evident by the difference in DT$_{50}$ of the evaluated clay and sandy soils observed in this study. This result can be attributed to the higher OM and clay content of the clay soil increasing the charges for diuron sorption and consequently lower availability for microbial degradation. This difference in diuron degradation may be related to increased CEC in the clay soil (100.2 mmol$_c$ kg^{-1}) about the sandy soil (35 mmol$_c$ kg^{-1}) (Table 2). A higher CEC improves the bioavailability of soil nutrients and may favor microbial activity, indirectly increasing diuron biodegradation [36]. Similar results were observed in the degradation of diuron applied to tropical soils at 156 d [37]. The authors followed that the
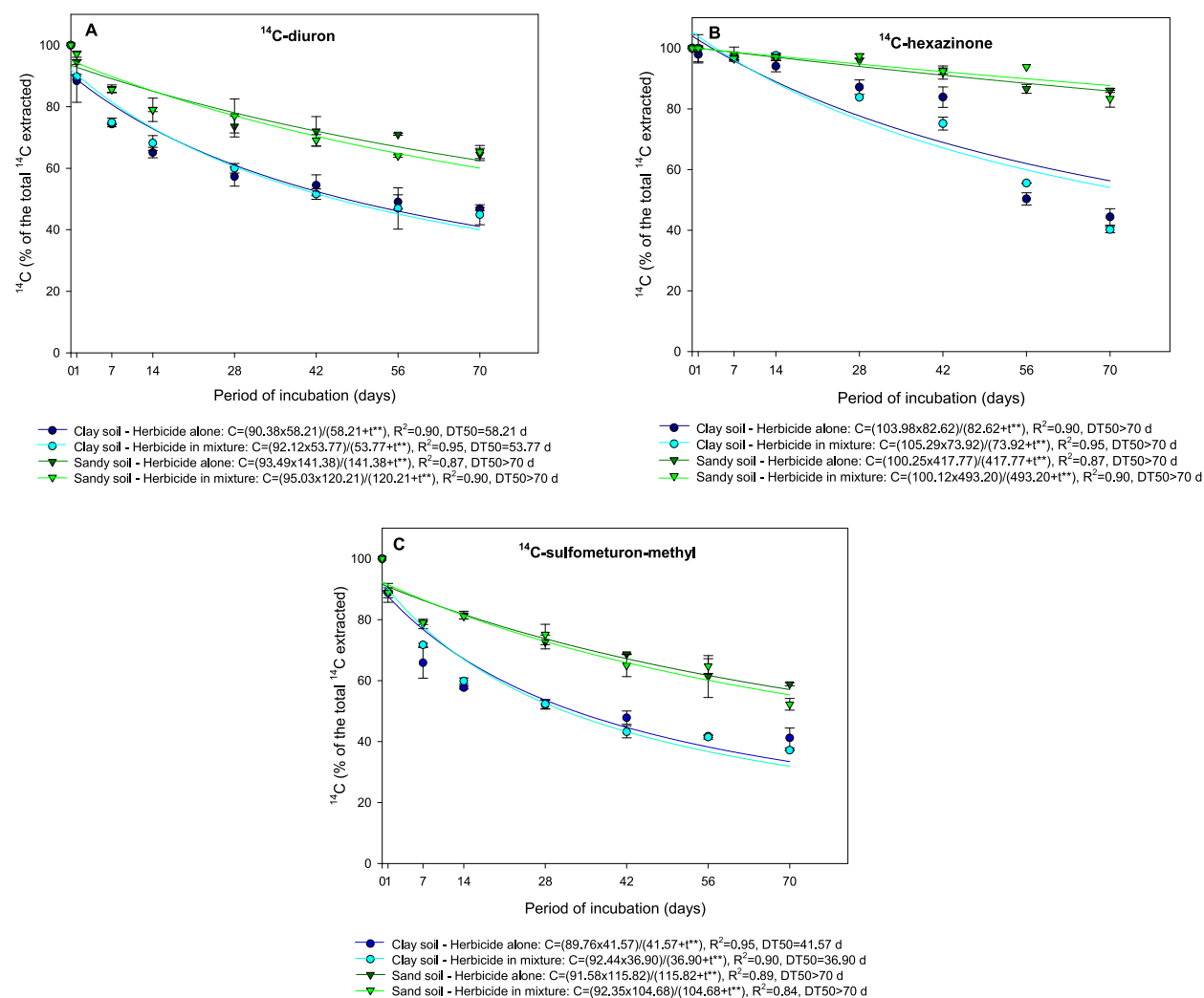
Fig. 4. Degradation of the ^{14}C-diuron (A), ^{14}C-hexazinone (B), and ^{14}C-sulfometuron-methyl (C) compared to the amount of each herbicide after extraction in time (extractable residue), separated by TLC plates in parental compound (%) when applied alone and in sand and clay soils, as a function of incubation time (70 d) in soil samples. The vertical bars inserted in the symbols and bars represent the standard deviation ($\pm$SD) of the mean ($n = 2$). $**p < 0.01$ by the F test.

diuron degradation was higher in clay soils ($DT_{50} = 46$ d) than in sandy ($DT_{50} = 91$ d). The degradation rate of diuron is not positively correlated with soil pH and OM content [38]. Biodegradation is the main route for diuron dissipation in the environment [39–41], and a higher OM content would increase the microbial population and herbicide degradation. However, a higher OM content may increase diuron sorption, making it unavailable for mineralization, but if the herbicide desorption process to soil solution occurs, microorganisms can easily degrade the herbicide.

Herbicides biodegradation in the soil is characterized by transforming the compound into mineralization products, microbial biomass, metabolites, and bound residue [42]. The bound residues is the result of physicochemical interactions between the original compound and its metabolites with soil OM [43,44]. The bound residues are defined as the residues that continue to bond in soil after extraction with methods which do not considerably modify their chemical nature [45,46]. As the herbicide remains in the soil, the physicochemical interactions and the amount of non-extracted residue increase. The decrease in herbicide bioavailability, or other organic compounds, depending on the time of soil contact, is often termed aging [47,48]. The bound residue of diuron formed was high (60% in the clay soil). OM is responsible for the more pronounced formation of bound diuron residues over time in Amazonian soils [49]. Diuron sorption in weathered soil was amended with adding OM to the soil [50]. It was observed by the authors to have a high percentage of bound residue in soils with high OM content. Values were similar to those followed by Guimarães et al. [36], who reported values ranging between 31.9% and 58.5% (70 d) of the total applied in five sugarcane cultivated soils. However, these authors noted that the diuron bound residues were released into the soil after 42 d. That is, after the formation of the bound residue of diuron, the herbicide can be returned (remobilization) of the herbicide into the soil solution and affect non-target organisms [51]. The remobilization was not studied in this study, but should be considered when assessing the environmental risk of herbicides.

The higher mineralization and degradation of hexazinone when in mixture with diuron and sulfometuron-methyl can be related to the higher bioavailability of hexazinone in the soil solution. Hexazinone exhibits high hydrophilicity ($S_w = 33,000$ mg L^{-1} and log K$_{ow}$ = 1.17) [14] compared to diuron and sulfometuron-methyl, enabling less competition with hydrophobic herbicides for available sorption sites in soil colloids. The study of hexazinone sorption in a mixture with diuron and sulfometuron-methyl in soil cultivated with sugarcane showed a reduction of hexazinone sorption by ~96% [18]. Hexazinone would become, by competitive sorption, more bioavailable in soil solution, and consequently, more susceptible to microorganisms, decreasing the DT$_{50}$ of hexazinone in soil. However, different results were observed for hexazinone in a mixture with diuron and sulfomethuron-methyl [19]. These authors reported that a mixture increased the DT$_{50}$ of hexazinone from ~30 to ~50 d, even though it showed lower sorption when applied in a mixture, which can be related to the acidic pH of the soil. Herbicides behavior in soil is very complex and depends on the interaction of several factors [5].

Hexazinone showed a higher degradation proportional to the ^{14}C–CO$_2$ produced in the clay soil [higher mineralization (35%) and shorter time to degradation (DT$_{50} > 70$ d)] compared to sandy soil. The higher OM content characteristics of the clay soil enhanced the soil microbial activity. Hexazinone degradation positively correlated with soil OM content [36]. A higher degradation rate of hexazinone was observed in soil with higher OM content, showing a higher bacterial community than the other soils, possibly the main reason for hexazinone degradation [52]. The amount of bound residue of hexazinone was similar between the two soils studied (~40%). Similar results (from 33.7 to 49.5%) were observed for hexazinone in soils that varied in clay content, CEC, pH, and OM [36]. The bound residue of hexazinone was evaluated in soils fertilized with various types of sugarcane residues, and the proportion of residues that remained as bound residues ranged from 23.3 to 48.1% [51].

Sulfometuron-methyl showed higher mineralization and slightly higher degradation when in a mixture with hexazinone and diuron. The presence of diuron may have directly affected the binding of sulfometuron-methyl to soil loads and, consequently higher bioavailability in the soil solution for microbial degradation. The mixture of sulfometuron-methyl with diuron and hexazinone was evaluated for sorption and desorption when applied in a mixture or alone [18]. These authors observed that the presence of diuron strongly affected the sorption process of sulfometuron-methyl, showing that this relationship between sulfometuron-methyl and diuron reinforces the evidence that herbicides with more remarkable similarity in molecular structure and hydrophobicity compete intensely for sorption sites.

The higher rate of mineralization of sulfometuron-methyl in clay soil suggests further degradation and, consequently a decrease in the amount of bioavailable herbicide in the soil solution. Trubey et al. [17] reported that the DT$_{50}$ of sulfometuron-methyl ranged from 12 to 25 d in a field study in different regions of the USA. Our data on the biodegradation of diuron, hexazinone, and sulfometuron-methyl were consistent with that on the mineralization and formation of bound and extractable residues in both soils, with an evident influence of soil texture on herbicide behavior. The sandy soil likely contained no microorganisms capable of sulfometuron-methyl mineralization. In addition, the sandy soil presented a lower OM content (11 g kg^{-1}) which is associated with a smaller microbial population that would be able to mineralize the herbicide. In sandy soils, OM is the main constituent for sorption, and in clay soils, OM has less importance in sorption capacity. Sulfometuron-methyl did not affect the microbial biomass or respiration rate in forest soils [53]. The influence of OM on sulfometuron-methyl biodegradation was observed in a bioassay conducted with corn roots [54]. It was followed by the authors that the sulfometuron-methyl degradation was higher in loam soil with a higher OM (40 g kg^{-1}) than in loam sandy soil (23 g kg^{-1}).

The high formation of bound residues means that the herbicide bioavailability will be reduced by sorption. When they return to the solution, they can cause carryover to successive crops. In a previous study, the percentage sulfometuron-methyl residue bound to soil colloids increased during the incubation period and peaked at 50 d at 56% [55]. Anderson and Dulka [16] noted that as the incubation time increased, the percentage bound residue of sulfometuron-methyl also increased. These authors reported that radiolabeled bound residues were 5% of the total applied at time zero and increased to 21% at the end of the incubation period (24 wk).

The results reported in this study show that care should be taken when choosing to apply herbicides in mixtures. Recent studies have shown that the a mixture of diuron, hexazinone, and sulfometuron-methyl decreased sorption, increased leaching, increased DT$_{50}$ of the herbicides, and affected soil microbial diversity [18,19,56]. However, regarding mineralization and degradation of the herbicides, the mixture increased the degradation of hexazinone and sulfometuron-methyl in this study. Despite the increased degradation when these herbicides were applied in a mixture, it should be taken into consideration that the other behavioral processes, such as leaching and sorption, are influenced and can potentially impact non-target organisms. On the other hand, high herbicide mineralization and degradation could mean a loss of agronomic efficiency, especially for hexazinone, due to a lower residual effect. Therefore additional studies to evaluate the persistence of herbicides applied in a mixture should be considered to predict the molecules final fate.

5. Conclusion

Hexazinone and sulfometuron-methyl mineralization to ^{14}C–CO$_2$ was higher when these herbicides were applied in a mixture than alone. It indicates a positive impact, as the herbicide will be bioavailable in the soil solution for a shorter period than when used alone. However, other behavioral processes of herbicides, such as sorption and leaching, must be considered when applying herbicides in mixtures, as they can directly impact non-target organisms and environmental contamination. The percentage of residue extracted decreased over time for all herbicides. Diuron had the highest percentage of bound residue. Herbicide degradation was higher in the clay soil regardless of the mode of application, which is related to the higher potential of the bacterial community in the clay soil to mineralize the herbicides.

Additional information

Supplementary content related to this article has been published online at [URL].

Author contribution statement

Data availability statement

No data was used for the research described in the article.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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